

NEUROMUSCULAR CHARACTERISTICS OF MULTIPLE SCLEROSIS PATIENTS

By

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DECLARATION

I declare that this thesis was composed by myself and that all the data were collected and analysed by myself. Neither the thesis nor the original work therein has been submitted to this or any other institution for a higher degree.

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ABSTRACT

Aim: The aim of this study was to describe the neuromuscular characteristics of Multiple Sclerosis (MS) patients. To help explain the neuromuscular characteristics physical activity levels, body composition and blood lipid profile were measured. In addition, Vitamin D was measured to determine if this was deficient in MS patients.

Hypothesis: We hypothesised that muscle fibre conduction velocity (MFCV) would be elevated and amplitude would be impaired in Multiple Sclerosis patients compared to an age and sex matched control group. In addition, we hypothesised that physical activity levels would be reduced, body composition would exhibit a higher percentage fat, blood lipid profile would be less favourable and Vitamin D levels would be reduced in the group of MS patients.

Methodology: 15 MS patients (53.8 ± 10.5 years) and 14 age and sex matched control participants (54.6 ± 9.6 years) were recruited for this study. Patients with a disability status (EDSS) (92) of between 4 and 6 were included in this study. All participants provided written informed consent after being fully informed of the procedures.

An array of 4, 8mm Ag-AgCl electrodes was placed in a hard plastic mould in a straight line, leaving 12.5mm between each electrode. The array was positioned on the vastus lateralis between the innervation zone and the distal tendon and was orientated to follow the muscle fibre pennation direction. Electromyographic (EMG) data was collected via the electrodes whilst the participant carried out each contraction. Muscle fibre conduction velocity (MFCV) and root mean square (RMS) were calculated from the raw EMG signal collected during each contraction.

The protocol was carried out on both legs. Isometric knee extensions were standardised using the Bio-Dex Systems 3 Isokinetic Dynamometer and executed with the knee at 60° angle of flexion. Participants were tested under four different contraction intensities; 20, 40, 60 and 80% of the peak value of their maximum voluntary contraction (MVC). Their MVC was established for both legs. For each test condition the participant was required to carry out 3 isometric contractions for 7 seconds; each contraction was separated by 14 seconds rest. During each set of contractions the target force was visible on the Bio-Dex monitor. Participants obtained visual feedback from the Bio-Dex throughout each contraction allowing the participant to adjust the force they were required to produce to maintain their target force.

Whole body composition (fat mass, lean mass and bone mineral content) was measured by Dual-energy X-ray Absorptiometry (DEXA) using a Lunar Prodigy DF+ 13643, GE medical systems scanner. Whole body and thigh composition were extrapolated from the digitalised results of the scan.

Habitual physical activity was measured for 7 days using the GT1M Actigraph accelerometer, which was worn on the participants' right hip during all waking hours. Data collected by and downloaded from the accelerometer was used to calculate each participant's total and mean accelerometer counts per day.

A fasted 10ml sample of venous blood was drawn from an antecubital vein using a 10ml syringe and a 15mm gauge needle. The blood samples were analysed for total cholesterol, triglyceride and high density lipoproteins (HDL), low density lipoprotein (LDL) and Vitamin D concentration.

Results: Initial findings revealed that the patient group exhibited a significantly faster ($P<0.05$) MFCV of the dominant leg than the control group. The difference in MFCV of the non dominant leg was approaching significance ($P=0.054$). However, after identifying a significant difference ($P<0.05$) in percentage fat of the thigh between the patient and the control group and revealing a strong positive correlation between MFCV and percentage fat of the thigh ($r=0.697$, $P<0.001$), percentage fat of the thigh was added into the analysis of variance for MFCV between the two groups. Findings revealed that there was no significant difference ($P>0.05$) in MFCV over all the contractions between the groups. There was no significant difference ($P>0.05$) in MFCV between the dominant and non-dominant leg within each group.

Analysis of the RMS data revealed that there was no significant difference ($P>0.05$) over all the contractions between the patient and the control group in either the dominant or the non-dominant leg. However there was a highly significant difference ($P<0.001$) in RMS between contraction intensity in both legs in both groups.

There was a significant difference ($P<0.05$) in maximum voluntary contraction (MVC) between the patient ($133.65\pm 54.20\text{nm}$, $115.21\pm 43.41\text{nm}$) and the control group ($175\pm 43.94\text{nm}$, $160.14\pm 47.55\text{nm}$). However, torque production per gram of muscle mass was not significantly different between the patient ($0.024\pm 0.0048\text{nm}\cdot\text{g}^{-1}$, $0.021\pm 0.0045\text{nm}\cdot\text{g}^{-1}$) and the control ($0.026\pm 0.0076\text{nm}\cdot\text{g}^{-1}$, $0.024\pm 0.0047\text{nm}\cdot\text{g}^{-1}$) group. No significant difference ($P>0.05$) was identified between the dominant and non-dominant leg within each group.

There was no significant difference ($P>0.05$) in absolute lean thigh mass (LTM) between the two groups however the difference in relative lean thigh mass (lean thigh mass/lean body mass*100) between the groups was approaching significance ($P=0.077$).

The patient group exhibited a significantly ($P<0.05$) higher whole body ($43.8\pm 7.2\%$, $34.28\pm 9.64\%$) and thigh fat ($44.46\pm 7.54\%$, $34.25\pm 10.34\%$) percentage than the control group. There was no difference ($P>0.05$) in whole body or thigh bone mineral density (BMD) between the groups.

The patient group was significantly ($P<0.05$) less physically active than the control group; this was evident from their total accelerometer counts/day (patients 167088 ± 113586 , control 316401 ± 108982) and mean accelerometer counts/day (patients 206.4 ± 139.5 , controls 364.5 ± 120.6).

There was no significant difference ($P>0.05$) in blood lipid profile (cholesterol, triglycerides, HDL or LDL) between patients and control group. However there was a significant difference ($P<0.05$) in serum Vitamin D concentration between the patient and the control group.

Conclusion: MS patients exhibited faster MFCV with a similar number of motor units recruited than healthy ages and sex matched controls. However, an increased quantity of subcutaneous fat artificially elevates MFCV (110) and MS patients in this study exhibit higher percentage fat than the controls. Therefore, when accounting for this confounding variable we then showed that there was in fact no difference in MFCV between the groups. However the patients MFCV responded differently to the increased contraction intensity than the controls which suggests altered neuromuscular processing within the patient group.

The MS group had reduced physical activity levels which caused a slight disuse atrophy of the lower limbs which did not compromise muscle quality. Therefore, similar quality of the muscle has resulted in unaltered neuromuscular recruitment in MS patients.

Overall, the results indicate that there was no difference in neuromuscular characteristics of MS patients compared to age and sex matched controls, even in the presence of altered body composition and physical activity levels.

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ABBREVIATIONS

Abbreviation	Description
Ag-AgCl	Silver to Silver Chloride
ANOVA	Analysis of Variance
BBB	Blood Brain Barrier
BDNF	Brain Derived Neurotrophic Factors
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
cLDL	Calculated Low Density Lipoproteins
CNS	Central Nervous System
DEXA	Dual Energy X-Ray Absorbtiometry
EDSS	Expanded Disability Status Scale
EDTA	Ethylenediaminetetraacetic acid
HDL	High Density Lipoproteins
IIDD	Idiopathic Inflammatory Demyelinating Disease
LBM	Lean Body Mass
LTM	Lean Thigh Mass
MANOVA	Multivariate Analysis of Variance
MFCV	Muscle Fibre Conduction Velocity
MS	Multiple Sclerosis
MVC	Maximum Voluntary Contraction
NGF	Nerve Growth Factors
PCr	Phosphocreatine
RMS	Root Mean Squared
sEMG	Surface Electomyography

1. Introduction

Multiple Sclerosis (MS) is a degenerative, demyelinating disease of the central nervous system (CNS) (154) characterised by spinal chord lesions which cause subsequent neuronal damage, resulting in marked physical disability (33). As a consequence of the neuronal damage of the CNS the delivery of neural activity to skeletal muscle is affected (148; 150). Therefore it is well accepted that the neural activity received by the muscle dictates the phenotypic expression of the muscle (133). Buller et al (1960) (21) demonstrated that when nerves from fast motor units are made to innervate a slow muscle, the muscle is transformed into a fast muscle and likewise slow motor units converted fast muscle to slow. In addition, Roy et al (1992) (143) demonstrated that after 6 months of spinal chord isolation mammalian skeletal muscle exhibited a significantly greater proportion of type II fibres. Concurrently, Castro et al (1999) (29) demonstrated a significant shift towards type IIx fibres in human patients within 6 months of complete spinal chord injury.

MS is characterised by spinal chord lesions which affect the neural transmission along the axon (154), therefore the neural activity received by the muscle of MS patients will be altered. Lack of neural innervation can also result from skeletal muscle inactivity as a result of bed rest, denervation, hind limb unloading, immobilisation or micro gravity (181).

Inactivity results in muscle atrophy which is characterised by a decrease in protein content, fibre diameter, force production, fatigue resistance (78) and a higher proportion of type II muscle fibres (101). As it has been demonstrated that a higher proportion of type II fibres can be indicative of disuse, it is reasonable to expect MS patients to exhibit a higher proportion of type II muscle fibres compared to equivalent controls. Kent-Braun et al (1998) (82) demonstrated that MS patients exhibited fewer type I muscle fibres in

the tibialis anterior than control participants. Kent-Braun et al (1994) (83) also revealed that phosphocreatine (PCr) resynthesis was slower in MS patients compared to controls; which indicates that MS patients exhibited a reduced oxidative capacity to generate ATP which is indicative of an increased expression of fatigable fast twitch muscle fibres. In addition, a strong positive correlation ($r=0.84$) between relative area of fast twitch fibres and muscle fibre conduction velocity (MFCV) measured using surface EMG was revealed by Sadoyama et al (1988) (145). Therefore the rate of the MFCV measured using surface EMG technique is indicative of the proportion of fast twitch fibres within the muscle.

It has been shown that elevated expression of type II fibres within the muscle is indicative of disuse (82). Differences in fibre type expression between MS patients and controls have been elucidated using histochemical techniques (82). However to our knowledge differences in MFCV between MS patients and controls using surface EMG technique have not been investigated.

Therefore the present study employed surface EMG technique to investigate if there was a difference in MFCV between MS patients and control participants. In order to explain any differences in MFCV physical activity and body composition was also measured.

2. Literature Review

2.1. Defining Multiple Sclerosis

Multiple Sclerosis (MS) is a degenerative, demyelinating disease of the central nervous system (CNS) (154) characterised by spinal chord lesions which cause subsequent neuronal damage, resulting in marked physical disability (33). Patients with MS experience numerous symptoms, including spasticity, fatigue, cognitive dysfunction, depression, bladder dysfunction, bowel dysfunction, sexual dysfunction and pain (33). The disease is characterised by episodes of relapse and remission however, the disease is inevitably progressive (154). About 85% of patients present with the relapsing-remitting form of MS, where symptoms suddenly deteriorate then remain static for a period of time. The remaining 15% present without relapses but show a slow progressive pattern where the symptoms steadily worsen over time. This is known as primary progressive MS (116).

Jean-Martin Charcot identified MS as a distinct entity in 1868 (161). Since then, research into the cause, nature, treatment and cure of MS has been ongoing. Today, understanding of the disease has improved greatly, however, the cause of the disease remains a mystery and there is still no known cure. This literature review aims to outline current opinion on the epidemiology and aetiology of MS, explore the affect the disease has on the neuromuscular system and investigate the benefits of physical activity for MS patients. The review also looks at the use of surface EMG techniques to determine muscle fibre conduction velocity.

2.2. Epidemiology of Multiple Sclerosis

2.2.1. Prevalence of MS

There is considerable variation in the prevalence of MS throughout the world however Scotland has the highest prevalence of Multiple Sclerosis (142) with an annual incidence of 12.2 per 100,000 in the South East of Scotland (142) which is almost double that reported for the whole of the UK (2). In addition, an annual incidence of MS of 7.2 per 100,000 was reported in Tayside (44); 5.7 per 100,000 in Glasgow (118) and 144 per 100,000 in Grampian (149). Clustering of MS cases has also been reported in Orkney and Shetland Islands as well as the Outer Hebrides (38). The prevalence rate of probable MS has been reported to be as high as 152 per 100,000 in the Shetland Islands (38); 258 per 100,000 in the Orkney Islands (137) and 82.1 per 100,000 for the Outer Hebrides (38). A number of suggestions have been made to explain why the prevalence of MS is elevated within island communities. A Dutch study by Hoppenbrouwers et al (2007) (73) suggests familial aggregation of MS in a genetically isolated population. As the gene pool within the island is largely closed, the genes which are susceptible to MS are more likely to be passed on to the next generation. Moreover, environmental factors (which will be discussed in the following sections) have been suggested to trigger the development of MS (7; 8) therefore the isolated environment of the islands may hold the key to what the environmental trigger is. Taylor et al (1980) (162) suggests that the effect of migration and social structure within the isolated communities may elevate the epidemiological findings. Taylor et al (1980) (162) suggests that lower emigration and higher immigration rates, of people with MS, to the island in addition to higher general rate of emigration from the islands may account in part to the high prevalence rates documented in the island communities in Scotland. In addition, Taylor et al (1980) (162)

postulated that lower rates of immigration to the islands may have influenced the elevated rates of MS recorded in the islands.

Furthermore, the disease presents with a bias towards woman, with the prevalence amongst women almost double that of men (44). A study by Orton et al (2006) (127) revealed a sex bias of MS towards females in Canada with a ratio of 3.2:1. In addition, Iversen (1982) (77) revealed that there were 296 male and 445 female deaths in Scotland attributed to MS between 1973 and 1980, which also highlights the sex bias that MS exhibits.

There is a consensus in many countries that the incidence and prevalence of MS is increasing (127; 180) however this may be attributed to an increase in diagnosis due to an increased number of neurologists (161) and more clearly defined diagnostic criteria (108; 136).

2.2.2. Effect of Latitude

The prevalence has been reported to increase as distance from the equator increases (25). One theory suggests that populations closer to the equator are exposed to more sunlight and therefore exhibit a higher level of serum Vitamin D, which is considered to have a protective effect to developing MS (7). This theory may not be substantiated as it has been shown that Vitamin D insufficiency is also high among populations with highly pigmented skin which make ultraviolet light less efficacious (71).

The prevalence of MS is greater in Caucasian populations than Asian or African populations (7). This may be attributed to the theory that MS is considered to be more prevalent the further you move away from the equator (25) as Caucasians generally exist

further away from the equator than African or Asians. It could however be due to a genetic predisposition within the Caucasian population towards developing MS (142).

Overall the prevalence of MS is increasing and prevalence in Scotland remains particularly high (44; 118; 142). Research into the cause, nature, treatment and cure of MS continues to be a high priority within Scotland due to the disproportionately high prevalence within Scotland (38; 44; 91; 142; 162; 180).

2.3. Aetiology of Multiple Sclerosis

MS is a complex disease and occurs because of a complex interaction between genetic and environmental factors (99).

2.3.1. Immigration Studies

Immigration studies have provided valuable information of the genetic risk of different ethnic groups to developing MS (36-42; 49; 50). The incidence of MS in migrants tends to be intermediate between that of their birth place and that of their residence, and close to the latter when migration occurs in childhood (58). However the shift in risk is not consistent between immigrants who move from low risk to high risk areas and those who move from high risk to low risk (58). High risk areas are considered to be geographical locations where the prevalence and incidence of MS is high, such as Scotland (44; 118; 142).

Dean and Elian (1993) (37) revealed that mortality rates from MS over a 22 year period of UK immigrants to South Africa were twice that of the white South African born population, but only a third of that of the population of England and Wales. Therefore

UK residents who immigrate to low risk areas lower their risk from what it was in the UK but not to the level of the indigenous population.

On the other hand, Dean et al (1976) (40) reported that few immigrants in Greater London from new commonwealth countries within Asia, Africa, America (including West Indies) and Europe (Gibraltar, Malta and Gozo) were discharged from hospital with the diagnosis of MS compared to people born in the UK. In addition Dean et al (1977) (36) concluded that MS is rare in immigrants resident in England and Wales who were born of Indian, Pakistani or African origin. Immigrants from new commonwealth countries exhibit one eighth of the risk of developing MS as UK born residents (58). Therefore immigrants to the UK from new commonwealth countries retain their low risk status although they reside in a high risk area.

Further studies examined whether the low risk of MS observed among immigrants to the UK from the West Indies, the Indian subcontinent, and Africa was shared by the UK born children of these immigrants. Elian and Dean (1987) (49) reported that the UK born children of West Indian immigrants had similar incidence and prevalence of probable MS to that of the indigenous population of Northern Ireland and the Irish Republic. In addition, Elian et al (1990) (50) concluded that children born in the UK of Asian, African and West Indian immigrants have a similar prevalence of MS as the UK population.

Although MS is a complex disease and occurs because of an interaction between genetic and environmental factors (99) these studies indicate that the prevalence and incidence of MS is equivalent across ethnic groups who are born and live in the UK. These findings provide strong evidence that the cause of the disease is primarily environmental and therefore it is potentially preventable (49).

Further evidence for this, Dean and Kurtzke (1971) (39) reported that white immigrants who arrived in South Africa before the age of 15 acquired the lower risk of their country of destination. In contrast white immigrants arriving after they were 15 years of age appeared to retain the higher risk of their country of origin. Therefore the age at immigration from an area of high risk to an area of low risk affected the likelihood of developing the disease. This suggests that children are more susceptible to the environmental factor which triggers MS. This has led to much research being carried out into the environmental conditions exposed to MS patients during their childhood (3; 76; 135; 170).

2.3.2. Environmental Factors

Several environmental factors have been suggested to play a role in the development of MS. However the two which have the most compelling evidence are: sunlight (62; 67; 76; 170; 171), and the Epstein-Barr virus (EBV) (3; 7; 9; 66; 67; 135). These factors can be categorised into infectious and non infectious factors (7; 8).

2.3.3. Sunlight

The inverse association between solar radiation and prevalence of MS was first observed in 1960 (1). More recently Sloka et al (2008) (152) demonstrated a significant negative correlation between MS incidence and level of sunlight. This indicates that MS incidence decreased with increased exposure to sunlight. These findings are consistent with the theory that MS prevalence increases with increasing distance from the equator (36-42; 49; 50) (as previously discussed). In addition, Islam et al (2007) (76) reported that sun avoidance seems to precede the diagnosis of MS when studying monozygotic twins. Goldacre et al (2008) (62) also reported that skin cancer was significantly less common in

people with MS, which indicated that MS patients have been exposed to less sunlight. Concurrently, van der Mei et al (2003) (170) concluded that sun exposure during childhood and early adolescence is associated with reduced risk of MS. Therefore insufficient ultraviolet light may influence the development of MS.

Sunlight plays an important role in Vitamin D metabolism (97). Vitamin D₃ is synthesised in the skin with exposure to sunlight and is then metabolised by the liver and the kidneys to produce the active form of Vitamin D (97). Munger et al (2006) (117) reported that high circulating levels of Vitamin D is associated with lower risk of MS. Vitamin D has a primary role in maintaining calcium homeostasis (45), however Vitamin D is also utilised as an immunomodulator (23; 96).

2.3.4. Epstein-Barr Virus

The Epstein-Barr virus (EBV) is a herpes virus and infects more than 90% of the population (141; 141). Infection with EBV is usually asymptomatic in childhood but can cause infectious mononucleosis in adolescents and adults (9). However only a small proportion of those infected with EBV develop MS (72). Haahr et al (2004) (66) documented an association between late EBV infection and an increased risk of developing MS. These findings have led to the theory known as the “Hygiene Hypothesis” according to which protection against MS is conferred by persistent effect on the immune system by repeated infection in childhood (6). A further study by Ponsonby et al (2004) (135) reported that high infant sibling exposure in the first 6 years of life was associated with a reduced risk of MS, possibly by altering childhood infection patterns and related immune response. Therefore if children are brought up too clinically they will be less likely to be exposed to infection at the critical stage of development.

Exposure to infection at this stage seems to have a protective effect against developing MS (135). Consequently, the association between the EBV and risk of MS is well documented; however the exact mechanism by which the EBV wields this effect remains to be elucidated.

In addition, a positive association has been found between higher education and MS risk (7); this finding is consistent with the hygiene hypothesis and with late infection of EBV. This suggests that children brought up with more affluent parents are less likely to experience repeated infections. This is also consistent with low rates of MS in developing countries (6), where living conditions are less sanitary and fresh water is not available hence children are more likely to experience infection at an early age.

2.3.5. Immune Response in MS

Current research suggests that proinflammatory cells and mediators may be triggered by environmental factors (as previously discussed) to mediate the disease in a genetically susceptible host (12). An agent triggers an immune response; autoreactive T cells in the periphery are attracted to the blood brain barrier (BBB). Activated T cells adhere to the endothelium of the BBB and release an enzyme (matrix metalloproteinase) which facilitates infiltration of activated T cells across the BBB into the CNS (12). Activated T cells within the CNS cause myelin damage and axonal injury; reactivation of these cells within the CNS mediates further damage (150).

Although several environmental factors have been suggested as triggers for the development of MS, no single factor has been identified as the causal factor. This could mean that the effect of each factor is small or that the factors exert an additive effect upon one another. Holmoy (2008) (72) suggests that Vitamin D protects against MS by

modulating the immune response to EBV and that low vitamin D status facilitates detrimental activation of the autoreactive T cells and skews the immune response to EBV in the proinflammatory direction. This suggests that more than one environmental factor must be present in order to develop MS. Therefore if the environmental risk factors are identified, there is the possibility of primary prevention of the disease (7; 72).

2.3.6. Treatment of Multiple Sclerosis

The treatment of MS has three components; disease modification, relapse treatment and symptom management (33). Immunomodulators or immunosuppressants such as beta interferon and mitoxantrone (56) are disease modifying agents. These drugs have been shown to reduce the number and frequency of relapses in patients with relapsing-remitting MS. The exact mechanism of how these drugs work is not certain, however it is known that they reduce the infiltration of the activated T cells across the BBB, thereby reducing the extent to which the T cells can cause damage within the CNS. Although these drugs are effective in reducing the number of relapses, they also produce side effects. Mitoxantrone can cause cardiotoxicity (56) therefore the function of the heart must be monitored when the drug is being administered and if any adverse adaptations occur the treatment would have to stop.

Corticosteroids are also used to treat acute exacerbation of MS. These are among the most potent anti-inflammatory and immunosuppressive drugs available and they work by restoring the BBB and induce T cell apoptosis (56), therefore reducing damage within the CNS.

Symptom management can vary extensively between patients as the symptoms reflect the neural pathways that have been damaged. Thus the clinical management of MS patients

is complex, requiring careful attention to multiple disease symptoms as well as symptoms arising from therapeutic interventions (68).

2.4. The Neuromuscular System

2.4.1. The Central Nervous System

The central nervous system (CNS) is a communication network which is composed of two different types of cells; neurons which form the communication pathway and glia cells which support the function of the neurons. Different glia cells have been identified within the CNS; astrocytes, oligodendrocytes and microglia (15). The oligodendrocyte is a myelin forming cell (15) and the myelin sheath is formed by the spiral wrapping of oligodendrocytes plasma membrane extensions around the axons of the CNS (151).

The oligodendrocyte produces myelin which is composed of cholesterol, phospholipids and glycolipids (15). This multilayered, myelin containing membrane insulates the axonal cytoplasm from the extracellular fluid (154) resulting in a faster transmission of the nerve impulse along the axon (154; 172). This method of nerve transduction, where the signal appears to jump from sheath to sheath is called saltatory conduction (15). Each myelin sheath is separated by a section of exposed axolemma, these are known as the nodes of ranvier (15; 151; 154; 172). The axolemma at the nodes of ranvier contain a high density of sodium channels to ensure a rapid transmission of the action potential along the neuron (15).

In MS the oligodendrocytes are damaged (15; 15; 43; 150) resulting in the exposure of the axolemma to the extracellular fluid. This reduces the speed of neural transmission at the point of the lesion (15; 154). The slowing down of the impulse at the lesion can have

a profound impact on the overall rate of signal transmission. When the signal is impaired or fails the effect is evident in co-ordinated movement in the MS patient, most notably in the patient's gait (125).

Blakemore and Patterson (20) demonstrated in 1978 that remyelination of demyelinated axon is made possible by remyelination competent cells generated by mitosis. In addition Carroll and Jennings (1994) have shown that remyelinating cells are generated soon after demyelination. Remyelination occurs in many MS lesions but becomes increasingly incomplete or inadequate and eventually fails in the majority of lesions (15) as a consequence patients experience an increase in MS symptoms; such as spasticity and ataxia (125). Efforts to understand the causes for this failure of regeneration have fuelled research into the biology of remyelination and the complex interdependent cellular and molecular factors that regulate this process (30).

In addition to demyelination, axonal degeneration plays an important role in the accumulation of physical disability in MS patients (53; 54; 150). Although MS is regarded primarily as a demyelinating disease, axonal loss is likely to contribute to much of the resulting disability (150). Irvine et al (2008) (75) demonstrated that prompt remyelination protects axons from demyelination-associated axon loss and that remyelination failure contributes to the axonal loss that occurs in multiple sclerosis. In MS remyelination occurs but it is incomplete and poorly maintained (138; 139) hence the axolemma is therefore exposed which results in increased disability experienced by the MS patient.

As previously discussed, the CNS is restricted in its capacity to support the re-extension and rearrangements of axonal connections (22), therefore maintaining function capacity

after axonal degradation is limited. The nature, location, extent and maturity of the neurological insult influences the degree and specificity of axonal growth necessary to improve function (22). Bareyre et al (2004) (13) demonstrated in adult rats that local growth of damaged corticospinal tract axon resulted in new synapse formation. However, axon re-growth remains limited within the mature CNS (22) therefore neuronal repair does not elicit functional capacity in multiple sclerosis patients (18; 22). Spillmann et al (1998) (158) identified that membrane proteins from CNS myelin were inhibitory to neurite outgrowth. Therefore a complex system of destruction and repair exists within the central nervous system. The exact mechanism remains to be elucidated.

Wujek et al (2002) (179) demonstrated with animal models of MS, a causal relationship between inflammation, axon loss, and irreversible neurological disability. Papadopolos et al (2006) (129) also concluded that axonal loss is the main determinant of irreversible neurological disability in rats. In addition the “axonal hypothesis” states that cumulative axonal loss causes irreversible disability during progressive multiple sclerosis (18).

Therefore, demyelination and axonal degradation together result in irreversible destruction of the CNS in multiple sclerosis (18; 27; 30; 129; 179).

2.4.2. Skeletal Muscle

As a consequence of the neuronal damage of the CNS, the delivery of neural activity to skeletal muscle is affected (148). Buller et al (1960) (21) demonstrated that when nerves from fast motor units are made to innervate a slow muscle, the muscle is transformed into a fast muscle and likewise slow motor units converted fast muscle to slow. In addition, Roy et al (1992) (143) demonstrated that after 6 months of spinal chord isolation (which has a similar effect to disuse of skeletal muscle) mammalian skeletal muscle exhibited a

significantly greater proportion of type II fibres. Concurrently, Castro et al (1999) (29) demonstrated a significant shift towards type IIx fibres in human patients within 6 months of complete spinal cord injury. Therefore it is well accepted that the neural activity received by the muscle dictates the phenotypic expression of the muscle (133).

MS is characterised by spinal chord lesions which effect the neural transmission along the axon (154), therefore the neural activity received by the muscle of MS patients will be altered. Reduced neural innervation can also result from skeletal muscle inactivity as a result of bed rest, denervation, hind limb unloading, immobilisation or micro gravity (181). The effect of reduced innervation to the muscle due to MS is similar to the effect of skeletal muscle inactivity.

Inactivity results in muscle atrophy which is characterised by a decrease in protein content, fibre diameter, force production fatigue resistance (78) and a higher proportion of type II muscle fibres (101). As it has been demonstrated that a higher proportion of type II fibres can be indicative of disuse it is reasonable to expect MS patients to exhibit a higher proportion of type II muscle fibres compared to equivalent controls. Kent-Braun et al (1998) (82) demonstrated that MS patients exhibit fewer type I muscle fibres in the tibialis anterior than control participants. In addition a strong positive correlation ($r=0.84$) between relative area of fast twitch fibre and muscle fibre conduction velocity (MFCV) measured using surface EMG was revealed by Sadoyama et al (1988) (145). In addition, Garner and Widrick (2002) (60) demonstrated that MS patients exhibited a subtle fast MCH isoform co expression which suggested a trend towards an increased expression of type II fibres with the skeletal muscle. Kent-Braun et al (1994) revealed slower phosphocreatine resynthesis (PCr) following exercise in MS patients, which is suggestive of impaired oxidative capacity within the skeletal muscle. These findings too

are indicative that MS patients exhibit a higher proportion of type II fibres within the muscle.

It is clear from the literature that there are alterations within skeletal muscle of MS patients (26; 74; 82), however it is difficult to quantify the cause of these changes as reduced innervation can arise for a number of reasons, such as inactivity, MS or bed rest. The problem is also confounded by the fact that MS patients are less active (122) than controls and hence their muscle would exhibit an effect of disuse before taking into account the effect of the disease. Therefore it is difficult to quantify how much of the alteration in skeletal muscle in MS patients is down to disuse or how much is down to the disease. This point remains to be elucidated.

2.4.3. Effect of Temperature on the neuromuscular system in MS

The effect of temperature on nerve transduction in pathological conditions is well documented (11; 31; 132; 154; 175). White et al (2000) (175) demonstrated improved walking performance and improvement in fatigue scores in MS patients after 30 minutes of lower body immersion in 16-17°C water. In addition, Capello et al (1995) (24) concluded that 45 minutes of cooling with a cooling device led to improvements in strength and fatigue for up to 2 hours in MS patients.

Heat worsens and cooling improves the negative symptoms of multiple sclerosis. Sitting in a cold bath can turn the “disease off” and gives an individual back the freedom to work or exercise (11). Temperature effects the disease by affecting the ability for the neural signal to be transmitted past the point of a lesion within the CNS (154). The increase in nodal widening (173) results in increased electrical capacitance of the node therefore the current required to depolarise the node to its firing threshold is increased (154). The

current received at the node is greater than the current required to depolarise the node in order to ensure that depolarisation occurs. The difference between the two currents is called the safety factor. In MS the safety factor is reduced due to widening of the nodal gap thereby allowing the current to be dissipated away from the node (154). If the current required by the node increases the safety factor is reduced. The more damaged the node becomes the more current is required to depolarise the node and continue the signal along the axon. Therefore the more damaged the node the smaller the safety factor and the smaller the safety factor the less likely the signal is to be propagated. Temperature can vastly influence the safety factor and it is because of this that cooling therapy has a beneficial effect on the symptoms of MS.

2.5. Habitual Physical Activity of MS Patients

Previous research supports the contention that MS patients are less active than control participants (60; 82; 122). Due to the debilitating nature of the disease it is not surprising that MS patients are habitually less active than healthy individuals.

2.5.1. Benefits of Physical Activity for MS patients

For many years MS patients were advised to avoid exercise because of excessive fatigue and thermosensitivity (131). Heat worsens and cooling improves negative symptoms of MS (11) therefore as patients exercise and body temperature increases symptoms worsen and they become more disabled. Exercise prescription must therefore be very specific for MS patients to ensure that they are given exercise that is going to benefit them whilst ensuring their safety and comfort. Numerous studies (17; 65; 119; 159; 163; 176; 178) have investigated the benefits of different modes and intensities of exercise programmes for MS patients. Bjarndottir et al (2007) (17) investigated the effect of a five week

exercise programme on patients with mild MS. After a 5 week programme of combined aerobic and resistance exercise, an increase of 14.7% in VO₂peak, 18.2% in peak workload and 27.3% in anaerobic threshold was observed in the exercise group. All these parameters were significantly different from the control group. The study also showed a tendency towards an improved quality of life in the exercise groups. This study concluded that a 5 week exercise programme of combined aerobic and resistance training was adequate to improve physical fitness in patients with mild MS.

Documented benefits of physical activity for MS patients include decreased oxygen consumption (119), reduced motor fatigue (159) improved gait kinematics (65) increased stepping performance (178), improved physical fitness (17) as well as having a positive effect on the psychological well being of the patient (107; 115).

Evidence is also emerging which suggests that physical activity may influence the mechanism of the disease. Gold et al (2003) (61) investigated the response of serum nerve growth factor (NGF) and brain derived neurotrophic (BDNF) factor concentrations to standardized acute exercise in MS patients and controls. They revealed that 30 minutes of moderate exercise induced significant BDNF production in both MS patients and controls. BDNF is thought to play a role in preventing neural cell death and favouring the recovery process, neural regeneration and remyelination (47). As a consequence of the previous study White and Castellano (2008) (174) suggested that since axonal loss and cerebral atrophy occur early in MS, exercise prescription in the acute stage could promote neuroprotection, neuroregeneration and neuroplasticity and reduce long-term disability. This remains a relatively new concept and further research is required on the volume of exercise required to induce the response and elucidate if the

benefits are long lasting in MS patients. However this theory does present a novel approach for exercise to be utilised as a disease modifying therapy in MS.

2.6. Surface Electromyography

The surface electromyography (sEMG) technique is a non invasive technique used to detect myoelectrical signals within the muscle (5). The sEMG technique involves at least two EMG signals, with surface electrodes aligned parallel to the muscle fibre and between the innervation zone and the tendon. Using surface electrodes, the recorded EMG signal is a summation of activities from numerous motor units containing a large number of fibres.

The application of sEMG has mainly been utilised to investigate the neuromuscular characteristics and adaptations in the field of sport and exercise science and in pathological conditions (52). The present study employed sEMG technique to investigate alterations in neuromuscular characteristics in a clinical population. Sadoyama et al (1988) (145) demonstrated a strong positive correlation ($r=0.84$) between relative area of fast twitch fibre and muscle fibre conduction velocity (MFCV) measured using surface EMG. Therefore the technique can be used to demonstrate non-invasively the proportion of type II fibre expressed within the muscle.

Nielson et al (2007) (124) documented that innervation zone distribution substantially influenced amplitude and characteristic spectral frequencies. However, Roy et al (1986) (144) previously reported that estimates of conduction velocity were most stable between the distal tendon and adjacent distal tendon. In addition, the level of subcutaneous fat between the sacrolemma and the electrode has been documented as having an affect on estimated MFCV (110). Neilson et al (2007) (124) also demonstrated that MFCV was

elevated under conditions with elevated subcutaneous fat, which is as a consequence of a special filtering effect. Therefore higher levels of subcutaneous fat appear to artificially elevate the MFCV estimate.

Masuda and Sadoyama (1987) (103) investigated which skeletal muscles were most appropriate for the use of sEMG and concluded that mostly the biceps brachii, vastus lateralis and the tibialis anterior were used. The present study utilised the vastus lateralis for sEMG data collection.

2.7. Present Study

Aim: The aim of this study was to describe the neuromuscular characteristics of Multiple Sclerosis (MS) patients. To help explain the neuromuscular characteristics physical activity levels, body composition and blood lipid profile were measured. In addition, Vitamin D was measured to determine if this was deficient in MS patients.

Hypothesis: We hypothesised that muscle fibre conduction velocity (MFCV) would be elevated and amplitude would be impaired in Multiple Sclerosis patients compared to an age and sex matched control group. In addition, we hypothesised that physical activity levels would be reduced, body composition would exhibit a higher percentage fat, blood lipid profile would be less favourable and Vitamin D levels would be reduced in the group of MS patients.

3. Methodology

3.1. Participants

In this study a group of MS patients were compared to a group of healthy age and sex matched control participants.

3.1.1. Characteristics of Participants

Originally 17 patients were recruited to participate in the study however one patient withdrew and another was excluded from the study. The MS group consisted of 15 patients, 8 women and 7 men, aged 53.72 years \pm 10.5 year (mean \pm SD; range 36-69) and all 15 were included for data analysis. The patients were recruited through local MS societies. To be included, patients had to suffer from MS, be aged between 18 and 70 years and have impaired mobility. All patients were considered to fall between 4 and 6 on Kurtzke expanded disability status scale (EDSS)(92). A rating of 4 describes a patient fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability whereas a rating of 6 describes a patient who needs Intermittent or unilateral constant assistance (cane, crutch, brace) to walk.

18 control participants were recruited and tested however 4 participants were rejected from the study due to incorrect electrode placement. Therefore 14 participants; 6 women and 8 men, aged 54.59 years \pm 9.6 years (mean \pm SD; range 37-70) were included for data analysis. These participants were recruited through local MS societies, posters throughout the university and local public buildings and the university web site. To be included in the study, participants had to be within the age range of the MS group and have an equivalent BMI.

Table 3-1 Descriptive Characteristic of Participants

	Patients	Control	P Value
Participants (<i>n</i>)	15	14	0.707
Age (yr)	53.72±10.5	54.59±9.6	0.752
Weight (Kg)	77.97±22.02	77.85±15.54	0.987
Height (M)	1.66±0.09	1.71±0.09	0.227
BMI	27.73±6.11	26.51±4.01	0.080

The laboratory procedures were carried out at the University of Stirling physiology laboratory within the Gannochy Sports Centre. Participants reported to the lab for 8:30am having fasted for 12 hours overnight prior to their visit. Participants read the participant information sheet and had the opportunity to ask questions before being asked to complete two consent forms and complete a pre-exercise risk evaluation questionnaire.

3.1.2. Exclusion Criteria

Participants were excluded from the trial if their pre-participation questionnaire revealed any major contraindications to exercise; such as high blood pressure, recent cardiac problems. No-one was excluded from the trial on these bases. Women who were pregnant or potentially pregnant or those who were breast feeding were excluded from the trial. One patient was excluded from the trail on the basis that she became pregnant.

3.1.3. Consent

All participants were fully informed of the procedures and risks involved in the study before providing written informed consent. Participants were free to withdraw from the study at any point and without giving a reason.

3.1.4. Ethical Approval

This study was performed according to the Declaration of Helsinki and was approved by the University of Stirling, Department of Sport Studies Ethics Committee (Reference: #161- See Appendix A)

3.2. Laboratory Procedures

3.2.1. Blood Sample

A 10ml sample of venous blood was drawn from the antecubital vein using a 10ml syringe and a 15mm gauge needle. The blood was decanted into one 5ml container and left to clot for one hour. The remaining 5ml of blood was separated into two 2.5ml containers containing EDTA. The EDTA is as an anticoagulant which stops the blood sample from clotting. Both samples were centrifuged at 5000g for 10 minutes. The clear serum was then separated from the sample using a pipette and emptied into the appropriate vial (yellow for lipid analysis and white for vitamin D analysis) and stored in a secured freezer at -80°C.

All samples were transported, in dry ice, to Glasgow Royal Infirmary where the samples were analysed by the NHS Greater Glasgow and Clyde Clinical Biochemistry Service (34).

The blood samples were analysed for total cholesterol, triglyceride and high density lipoproteins (HDL) using enzymatic techniques. Low density lipoprotein (LDL) concentration was calculated using the equation: $\text{cholesterol} - \text{HDL} - (\text{Trig} \times 0.46)$. Fasted blood samples were used for this analysis as it has been shown that lipoprotein cholesterol concentration measured in the fed state differs significantly to those measured

in the fasted state (32). Vitamin D content was determined using Tandem Mass Spectrometry as described by Maunsell et al (106).

3.2.2. Initial Measurements

Height was measured with bare feet with the participant standing with their feet shoulder width apart. Participants were asked to stand up straight with their back to the wall and look directly ahead. Height was recorded using a stationary stadiometer.

Total body weight, percentage body fat and percentage fat was measured using bioelectrical impedance monitor. Participants' height and gender were programmed into the monitor; they were then instructed to stand on the scales in their bare feet with their heels and balls of their feet in contact with the metal plates, ensuring that their knees were not touching.

Blood pressure was measured using an automated blood pressure cuff. The cuff of the blood pressure monitor was applied securely to the upper section of the left arm. The monitor was then initialised and readings were recorded.

3.2.3. Standardised Breakfast

After the blood sample was drawn, participants received a standardised breakfast. Participants were supplied with one white bread roll with a choice of butter or margarine and jam. In addition to this participants received a cup of tea or coffee. As well as providing food for participants after their fast this standardised breakfast ensured that all participants were in a similar metabolic state before commencing the test.

3.2.4. The Contraction Protocol

Contractions were standardised by using the Bio-Dex Systems 3 Isokinetic Dynamometer (Bio-Dex Medical Systems, New York). Subjects were asked to sit on the dynamometer chair with the back of their knees in contact with the chair. The back of the chair was adjusted to hold the subject comfortably in that position. The subjects' hips, thighs and upper body were firmly strapped into position. In this position their hip angle was held at 100° angle of flexion. The chair was raised to the point where the axis of rotation of the dynamometer arm was aligned with the lateral femoral condyle. The lower leg was then attached to the lever arm of the dynamometer at a level slightly above the lateral malleolus of the ankle joint. The dynamometer arm was set to hold the leg at 60° of flexion during each isometric contraction, with 0° constituting a full extension with a straight leg.



Figure 3-1 Protocol set up on the Bio-Dex Systems 3 Isokinetic Dynamometer

Participants were tested under four different contraction intensities, in the order; 20, 40, 60 and 80% of the peak value of their maximum voluntary contraction (MVC). Their MVC was established for both legs. For each test condition the participant was required

to carry out 3 isometric contractions for 7 seconds; each contraction was separated by 14 seconds rest. During each set of contractions the target force was visible on the Bio-Dex monitor. Participants obtained visual feedback from the Bio-Dex throughout each contraction allowing the participant to adjust the force they were required to produce to maintain the target force.

Participants were instructed to perform a contraction by attempting to straighten their leg whilst keeping the back of their knee against the Bio-Dex chair. Each participant performed two sets of sub-maximal isometric contractions to warm up the muscle and to familiarise them with the protocol before carrying out the MVC. The first warm up set was carried out at 50% of the participants perceived maximum effort; the second warm up set was carried out at 75% of their perceived maximum effort. They were then asked to perform one set of maximal voluntary contractions (MVC); all participants were encouraged verbally to exert maximal effort during the maximal contraction of both legs. During the MVC and the four test contraction intensities the EMG signal was recorded, captured and stored on a host computer for future analysis.

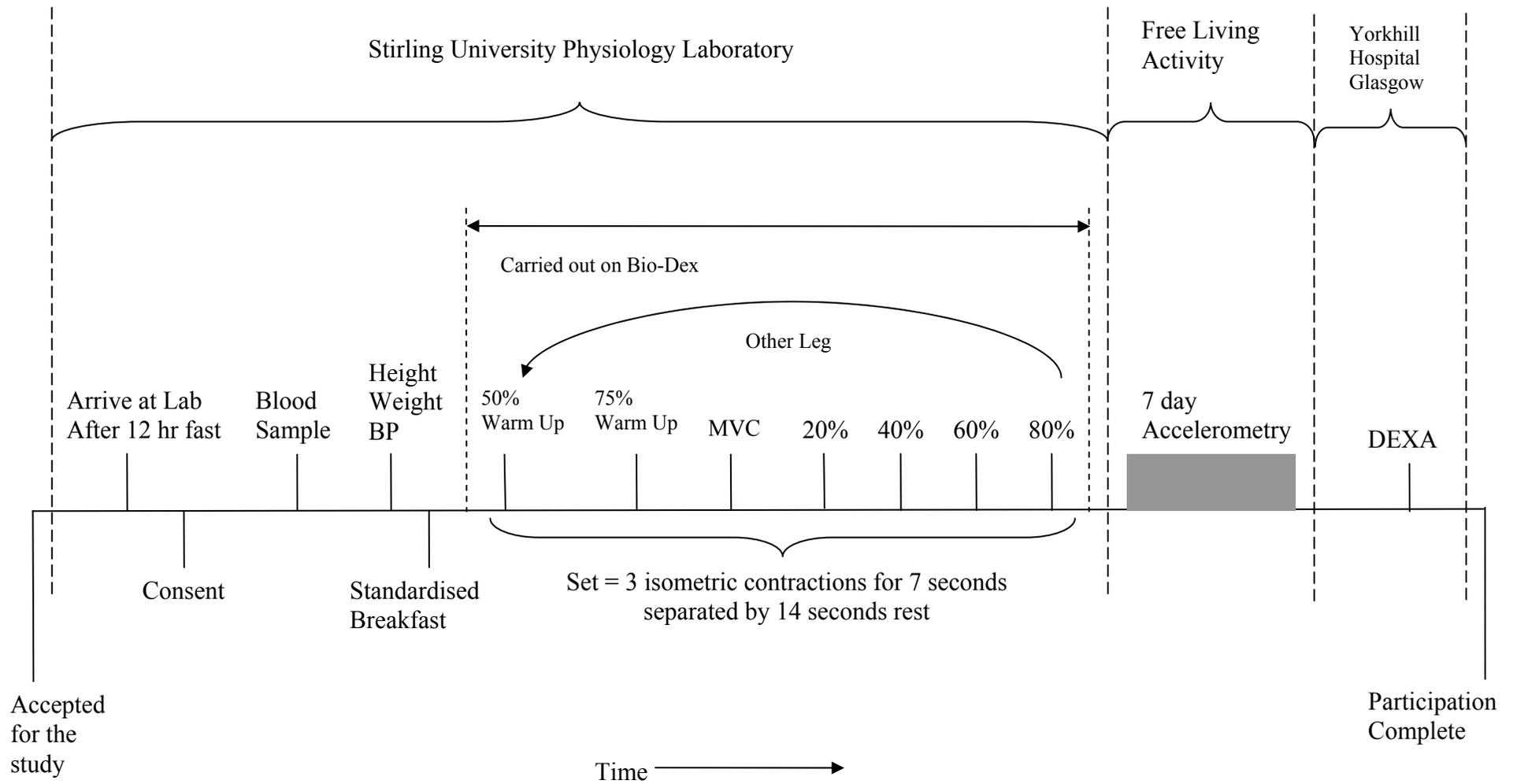


Figure 3-2 Illustration of Protocol

3.3. Surface Electromyography Technique

3.3.1. Bio-Pac Equipment

Surface EMG (sEMG) technique was employed to record and measure both EMG amplitude and muscle fibre conduction velocity (MFCV) of the vastus lateralis muscle throughout each contraction.



Figure 3-3 BioPac Silver to Silver Chloride Electrodes

4 silver to silver chloride (Ag-AgCl) EL258S shielded electrodes (Biopac, USA) were inserted into a hard plastic mould in a straight line next to one another allowing a distance of 12.5mm apart from the signal detection area and configured to record 3 parallel EMG signals as described by Lowery et al (2002) (98). The electrode array, with dry round silver inserts, was then positioned on cleansed and shaven skin along the approximate pennation angle of the muscle fibres half way between the main belly of the Vastus Lateralis and its distal end. A variety of different locations on the muscle was used until there was a clear propagation in one direction of the action potentials without change in shape in all of the 3 electromyography (EMG) signal channels. Then the electrode array position was marked with a permanent marker pen, after which the dry silver inserts were subsequently removed and the electrode wells were filled with conductive gel (20-30 μ l). The electrode array was then placed within the marked area on the skin and secured with 2 sections of Tegaderm. This electrode array was linked to the BioPac EMG apparatus (Biopac Systems, USA) and host computer. The EMG data was automatically anti-aliased by the hardware (Biopac Systems, USA). Each activity was sampled at a 2000 Hz

capture rate. This gave root mean square (RMS) of the EMG signal, giving a measure of the power of the signal, which was used for subsequent analyses.

MFCV was estimated by doing a cross-correlation between the temporal EMG signals measured at the three electrodes. The cross-correlation of two signals x and y is given by:

$$R_{xy}(m) = E\{x_{n+m}y_n^*\} = E\{x_n y_{n-m}^*\}$$

Where $E\{\cdot\}$ is the expectation operator. The cross-correlation function of two similar signals will peak where the two signals are maximally similar. In our case the signals are pulsed signals where one signal is a delayed and path distorted version of the other signal, so that their cross-correlation should peak at a number of samples equivalent to the time-delay between them. We make use of this property to estimate the delay between the signals measured at the electrodes 1 and 2, 2 and 3 and 1 and 3. With the distance between the electrodes known, we found the velocities of the signals from:

$$\text{velocity}_{nm} = \text{electrode pair distance}_{nm} / \text{estimated time delay}_{nm} \quad (\text{m/s})$$

MFCV was estimated using the `xcorr` function of Matlab where `xcorr` estimates the cross-correlation sequence of a random process. The average velocity was then found from the velocities V_{12} , V_{23} and V_{13} . Estimates of MFCV were accepted only when cross-correlation values were higher than 0.9.

3.4. Analysis of EMG Data

3.4.1. Analysis of Conduction Velocity from EMG signal

Two second segment of plateau data was selected. Wave form data was copied and pasted into word pad. This data was then entered into a specifically designed software programme run on the mathematical software Matlab (164). The software analysed the wave form data and established the MFCV of the signal.

The mat lab programme generated 4 MFCV velocity values. Values were for electrode 1-2, 2-3, 1-3 and an average value of the three values. The average value was utilised for analysis. Where the average value was invalid the value for electrode 1-3 was accepted (n=2).

If the average MFCV for any one contraction was greater than or equal to $30\text{m}\cdot\text{s}^{-1}$ that data point was omitted from the study. A total of 4 control participants were completely rejected from the study on this basis. A further 2 control participants and 2 patients had data of one leg rejected. However; only when data from both legs were rejected was the participant completely rejected from the study. Therefore no data gathered for these participants was considered for any analysis within this study.

3.5. Measurement of Habitual Physical Activity Level

3.5.1. Accelerometry Data Collection

Participants were supplied with a uniaxial accelerometer, model GT1M (Actigraph™, LLC, Fort Walton Beach, FL). The GT1M accurately and concisely measures time varying acceleration ranging in magnitude from 0.05 to 2.0 G's (according to

manufacturers technical details) in order to measure level of habitual physical activity. The actigraph accelerometers were activated and downloaded onto a PC using ActiLife Monitoring Systems Software version 3.2.8.



Figure 3-4 GT1M Actigraph™ Accelerometer

The accelerometer was secured over the participants' right hip using an elastic belt. The monitor was worn during all waking hours for 7 days, except during water activities (e.g. bathing, showering and swimming). Each accelerometer was set to record activity counts in 1 minute epochs (as recommended by manufactures instructions).

All participants completed a 7-day diary. Each day participants reported the time they attached the accelerometer, time they removed the accelerometer in the evening and highlighted any periods of time during the day when the accelerometer was removed (e.g. showering).

3.5.2. Analysis of Accelerometry Data

Data was rejected if there was less than 480 minutes (8 hours) of activity captured in a single day (167). Where this occurred that single days data was rejected. Accelerometry data set was discounted where less than 5 days of valid data was collected (167). Data was downloaded onto the PC and converted into an excel file and was split into seven single days worth of data. The data was screened and any strings of zeros of 60 minutes or more were removed (167). In addition to this data was removed if

the participant had indicated in their physical activity log book that they had removed their accelerometer. Total counts for the week and mean counts per day were calculated from the data collected.

3.6. Body Composition Analysis

3.6.1. Dual-energy X-ray Absorptiometry (DEXA)

A Whole Body Dual-energy X-ray Absorptiometry (DEXA) scan was carried out on each participant. This scan took place at Yorkhill Children's Hospital, Glasgow and was carried out on a Lunar Prodigy DF+ 13643, GE medical systems. The DEXA scan distinguishes between bone density, fat mass and lean mass. Participants were asked to remove any jewellery containing metal and take off any clothes that contained metal; such as jeans with metal zips and button. Hospital gowns were available for participants use. Participants lay on their back on the base unit and were manoeuvred into position. Participants were asked to remain as still as possible throughout the duration of the scan; which took between 5-10 minutes. All scans were carried out by a trained research technician who carries out scans regularly.

3.7. Analysis of Body Composition

3.7.1. Whole Body

The default setting was utilised to produce whole body values for total body fat, total body lean mass, total body BMD.

3.7.2. Thigh

Using a standard procedure thigh measurements were obtained by custom analysis to define each thigh and obtain lean thigh mass (LTM) and Bone Mineral Density (BMD) of each thigh. A standard rectangle was used to mark off the thigh at the point of the greater trochanter of the femur and the medial femoral condyle. The sides of the rectangle were altered to incorporate all the mass of the thigh.

3.8. Statistical Analysis

3.8.1. Determination of Sample Size

The sample size was determined using data from a pilot study which compared MFCV between MS patients and controls. Sample size was calculated from estimated size of effect from pilot data.

In addition, a minimum sample size of 35.2 was calculated was using the size formula $n = 2(1-r)N$, where $N = 800$ according to www.sportsci.org and the r value of 0.978 ($P < 0.001$) was provided by Moritani et al (1993) (114). The r value for the reliability is the reliability of the surface EMG measurement to pick up an accurate reading.

$$N = 2(1 - 0.978)800 = 35.2$$

Therefore in order to detect a difference between the two groups the study must recruit 36 subjects.

As a result we aimed to recruit 40 subjects in total, 20 MS patients and 20 controls subjects. This slightly larger sample size would have accounted for any outliers;

equipment failure; lack of attendance from MS patients as a result of the relapsing nature of the disease or any unforeseen circumstances.

3.8.2. Conduction Velocity and Amplitude Data

All conduction velocity and amplitude data collected for the patient and the control group was compared using a multivariate analysis of variance (MANOVA) with repeated measures. Subsequent analysis of MFCV data included percentage thigh fat as a covariant. Significance was accepted at $P \leq 0.05$. Data are expressed as mean \pm SD.

3.8.3. Accelerometry, Blood Sample and DEXA Data

Data was checked for normality using a histogram with a normal curve to identify any discrepancy to the normality of the data. When normality was assumed an independent T-Test was used to compared the patient group to the control group. Significance was accepted at $P \leq 0.05$. Data is expressed mean \pm SD.

4. Results

4.1. Muscle Fibre Conduction Velocity

4.1.1. Muscle Fibre Conduction Velocity of the Dominant Leg

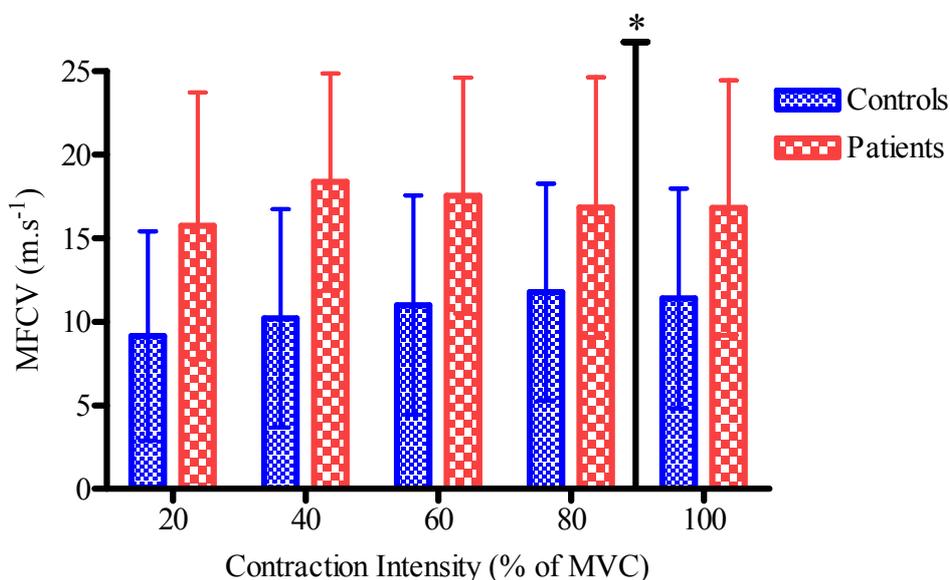


Figure 4-1 Muscle Fibre Conduction Velocity of the Dominant Leg for the Patient and Control Group

Over 20, 40, 60, 80 and 100% MVC

The patients exhibited a significantly faster ($P < 0.05$) MFCV than controls over all contractions

* Indicate that contractions were not carried out consecutively. 100% contraction was carried out first.

Initial analysis indicated that there was a significant difference in MFCV of the dominant leg between patient and control group ($P < 0.05$). However, a significant difference ($P < 0.05$) was also identified in the percentage fat of the dominant thigh between the two groups, from which a highly significant positive correlation ($P < 0.001$, $r = 0.697$) was observed between percentage fat of the thigh and MFCV. Therefore, percentage fat of the thigh was included as a covariant in the analysis of variance of MFCV of the dominant leg between the two groups. When the covariant was considered in the analysis no significant difference ($P > 0.05$) was identified between the two groups.

Nevertheless, there is a different interaction effect over the contractions from 20-80% between the two groups. MFCV of the control groups incrementally increases from 20%

through to 80% of MVC where as the patient group response does not follow this pattern. The MFCV of the patient group peaked at 40% of MVC and then decreased again during the 60% and the 80% contraction.

There was no significant difference in MFCV between contraction intensities ($P>0.05$).

There was also no interaction identified between the patient and the control group ($P>0.05$).

Table 4-1 Muscle Fibre Conduction Velocity Values of the Dominant Leg for the Patient and the Control Group

	Contraction Intensity (% of MVC)	N	Mean MFCV (m.s⁻¹)	SD
Controls	20%	12	9.15	6.26
	40%	14	10.21	6.54
	60%	14	11.00	6.57
	80%	14	11.78	6.48
	100% (MVC)	14	11.39	6.58
Patients	20%	15	15.75	7.98
	40%	14	18.38	6.49
	60%	13	17.55	7.07
	80%	14	16.84	7.79
	100% (MVC)	15	16.81	7.63

4.1.2. Muscle Fibre Conduction Velocity of the Non-Dominant Leg

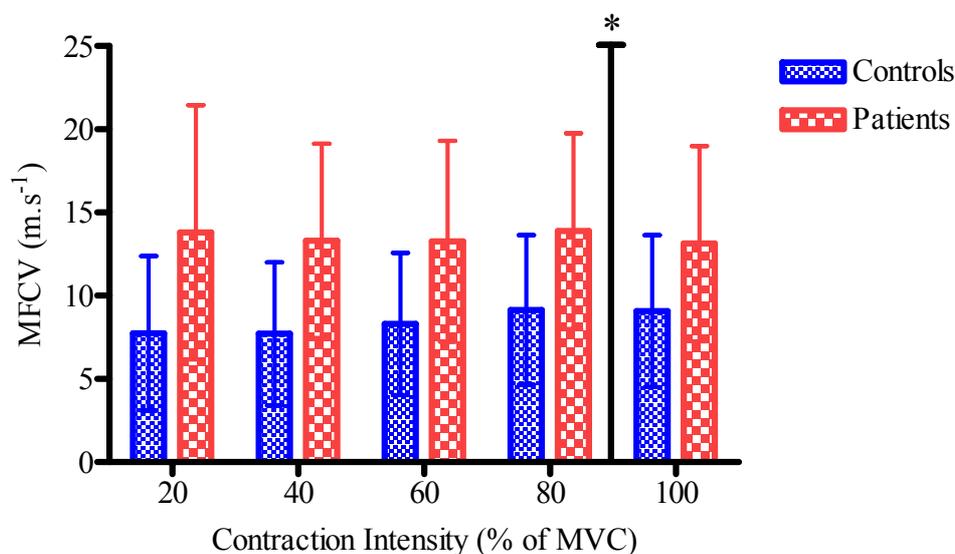


Figure 4-2 Muscle Fibre Conduction Velocity for the Non Dominant Leg of the Patient and the Control Group

Over 20, 40, 60, 80 and 100% MVC

The patients exhibited a no significant difference ($P>0.05$) in MFCV compared to the control group over all contractions

* Indicate that contractions were not carried out consecutively. 100% contraction was carried out first.

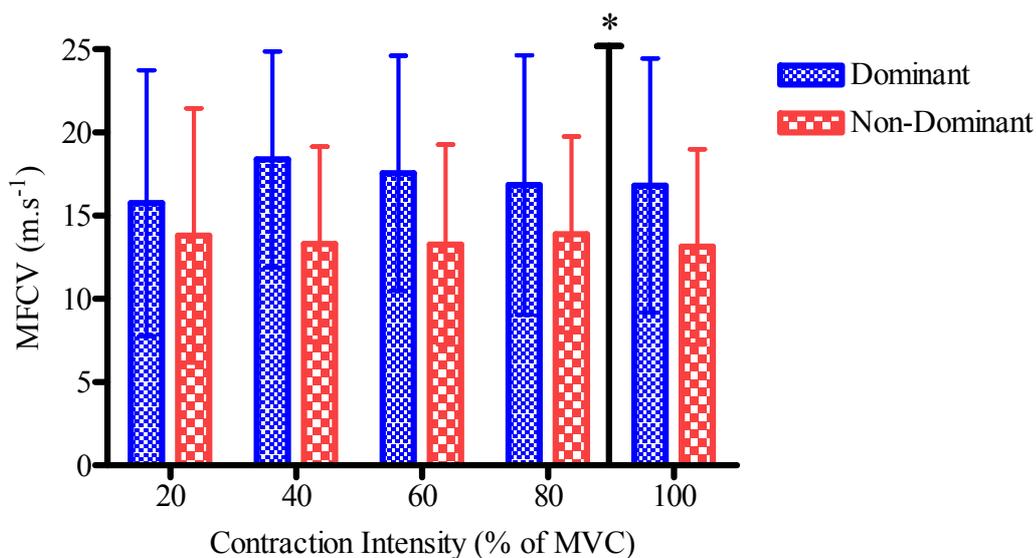
The initial analysis of the non dominant leg indicated that there was almost a significant difference ($P=0.054$) in the MFCV between the patients and the control groups. As with the dominant leg a significant difference ($P<0.05$) was found in the percentage fat of the non dominant thigh between the groups, from which a highly significant positive correlation ($P<0.001$, $r=0.697$) was observed between and percentage fat of the thigh and MFCV. Therefore, percentage fat of the non dominant thigh was included as a covariant in the analysis of MFCV of the non dominant leg between the groups. The results found that there was no significant difference ($P>0.05$) in MFCV of the non dominant leg between the patient and the control group.

There was also no significant difference ($P>0.05$) in MFCV between the contraction intensities. In addition no interaction effect was identified between the patient and the control group ($P>0.05$).

Table 4-2 Muscle Fibre Conduction Velocity Values for the Non-Dominant Leg of the Patient and the Control Group

	Contraction Intensity (% of MVC)	N	Mean MFCV (m.s ⁻¹)	SD
Controls	20 %	11	7.73	4.65
	40 %	12	7.71	4.31
	60 %	12	8.30	4.28
	80 %	12	9.14	4.49
	100 % (MVC)	12	9.08	4.56
Patients	20 %	13	13.81	7.64
	40 %	12	13.30	5.85
	60 %	12	13.27	6.02
	80 %	12	13.88	5.89
	100 % (MVC)	13	13.13	5.86

4.1.3. Muscle Fibre Conduction Velocity for the Patient Group

**Figure 4-3 Muscle Fibre Conduction Velocity of the Dominant and Non-Dominant Leg for the Patient Group**

Over 20, 40, 60, 80 and 100% MVC

There was no significant difference ($P > 0.05$) in MFCV between the dominant and non-dominant leg of the patient group

* Indicate that contractions were not carried out consecutively. 100% contraction was carried out first.

There was no significant difference in MFCV between the dominant and non-dominant leg of MS patients ($P > 0.05$). Neither was there a significant difference in MFCV

between contraction intensities ($P>0.05$). There was no interaction effect identified for MFCV between the dominant and non-dominant leg in MS patients ($P>0.05$).

Table 4-3 Muscle Fibre Conduction Velocity Values of the Dominant and Non-Dominant Leg for the Patient Group

	Contraction Intensity (% of MVC)	N	Mean MFCV ($\text{m}\cdot\text{s}^{-1}$)	SD
Dominant	20 %	15	15.75	7.98
	40 %	14	18.38	6.49
	60 %	13	17.55	7.07
	80 %	14	16.84	7.79
	100 % (MVC)	15	16.81	7.63
Non-Dominant	20 %	13	13.81	7.64
	40 %	12	13.30	5.85
	60 %	12	13.27	6.02
	80 %	12	13.88	5.89
	100 % (MVC)	13	13.13	5.86

4.1.4. Muscle Fibre Conduction Velocity for the Control Group

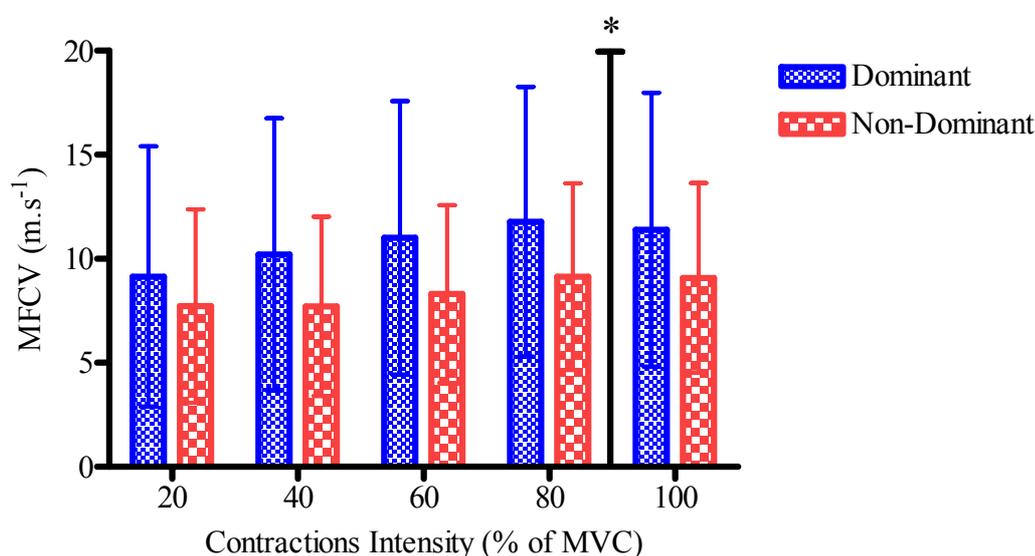


Figure 4-4 Muscle Fibre Conduction Velocity of the Dominant and Non-Dominant Leg for the Control Group

Over 20, 40, 60, 80 and 100% MVC

There was no significant difference ($P>0.05$) in MFCV between the dominant and non-dominant leg of the control patient group

* Indicate that contractions were not carried out consecutively. 100% contraction was carried out first.

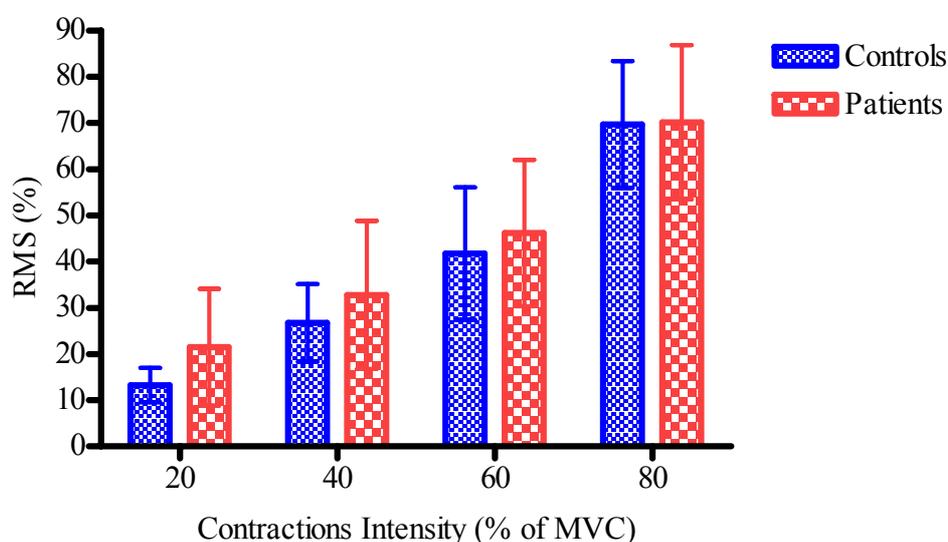
No significant difference in MFCV between the dominant and non-dominant leg of control group were identified ($P>0.05$). However there was a significant difference in MFCV between contraction intensities ($P<0.05$); indicating that the neuromuscular response of the control participant changed as contraction intensity increased. In addition to this these results illustrate that both the dominant and non-dominant legs of the control participant exhibit same response to increased contraction intensity; that is the conduction velocity increases in both legs in response to increased contraction intensity. No interaction effect was identified when comparing the MFCV of the dominant and non-dominant leg of control participants ($P>0.05$) therefore both legs responded in the similar way to the increase in contraction intensity.

Table 4-4 Muscle Fibre Conduction Velocity Values of the Dominant and Non-Dominant Leg for the Control Group

	Contraction Intensity (% of MVC)	N	Mean MFCV (m.s ⁻¹)	SD
Dominant	20 %	12	9.15	6.26
	40 %	14	10.21	6.54
	60 %	14	11.00	6.57
	80 %	14	11.78	6.48
	100 % (MVC)	14	11.39	6.58
Non-Dominant	20 %	11	7.73	4.65
	40 %	12	7.71	4.31
	60 %	12	8.30	4.28
	80 %	12	9.14	4.49
	100 % (MVC)	12	9.08	4.56

4.2. Amplitude

4.2.1. Amplitude of the Dominant Leg for the Patient and the Control Group

**Figure 4-5 Amplitude of the Dominant Leg for the Patient and the Control Group**

Over 20, 40, 60 and 80% of MVC

There was no significant difference ($P > 0.05$) in amplitude of the dominant leg between patient and control participants.

There was no significant difference in RMS of the dominant leg between patient and control participants ($P > 0.05$). There was however a significant increase in RMS over the contraction intensities ($P < 0.001$). No interaction effect was identified ($P > 0.05$) therefore

both patients and controls have shown a similar response throughout the contractions for their dominant leg.

Table 4-5 Amplitude Values of the Dominant Leg for the Patient and the Control Group

	Contraction Intensity (% of MVC)	N	Mean RMS (%)	SD
Controls	20%	12	13.27%	3.70%
	40%	14	26.73%	8.39%
	60%	14	41.73%	14.31%
	80%	14	69.63%	13.75%
Patients	20%	15	21.46%	12.62%
	40%	14	32.75%	16.00%
	60%	13	46.15%	15.81%
	80%	14	70.12%	16.66%

4.2.2. Amplitude of the Non-Dominant Leg for the Patient and the Control Group

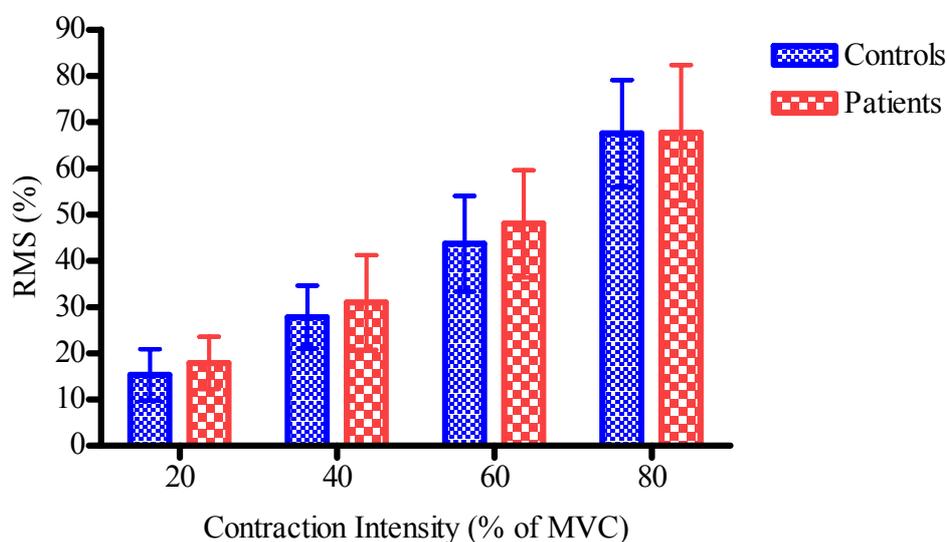


Figure 4-6 Amplitude of the Non Dominant Leg for the Patient and the Control Group
Over 20, 40, 60 and 80% of MVC
There was no significant difference ($P>0.05$) in amplitude of the non -dominant leg between patient and control participants.

There was no significant difference in RMS of the non-dominant leg between the patient and control group ($P>0.05$). There was however a significant increase in RMS over the contraction intensities ($P<0.001$) in both groups. Like the dominant leg, this indicates

that the non-dominant leg of both patients and controls received more increased neural activity as contraction intensity increased; therefore the dominant and non-dominant leg exhibited equivalent responses to the increased contraction intensity. No interaction effect was identified ($P>0.05$) therefore patients and controls have shown similar RMS response throughout the contractions of the non-dominant leg.

Table 4-6 Amplitude Values for the Non Dominant Leg of the Patient and the Control Group

	Contraction Intensity (% of MVC)	N	Mean RMS (%)	SD
Controls	20%	11	15.36%	5.54%
	40%	12	27.82%	6.78%
	60%	12	43.68%	10.37%
	80%	12	67.52%	11.57%
Patients	20%	16	17.91%	5.65%
	40%	15	30.98%	10.22%
	60%	15	48.02%	11.58%
	80%	15	67.67%	14.62%

4.2.3. Amplitude of the Patient Group

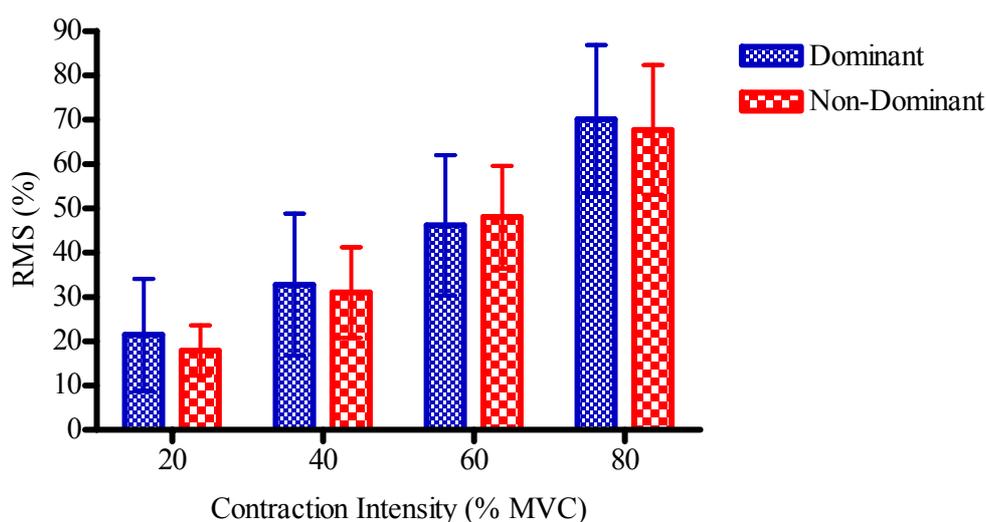


Figure 4-7 Amplitude of the Dominant and Non-Dominant Leg within the Patient Group
Over 20, 40, 60 and 80% of MVC
There was no significant difference ($P>0.05$) in amplitude between the dominant and non-dominant leg of the patient group.

There was no significant difference in RMS between MS patients dominant and non-dominant leg over all the contractions ($P>0.05$). There was nevertheless a highly significant increase in RMS between contractions intensities ($P<0.001$). This shows that both the dominant and the non-dominant leg of MS patients show an increase in neural activity received by the muscle as contraction intensity increased. There was no interaction effect identified between the dominant and non-dominant leg in the patient group ($P>0.05$); therefore both leg responded similarly to the increase in contraction intensity.

Table 4-7 Amplitude Values of the Dominant and Non-Dominant Leg within the Patient Group

	Contraction Intensity (% of MVC)	N	Mean RMS (%)	SD
Dominant	20%	15	21.46%	12.62%
	40%	14	32.75%	16.00%
	60%	13	46.15%	15.81%
	80%	14	70.12%	16.66%
Non-Dominant	20%	13	17.91%	5.65%
	40%	12	30.98%	10.22%
	60%	12	48.02%	11.58%
	80%	12	67.67%	14.62%

4.2.4. Amplitude of the Control Group

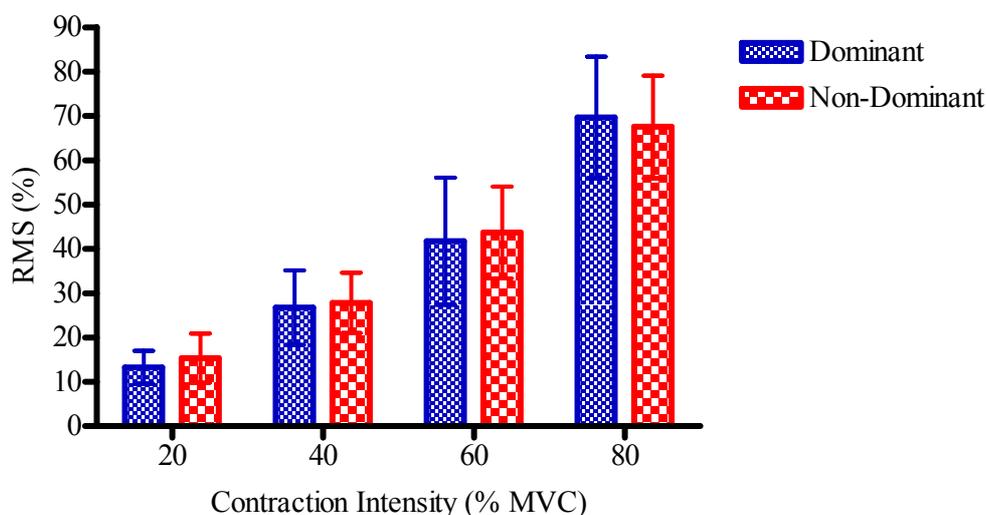


Figure 4-8 Amplitude of the Dominant and Non-Dominant Leg within the Control Group
Over 20, 40, 60 and 80% of MVC
There was no significant difference ($P>0.05$) in amplitude between the dominant and non-dominant leg of the control group.

There was no significant difference in RMS between the dominant and non-dominant leg of control group ($P>0.05$). As seen in the patient group; there was a significant increase ($P<0.001$) in RMS as the contraction force increased within the control group. There was no interaction effect ($P>0.05$), therefore there was no difference in the RMS response throughout the contraction between the dominant and non-dominant leg of the control group.

Table 4-8 Amplitude Values of the Dominant and Non-Dominant Leg within the Control Group

	Contraction Intensity (% of MVC)	N	Mean RMS (%)	SD
Dominant	20%	12	13.27%	3.70%
	40%	14	26.73%	8.39%
	60%	14	41.73%	14.31%
	80%	14	69.63%	13.75%
Non-Dominant	20%	11	15.36%	5.54%
	40%	12	27.82%	6.78%
	60%	12	43.68%	10.37%
	80%	12	67.52%	11.57%

4.2.5. RMS Relative to Fat Mass

Raw RMS data for each contraction was multiplied by the mass of fat in the thigh carrying out the contraction. This analysis reveals whether the higher level of body fat apparent in the patient group affected the RMS data.

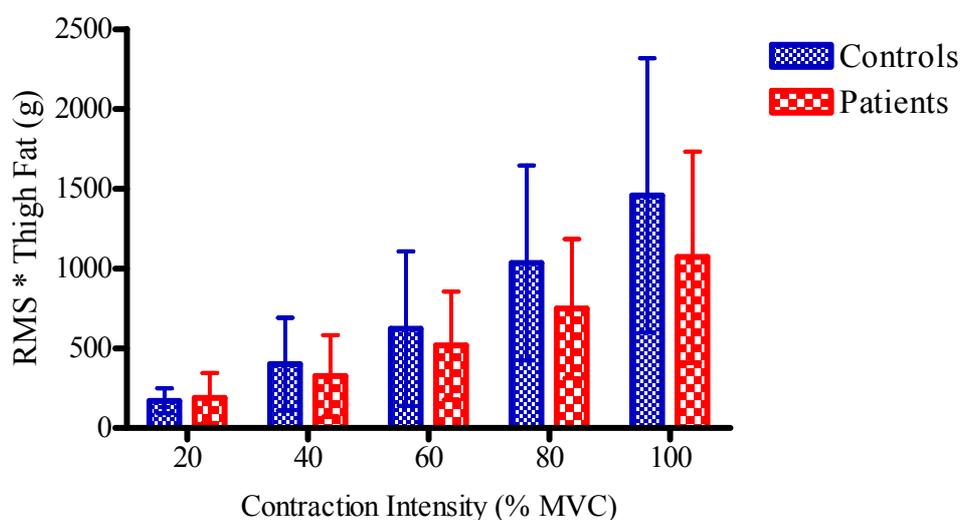


Figure 4-9 RMS relative to Mass of Fat in the Thigh
Over 20, 40, 60 and 80% of MVC
There was no significant difference ($P>0.05$) in RMS*Thigh Fat groups. There was a significant difference ($P<0.05$) between contraction intensity.

There was no significant difference ($P>0.05$) revealed between the groups when expressing RMS relative to mass of fat in the thigh. However there was a significant difference ($P<0.05$) between contraction intensity as had previously been elucidated from the percentage RMS data. A similar result emerged between the dominant and non dominant legs. Data is shown in tables below.

Table 4-9 Patients RMS * Thigh Fat Data

		N	Mean RMS*Thigh Fat(g)	SD
Dominant	20%	15	188.61	156.31
	40%	14	326.68	254.78
	60%	13	516.67	339.15
	80%	14	748.59	436.65
	100%	15	1071.20	661.31
Non-Dominant	20%	13	141.75	61.29
	40%	12	281.28	197.76
	60%	12	490.92	498.43
	80%	12	703.83	713.03
	100%	13	942.38	733.87

Table 4-10 Controls Patients RMS * Thigh Fat Data

		N	Mean RMS*Thigh Fat(g)	SD
Dominant	20%	11	170.56	78.26
	40%	12	400.61	290.89
	60%	12	622.88	485.21
	80%	12	1034.09	609.93
	100%	12	1458.12	860.14
Non-Dominant	20%	10	203.96	104.43
	40%	11	342.60	173.16
	60%	11	541.99	310.78
	80%	11	830.21	486.94
	100%	11	1224.33	634.44

4.3. Torque Production

4.3.1. Performed Contraction Intensity

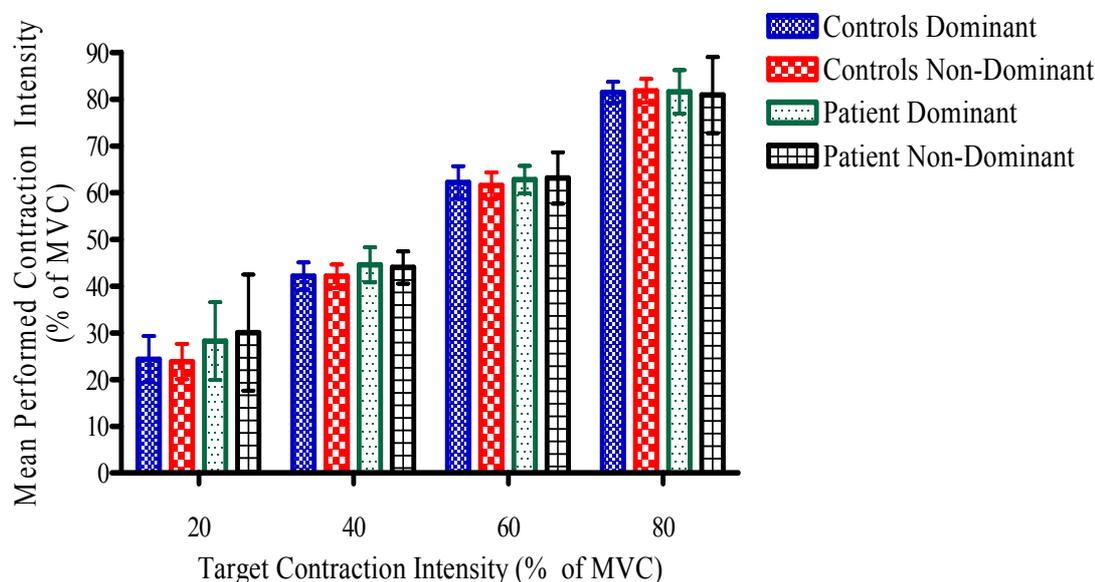


Figure 4-10 Intensity of Contraction Performed at each Target Intensity

Over 20, 40, 60 and 80% of MVC

There was no significant difference in performed contraction intensity within the patient ($P>0.05$) or the control ($P>0.05$) group

There was no significant difference in performed contraction intensity within the patient ($P>0.05$) or the control ($P>0.05$) group when comparing the dominant and non-dominant leg within each group at each contraction intensity. Neither was there a significant difference in performed contraction intensity between the patient and the control groups for the dominant leg ($P>0.05$) or the non-dominant leg ($P>0.05$) at each contraction intensity.

There was a highly significant difference between the contraction intensities when compared between groups and between dominant and non-dominant leg ($P<0.001$). Therefore, each target contraction was carried out at significantly different intensities than the other target intensity in both groups. This was the intended outcome as the purpose of the study was to investigate neuromuscular characteristics at different

contraction intensities. This proves that the contractions were in fact carried out at increasing intensity.

However, there was a significant difference when performed contraction intensity was compared to target intensity for the dominant ($P<0.05$) and non-dominant ($P<0.05$) leg. There was a highly significant difference in performed contraction intensity and target contraction intensity for both the patient ($P<0.001$) and the control ($P<0.001$) group. Consequently the performed contraction was carried out at a significantly different intensity to the intended contraction intensity however there was no significant difference in the contraction intensity carried out between the two groups or between the dominant and non dominant leg within each group therefore comparison of the results remains valid.

Table 4-11 Intensity at which Target Contractions were Performed

		Target Contraction Intensity (% of MVC)	N	Actual Mean Contraction Intensity (% of MVC)	SD
Patients	Dominant	20 %	15	28.24%	8.32%
		40 %	14	44.54%	3.77%
		60 %	13	62.79%	2.98%
		80 %	14	81.58%	4.62%
	Non-Dominant	20 %	13	30.03%	12.42%
		40 %	12	43.98%	3.47%
		60 %	12	63.13%	5.49%
		80 %	12	80.88%	8.16%
Controls	Dominant	20 %	12	24.34%	4.93%
		40 %	14	42.10%	2.97%
		60 %	14	62.20%	3.48%
		80 %	14	81.47%	2.28%
	Non-Dominant	20 %	11	23.82%	3.79%
		40 %	12	42.10%	2.50%
		60 %	12	61.50%	2.85%
		80 %	12	81.79%	2.52%

4.3.2. Absolute Torque - Maximum Voluntary Contraction (MVC)

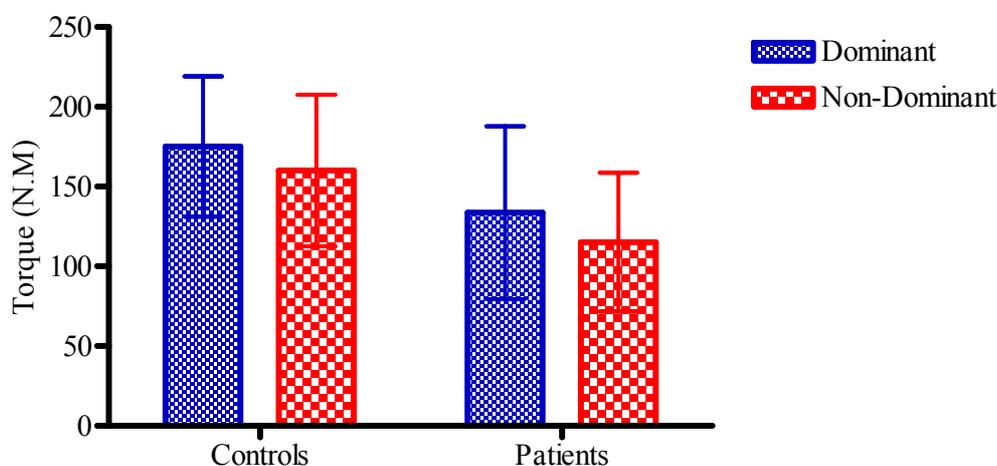


Figure 4-11 Maximum Voluntary Contraction of the Patient and Control Group
Maximum torque produced during participants MVC for both legs
The MVC of the control group was significantly greater ($P < 0.05$) than the MVC of the patient group for both legs.

The MVC of the control group was significantly greater than the MVC of the patient group when comparing the dominant (95% CI 3.67, 79.20; $P < 0.05$) and non-dominant (95% CI 7.31, 82.56; $P < 0.05$) leg. The control group produced significantly more torque with both their dominant and non-dominant leg than the patient group. Therefore the control group exhibited greater torque production than the patient group for both legs.

Table 4-12 Maximum Voluntary Contraction for the Dominant and Non-Dominant Leg of both Groups

Groups		N	Mean Force Produced (N.M)	SD
Dominant	Controls	14	175.09	43.94
	Patients	15	133.65	54.20
Non-Dominant	Controls	12	160.14	47.55
	Patients	13	115.21	43.41

There was no significant difference between dominant and non-dominant leg in the patient (95% CI -20.12, 57.01; $P > 0.05$) or the control (95% CI -22.09, 52.00; $P > 0.05$) group. Hence within each group the dominant leg was not significantly stronger than the non-dominant leg.

4.3.3. Relative Torque

Relative torque was used to express the torque produced in terms of muscle mass. Therefore this analysis indicates how much torque each gram of muscle produced during the MVC. This allows a more direct comparison of muscle quality to be made between the groups (100).

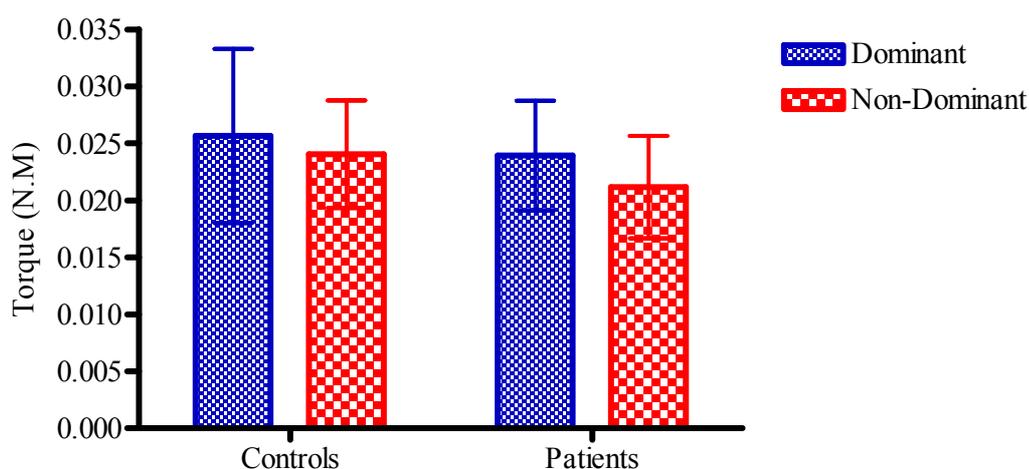


Figure 4-12 Relative Torque Production in the Patient and Control Group
Torque produced per gram of muscle mass during participants MVC with both legs
There was no significant difference ($P>0.05$) in torque production per gram of muscle between groups.

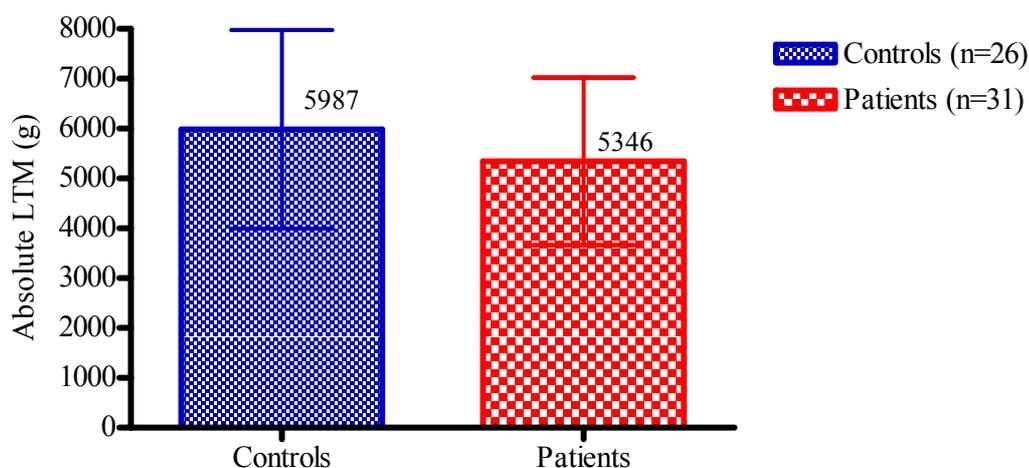
There was no significant difference in torque production per gram of muscle between groups for the dominant (95% CI -0.0007, 0.0073; $P>0.05$) and the non-dominant (95% CI -0.0010, 0.0068; $P>0.05$) leg.

The torque produced per gram of muscle in the dominant leg is not significantly different from that produced in the non dominant for both the patient (95% CI -0.0008, 0.0064; $P>0.05$) and the control (95% CI -0.0012, 0.0075; $P>0.05$) group.

Table 4-13 Relative Torque Production in the Patient and Control Group

	Groups	N	Mean	SD
Dominant	Controls	14	0.026	0.0076
	Patients	15	0.024	0.0048
Non Dominant	Controls	11	0.024	0.0047
	Patients	13	0.021	0.0045

4.3.4. Absolute Lean Mass of the Thigh

**Figure 4-13 Absolute Lean Mass of Thigh for both Patient and Control Group**

Lean mass of both legs considered within each group

A significant difference ($P < 0.05$) was identified between the groups when the lean thigh mass of both the dominant and the non dominant leg were considered within each group.

There was no significant difference in absolute LTM when comparing solely the dominant (95% CI -408, 2231; $P > 0.05$) or the non dominant (95% CI -2487, 425; $P > 0.05$) leg between groups. A significant difference was identified between the groups when the lean thigh mass of both the dominant and the non dominant leg were considered within each group (95% CI -1899, -34.86; $P < 0.05$).

In addition, no significant difference was identified between the dominant and non dominant leg within the patient group (95% CI -1249, 1454; $P > 0.05$) or the control (95% CI -1420, 1386; $P > 0.05$) group.

4.3.5. Relative Lean Mass of the Thigh – Between Groups Comparison

Lean Thigh Mass (LTM) ratio is used to express the lean mass of the thigh in comparison to the lean mass of the whole body. This allows the lean mass of the thigh to be expressed as a relative percentage of the whole body lean mass.

Table 4-14 Lean Thigh Mass – Between Groups Comparison
 § LTM Ratio: LTM (Lean Thigh Mass)/LBM (Lean Body Mass)*100
 * Calculation includes mass of both thighs.
 † Mass of one thigh considered in these calculations

Lean Thigh Mass Ratio §	Controls		Patients		P Value
	Mean LTM (%)	N	Mean LTM (%)	N	
Total *	24.80±1.51	12	23.72±1.51	15	0.077
Dominant Leg †	12.37±0.79	12	11.86±0.79	15	0.103
Non-Dominant Leg †	12.43±0.86	12	11.86±0.88	15	0.108

No significant difference was identified in LTM ratio between the patient and control group for either dominant (95% CI -0.001, 0.11; P>0.05) or non-dominant leg (95% CI -0.001, 0.013; P>0.05). However the difference in LTM ratio when considering total lean thigh mass; which includes the mass of both the dominant and non-dominant thigh together, is approaching significance (95% CI -0.001-0.023; P=0.077).

4.3.6. Relative Lean Mass of the Thigh – Within Group Comparison

Table 4-15 Lean Thigh Mass – With Group Comparison

	Dominant		Non-Dominant		P Value
	Mean LTM (%)	N	Mean LTM (%)	N	
Controls	12.37±0.79	12	12.43±0.86	12	0.878
Patients	11.86%±0.79	15	11.86±0.88	15	0.976

There is no significant difference between dominant and non-dominant leg for the LTM ratio within the control (95% CI -0.008-0.006; P>0.05) or the patient (95% CI -0.006-0.006; P>0.05) group. This is in concurrence with the finding that there was no significant difference in the MVC between the dominant and non-dominant leg within the patient and control group.

4.3.7. Correlation of MFCV to Lean Thigh Mass Ratio

LTM of the dominant leg was correlated against MFCV from the 100% contraction (MVC) of the dominant leg for each participant; and likewise for the non dominant leg. Then both groups were considered together for both the dominant and the non dominant leg.

Table 4-16 Correlation of MFCV to Lean Thigh Mass Ratio
* Significance $P < 0.05$

Lean Thigh Mass		N	r	P	
Dominant	Patients	15	-0.144	0.610	Fig 4-16
	Controls	12	-0.629	0.028*	Fig 4-14
	Both Groups	27	-0.400	0.039*	Fig 4-15
Non-Dominant	Patients	13	-0.241	0.428	
	Controls	11	-0.457	0.158	
	Both Groups	24	-0.395	0.056	

A negative correlation was revealed throughout all of the correlations made regarding LTM ratio and MFCV; which indicated that as lean thigh mass ratio increased MFCV decreased. The comparison reached significance ($P < 0.05$) in two circumstances (Table 4-16); when considering the dominant leg of both groups (Fig 4-15) and when considering the control groups (Fig 4-14) dominant leg. Figure 4-16 shows that there is no correlation between MFCV and Lean Thigh Mass Ratio.

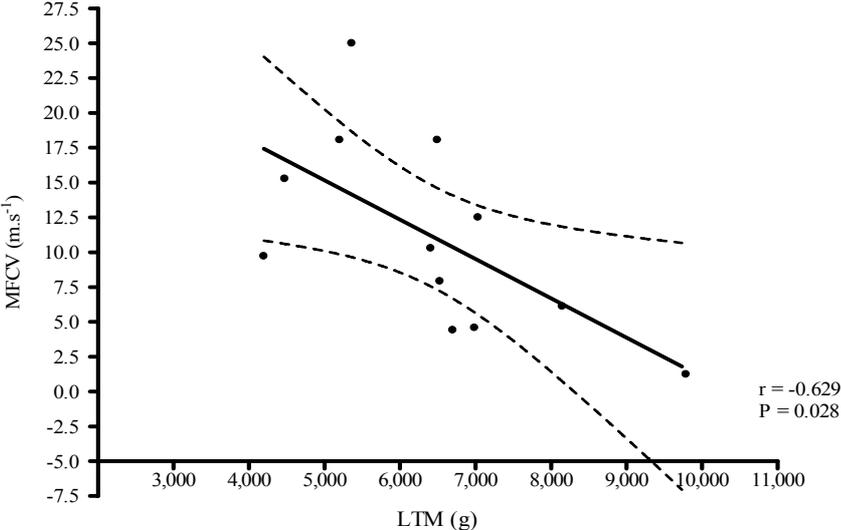


Figure 4-14 Correlation of MFCV to Lean Thigh Mass ratio for the Dominant Leg of the Control Group

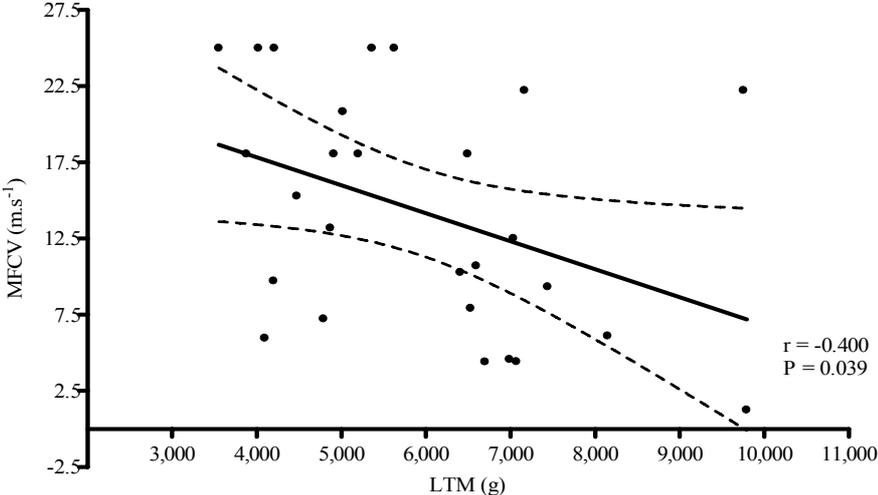


Figure 4-15 Correlation of MFCV to Lean Thigh Mass Ratio of the Dominant Leg of Both Groups

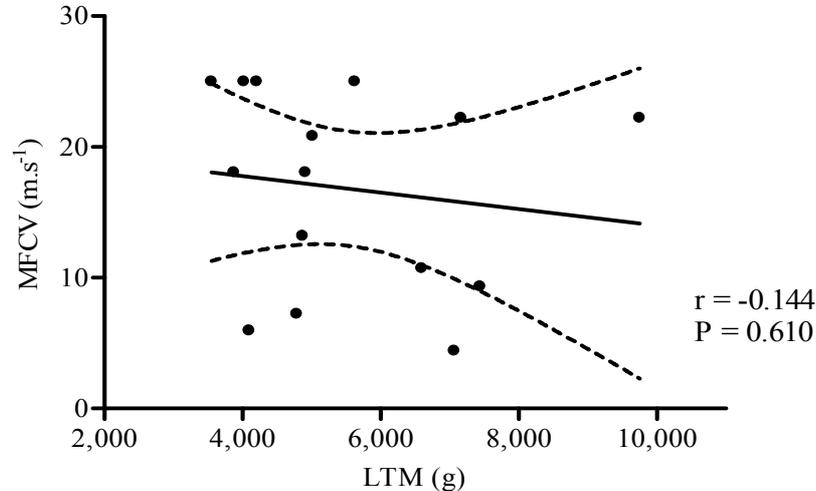


Figure 4-16 Correlation of MFCV to Lean Thigh Mass Ratio of the Dominant Leg of the Patient Groups

4.4. Body Composition Analysis

4.4.1. Total Percentage Body Fat

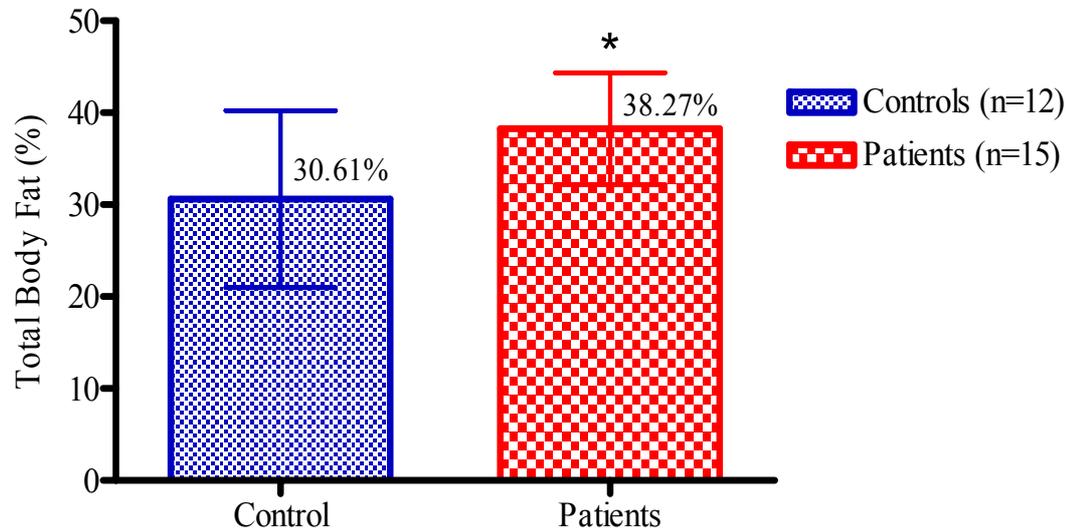


Figure 4-17 Total Body Fat (%) of Patients and Controls

The patient group exhibited a significantly higher ($P < 0.05$) percentage total body fat than the control group.

* Denotes significant difference

Figure 4-17 shows that there was a significant difference in total body fat percentage between the patient group and the control group (95% CI -0.139, -0.0140; $P < 0.05$).

4.4.2. Correlation of MFCV to Total Body Fat Percentage

Total body fat percentage for each participant was correlated with the MFCV from their MVC for both legs.

Table 4-17 Correlation of MFCV to Total Percentage Body Fat
* Significance $P < 0.05$

		N	r	P	
Dominant	Patients	15	0.516	0.049*	Fig 4-18
	Controls	12	0.526	0.079	
	Both Groups	27	0.564	0.002*	Fig 4-19
Non-Dominant	Patients	13	0.394	0.178	
	Controls	11	0.398	0.231	
	Both Groups	24	0.437	0.033*	Fig 4-20

The relationship between total percentage body fat and MFCV shows a positive correlation throughout all comparisons (Table 4-17); this signifies that as the participants' level of body fat increased so did their MFCV. A significant ($P < 0.05$) correlation was identified when considering the dominant leg of the patient group (Fig 4-18), the dominant leg of both groups (Fig 4-19) and the non dominant leg of both groups (Fig 4-20).

This correlation may indicate a physiological response to the increased level of body fat however it may also be a methodological problem as increased levels of adipose tissue are known to artificially increase the MFCV reading from surface electrodes (110).

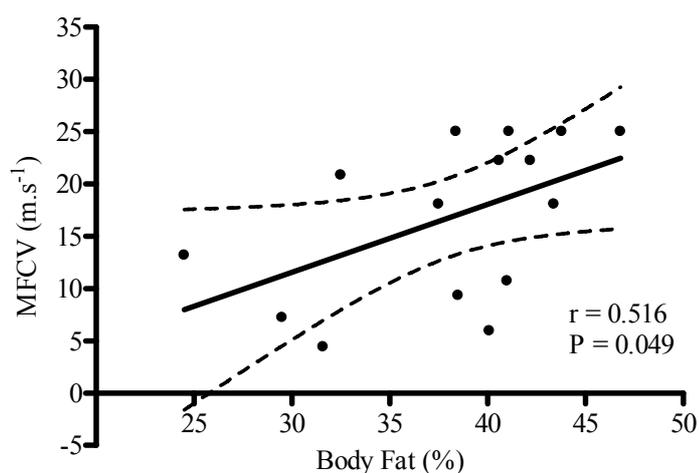


Figure 4-18 Correlation of MFCV to Total Percentage Body Fat for the Dominant Leg of the Patient Group

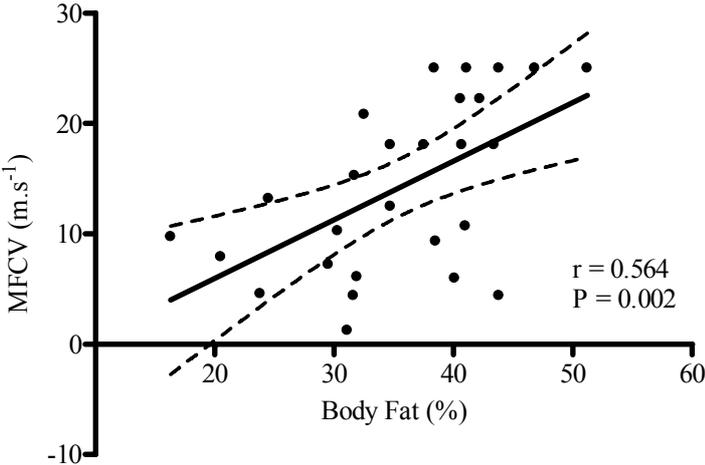


Figure 4-19 Correlation of MFCV to Total Percentage Body Fat for the Dominant Leg of both Groups

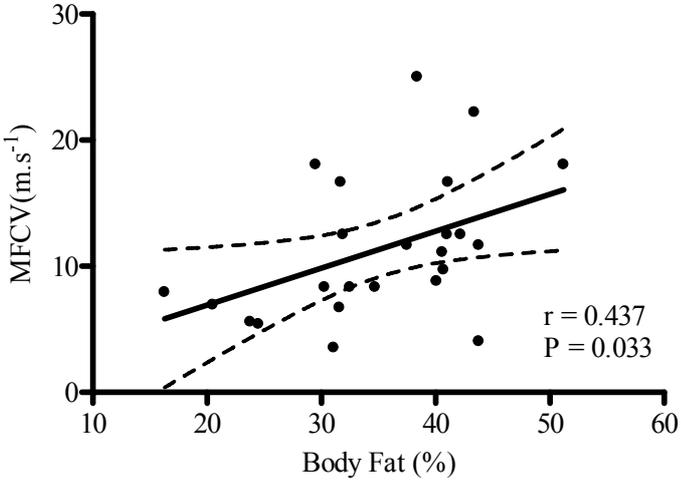


Figure 4-20 Correlation of MFCV to Total Percentage Body Fat of the Non-Dominant Leg of both Groups

4.4.3. Percentage Fat of the Thigh

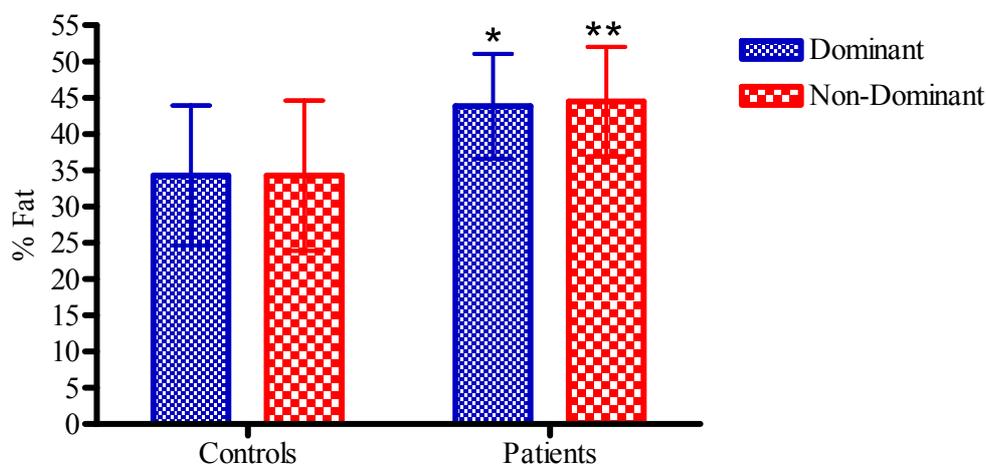


Figure 4-21 Percentage Fat of the Dominant Thigh for the Patient and the Control Group
 The patient group exhibited a significantly higher ($P < 0.05$) percentage fat of the thigh than the control group.
 * Denotes significant difference between dominant legs.
 ** Denotes significant difference between non dominant legs.

A significant difference in percentage fat of the thigh of the dominant leg was identified between the patient and the control group; (95% CI 0.02569, 0.16231; $P < 0.05$).

A significant difference in percentage fat of the thigh of the non dominant leg was identified between the patient and the control group; (95% CI 0.02622, 0.17792; $P < 0.05$).

Table 4-18 Percentage Fat of the Dominant Thigh for the Patient and the Control Group

		N	Mean Fat (%)	SD
Control	Dominant	12	34.28	9.64
	Non Dominant	11	34.25	10.34
Patient	Dominant	15	43.83	7.22
	Non Dominant	13	44.46	7.54

In addition there was no significant difference in percentage fat of the thigh between the two legs within the control (95% CI -8.63, 8.63; $P > 0.05$) and the patient (95% CI -6.37, 5.11; $P > 0.05$) group.

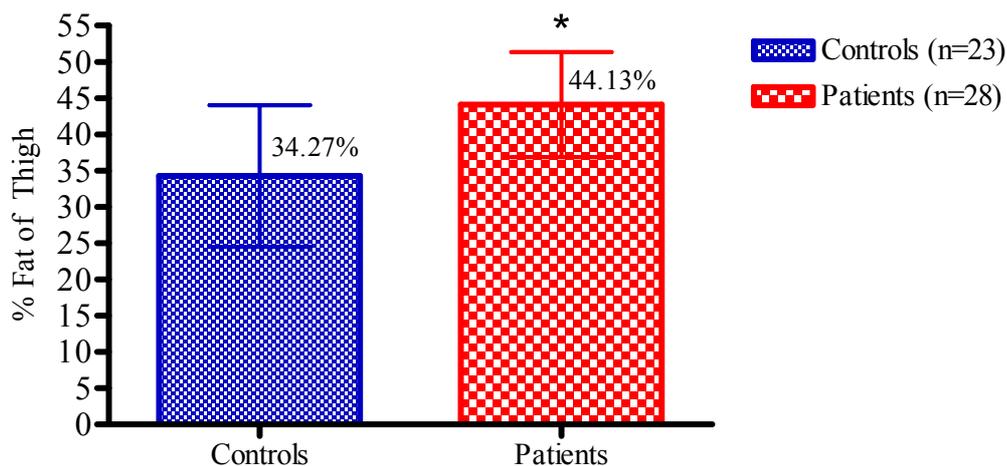


Figure 4-22 Percentage Fat of the Thigh when considering both the Dominant and Non-Dominant Leg within each Group

The patient group exhibited a highly significantly higher ($P < 0.001$) percentage fat of the thigh than the control group.

* Denotes significant difference.

A highly significant difference in percentage fat of the thigh was identified between the groups when considering both the dominant and non dominant leg together within each group; (95% CI 0.05069, 0.14642; $P < 0.001$).

4.4.4. Correlation of MFCV to Percentage Fat of the Thigh

Percentage fat of the thigh for each participant was correlated against their MFCV from their 100% contraction (MVC). Both the dominant and the non dominant leg were considered in both the patient and the control group.

Table 4-19 Correlation of MFCV to Percentage Fat of the Thigh

*Significance $P < 0.05$

**Significance $P < 0.001$

	N	r	P	
Patients	28	0.532	0.004*	Fig 4-23
Controls	23	0.795	<0.001**	Fig 4-24
Both Groups	51	0.0697	<0.001**	Fig 4-25

A positive correlation existed between MFCV and percentage fat of the thigh when considering patient and controls alone and when considering both groups together; all three correlations were significant. A highly significant ($P < 0.001$) correlation was

identified when looking at the correlation between MFCV and percentage fat of the thigh of the control group and for both groups together. A significant correlation was identified when looking at the relationship between patients MFCV and patients' thigh fat.

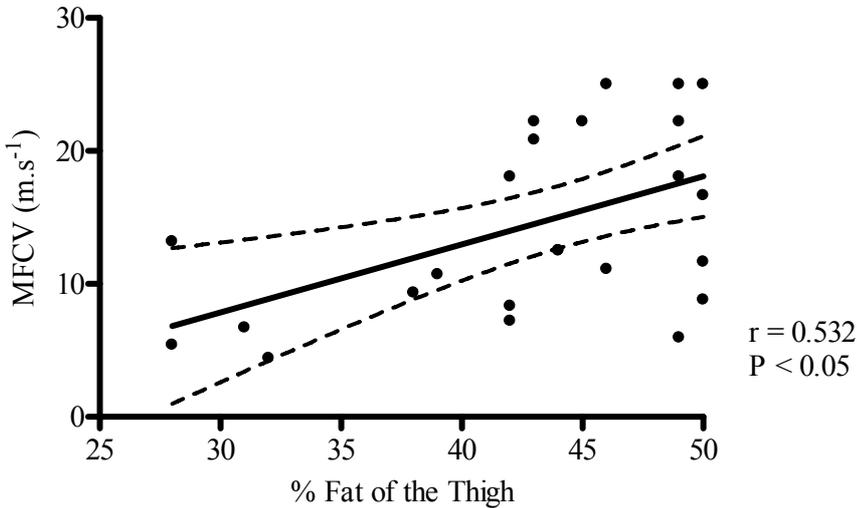


Figure 4-23 Correlation of MFCV to Percentage Fat of the Thigh in the Patient Group

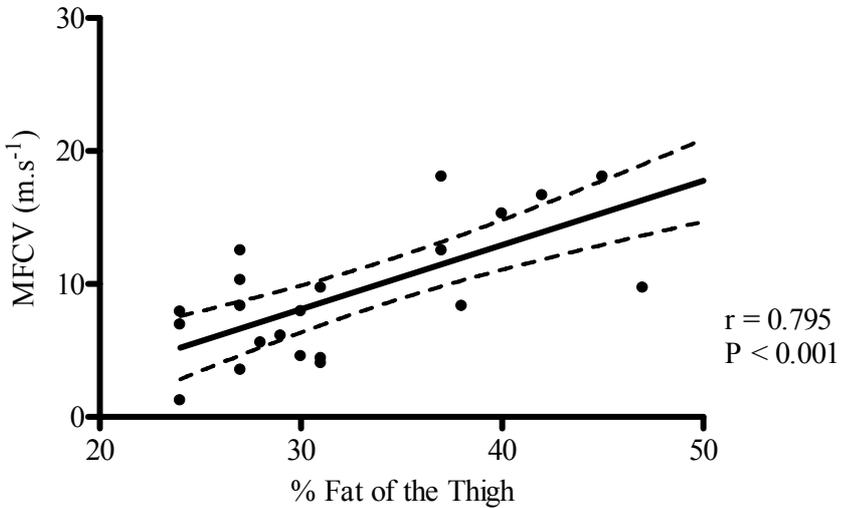


Figure 4-24 Correlation of MFCV to Percentage Fat of the Thigh in the Control Group

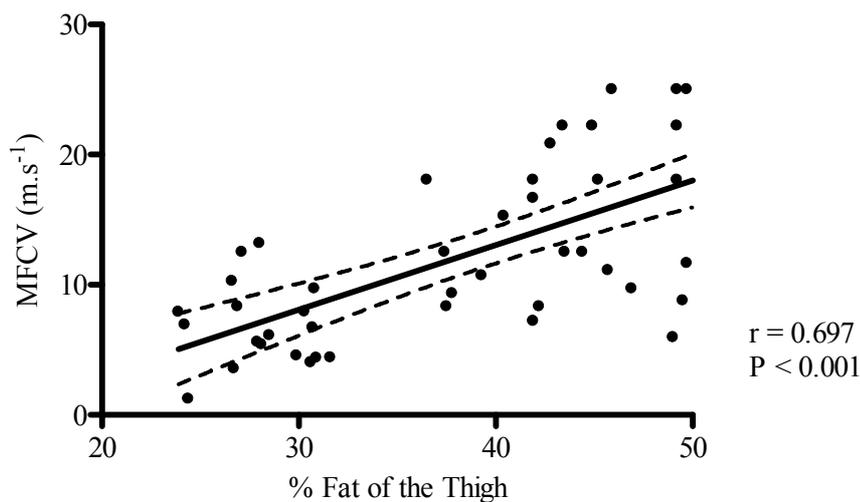


Figure 4-25 Correlation of MFCV to Percentage Fat of the Thigh in both the Patient and the Control Group

4.4.5. Bone Mineral Density – Between Groups Comparison

Table 4-20 Bone Mineral Density – Between Groups Comparison

	Controls			Patients			P value
	Mean BMD (g/cm ²)	SD	N	Mean BMD (g/cm ²)	SD	N	
Whole Body	1.185	0.132	12	1.144	0.136	15	0.476
Dominant	1.381	0.166	12	1.289	0.212	15	0.234
Non-Dominant	1.394	0.151	12	1.275	0.235	15	0.141

There was no significant difference in BMD between patient and control group for either whole body (95% CI -0.08, 0.16; $P > 0.05$), dominant (95% CI -0.06, 0.25; $P > 0.05$) or non-dominant (95% CI -0.04, 0.28; $P > 0.05$) thigh.

4.4.6. Bone Mineral Density – Within Group Comparison

Table 4-21 Bone Mineral Density – Within Groups Comparison

	Dominant			Non-Dominant		
	N	Mean BMD (g/cm ²)	SD	Mean BMD (g/cm ²)	SD	P value
Controls	12	1.381	0.166	1.394	0.151	0.841
Patients	15	1.289	0.212	1.275	0.234	0.862

There was no significant difference in BMD between dominant and non dominant leg for either the control (95% CI -0.15, 0.12; P>0.05) or the patient (95% CI -0.15, 0.18; P>0.05) group. This result supports previous findings in this study to suggest that there are no differences between the dominant and non-dominant leg for other variables such as LTM and percentage fat of the thigh.

4.5. Physical Activity Levels

4.5.1. Total Physical Activity

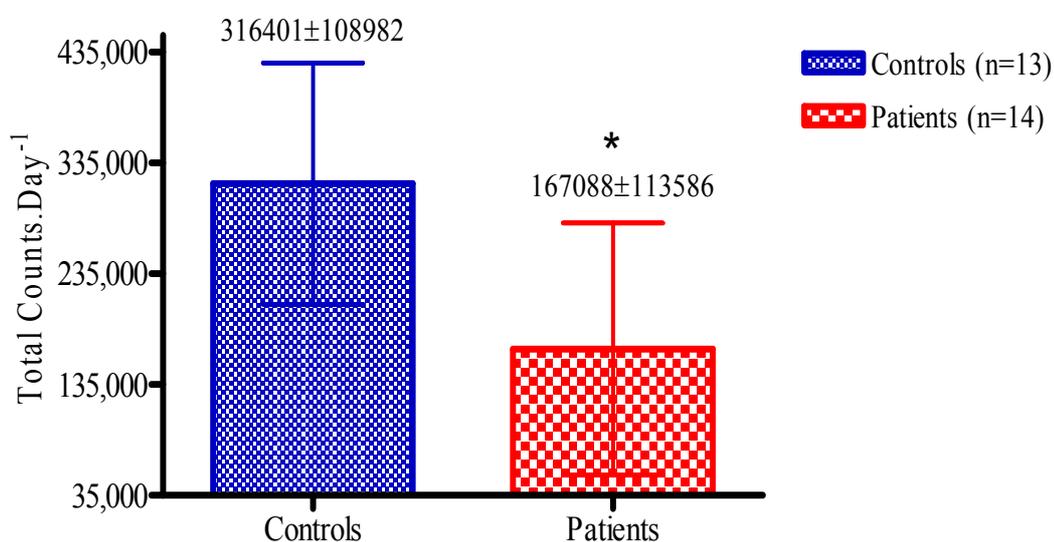


Figure 4-26 Total Physical Activity Counts for the Patient and the Control Group
The patient group was significantly (P<0.05) less active than the control group
* Denotes significant difference.

The control group show significantly higher total counts than the patients group (95% CI 60944.46, 237682.7 P<0.05). This demonstrates that control group exhibited a significantly higher level of habitual physical activity than the patient group.

4.5.2. Relationship of MFCV to Total Physical Activity

Total physical activity counts for each participant were correlated to their MFCV from the 100% contraction (MVC) of both the dominant and non-dominant leg.

Table 4-22 Correlation of MFCV to Total Physical Activity Counts
** Significance P<0.001

Total Physical Activity		N	r	P	
Dominant	Patients	14	-0.106	0.718	
	Controls	13	0.014	0.964	
	Both Groups	27	-0.254	0.202	
Non-Dominant	Patients	12	0.826	0.001**	Fig 4-27
	Controls	12	0.005	0.987	
	Both Groups	24	0.180	0.399	

The correlation of MFCV to total physical activity was not consistent throughout all comparisons. Two of the comparisons exhibited a negative correlation (Table 4-22) suggesting that as total physical activity level increased MFCV decreased, although this correlation did not reach significance. Whereas the other four comparisons reveal a positive correlation signifying that as total physical activity level increased so did MFCV. This correlation reached significance in one instance (P=0.001); Figure 4-27 shows how total physical activity level increased and MFCV increased when the non-dominant leg of the patient group was correlated to total physical activity level of the patient group.

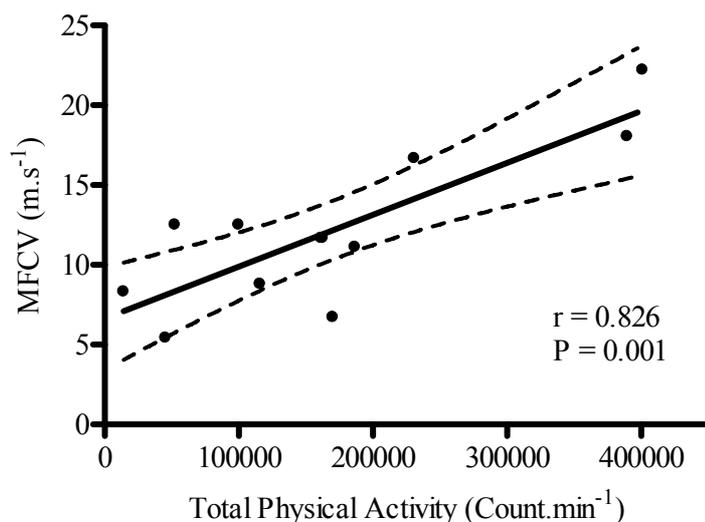


Figure 4-27 Correlation of MFCV to Total Physical Activity for Patients with the Non-Dominant Leg

4.5.3. Mean Physical Activity

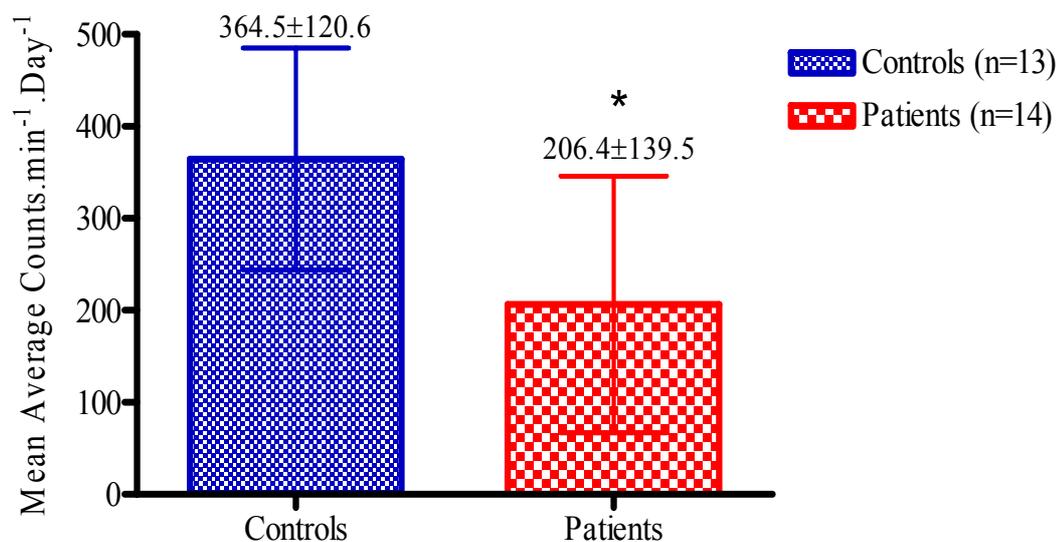


Figure 4-28 Mean Average Counts/Day for the Patient and the Control Group
The patient group was significantly ($P < 0.05$) less active than the control group
* Denotes significant difference.

The control group show a significantly higher mean average counts per day than the patient group (95% CI 54.35, 261.87; $P < 0.05$). This substantiates the findings from the total physical activity level results; that the control group show evidence of a higher level of habitual physical activity than the patient group.

4.5.4. Correlation of MFCV to Mean Physical Activity

Mean physical activity counts for each participant was correlated to their MFCV from the 100% contraction (MVC) of both the dominant and non-dominant leg

Table 4-23 Correlation of MFCV to Mean Physical Activity Counts
* Significance P<0.001

Mean Physical Activity		N	r	P	
Dominant	Patients	14	-0.096	0.743	
	Controls	13	0.023	0.942	
	Both Groups	27	-0.227	0.256	
Non-Dominant	Patients	12	0.834	0.001*	Fig 4-29
	Controls	12	0.048	0.882	
	Both Groups	24	0.235	0.269	

When taking into consideration mean physical activity, similar findings to the correlation between total physical activity and MFCV were revealed. A negative correlation was exposed when the correlation was made between dominant leg of both the groups together as well as for the patient group alone. This suggests that the MFCV decreased as mean physical activity level increased; however this correlation did not reach significance. The further four correlation made between mean physical activity level and MFCV exhibited a positive correlation suggesting that as mean physical activity level increased so did MFCV. Three out of the four correlations show a very weak positive relationship where as one comparison reached significance.

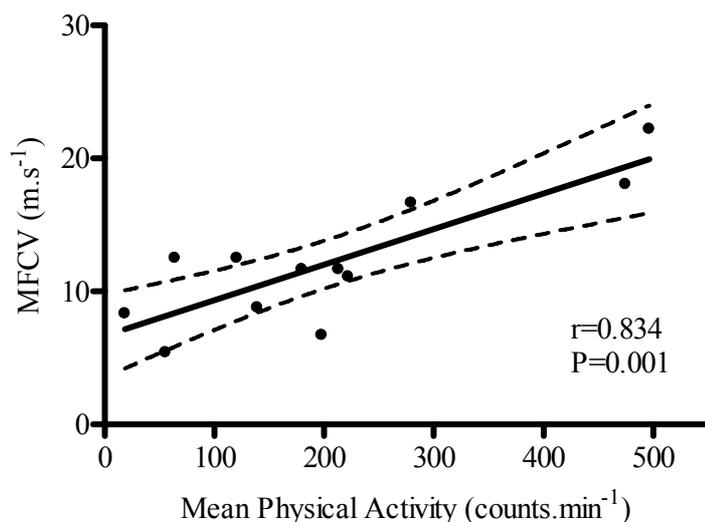


Figure 4-29 Relationship of MFCV to Mean Physical Activity for patients for the Non-Dominant Limb

4.6. Blood Sample Results

4.6.1. Blood Lipid Profile

Table 4-24 Blood Lipid Profile Results for the Patient and the Control Group

	Control Group			Patient Group			P Value
	N	Mean Concentration (mmol ⁻¹)	SD	N	Mean Concentration (mmol ⁻¹)	SD	
Cholesterol	17	5.18	1.04	14	5.30	0.61	0.715
Triglyceride	17	1.30	0.42	14	1.25	0.48	0.798
HDL	17	1.45	0.37	14	1.43	0.33	0.880
cLDL	17	3.13	0.92	14	3.29	0.73	0.615

There was no significant difference identified between the patient and control group for cholesterol (95% CI -0.52635, 0.75761; $P > 0.05$), triglycerides (95% CI -0.37143, 0.28824; $P > 0.05$), HDL (95% CI -0.28253, 0.24337; $P > 0.05$) or calculated LDL (95% CI -0.46664, 0.77597; $P > 0.05$) levels.

4.6.2. Vitamin D Concentration

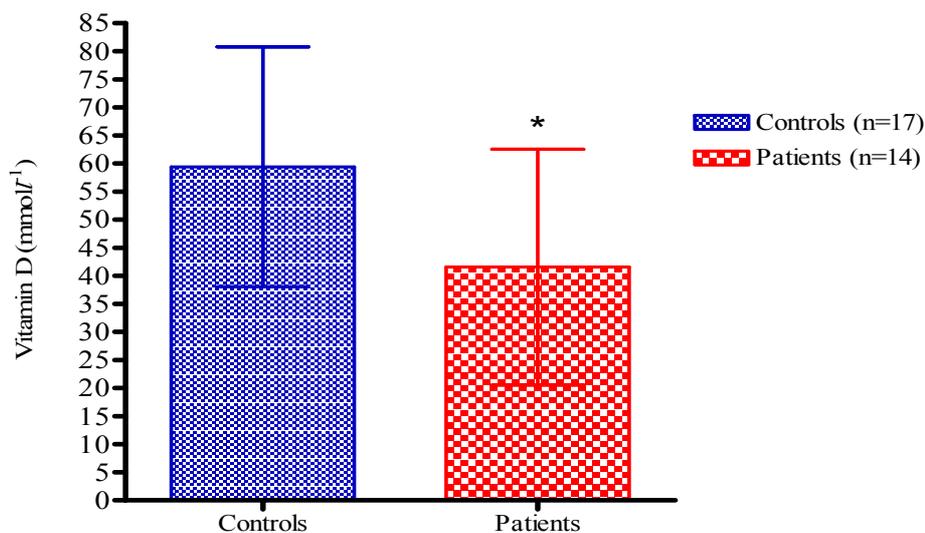


Figure 4-30 Vitamin D Concentration of the Patient and the Control Group
 The patient group exhibited significantly ($P < 0.05$) lower vitamin D levels than the control group.
 * Denotes significance

There was a significant difference in serum Vitamin D concentration between the patient and the control group (95% CI -33.49702, -2.18365; $P < 0.05$).

Table 4-25 Vitamin D Concentration of the Patients and the Control Group

	N	Mean Vitamin D Concentration (mmol ⁻¹)	SD
Controls	17	59.41	21.38
Patients	14	41.57	20.98

MS patients exhibit a significantly lower level of serum vitamin D than the control group.

5. Discussion

5.1. Study Objectives

Aim: The aim of this study was to describe the neuromuscular characteristics of Multiple Sclerosis (MS) patients. To help explain the neuromuscular characteristics physical activity levels, body composition and blood lipid profile were measured. In addition, Vitamin D was measured to determine if this was deficient in MS patients.

Hypothesis: We hypothesised that muscle fibre conduction velocity (MFCV) would be elevated and amplitude would be impaired in Multiple Sclerosis patients compared to an age and sex matched control group. In addition, we hypothesised that physical activity levels would be reduced, body composition would exhibit a higher percentage fat, blood lipid profile would be less favourable and Vitamin D levels would be reduced in the group of MS patients.

5.2. Adherence to Protocol

5.2.1. Comparable Groups

The patient and the control group were well matched; there was no difference between the groups in age, sex, height, weight or BMI. There were also an equivalent number of males and females in each group.

Data generated from the present study confirms that the two groups were well matched. The blood sample results show that the groups were well matched because there was no difference in blood lipid profile between the groups. If blood lipid profile can be taken as an indication of health status both groups are equivalent for risk of various medical

conditions such as coronary heart disease, obesity and type 2 diabetes. It also indicates a similarity in lifestyle between the two groups.

Physical activity results, although they show a significant difference between the groups, the absolute values from both the groups remain relatively low. Hence the control group were a relatively sedentary group; therefore there were no participants in the group who are highly active who could potentially skew the results.

5.2.2. MS Patients' Disability Status

All patients had a positive diagnosis of MS and had been diagnosed for a minimum of 3 years. The disability status of patients ranges between 4 and 6 on the expanded disability status scale (92). All patients presented with a degree of disability in the lower extremities. All patients completed the contraction protocol apart from one person who was unable to carry out the contraction with one of their legs. The leg that was used to carry out the contractions was considered the dominant leg for analysis.

5.2.3. Contraction Protocol

The contractions within the protocol were carried out at 20%, 40%, 60% and 80% of participants MVC. The target contraction intensities were calculated from each individual MVC; this was calculated for each leg. As the participants contracted their leg they received visual feedback, from a PC monitor, on how closely they maintained the force to their target contraction intensity, which was indicated on the screen. They were encouraged to adjust their contraction in line with the visual feedback in order to return to the target intensity indicated on the screen.

There was no difference in performed contraction intensity when comparing the dominant and non-dominant leg within each group. Neither was there a difference in performed contraction intensity between the patient and the controls groups for the dominant leg or the non-dominant leg. This reaffirms that the study was well controlled and highlights that comparisons made between the groups and within the groups are valid as no significant differences exists between the groups or within the groups for the relative contraction intensity.

There was a difference in contraction intensity between the target contraction intensities when compared between groups and within groups. Therefore, each contraction was carried out at a different intensity to the other contractions. In addition, within each contraction intensity i.e. (40%) the intensity carried out was not different between groups or within groups. This shows that comparison of individual contraction intensity between the two groups is valid as no difference in contraction intensity was identified between groups.

However, there was a difference when performed contraction intensity was compared to target intensity for the dominant and non-dominant leg. There was also a difference in performed contraction intensity and target contraction intensity for both the patient and the control group. Consequently the performed contractions were carried out at a different intensity than was intended. However, there was no difference in the contraction intensity carried out between the two groups or between dominant and non-dominant legs, therefore comparisons can still be made.

5.2.4. Vastus Lateralis

In the present study the vastus lateralis (VL) was measured in all patients regardless of what symptoms they presented with. This may have underestimated the degree of difference in MFCV between patients and controls as VL is not necessarily affected by the disease. Therefore, had the affected area of each patient been measured and compared to the equivalent muscle of a controls subject this may have shown more of a difference.

Nevertheless, it is the norm for patients with MS to experience some form of impaired gait and therefore it was assumed that using the vastus lateralis muscle, which is used for locomotion, as a means of comparison was as valid as any. In addition the vastus lateralis tends to be situated closer to the surface of the skin than other quadriceps muscle and therefore was more easily identifiable. This was also an advantage as the electrodes were more likely to make direct contact with the muscle.

5.3. Neuromuscular Characteristics

5.3.1. Muscle Fibre Conduction Velocity

The primary aim of this study was to identify if neuromuscular characteristics were different in MS patients compared to age and sex matched control participants. Initial findings indicated that the MFCV was faster in the patient group than it was in the control group; this difference reached significance when comparing the dominant leg of the two groups and just missed significance when comparing the non dominant leg. This suggests altered neuromuscular characteristics in the MS group as it may indicate a predominance of type 2 fibres within the muscle as a result of disuse (165; 166) or as a consequence of MS (82). However a significant difference was also revealed for the

dominant and the non dominant leg when comparing percentage fat of the thigh between groups. A highly significant ($P < 0.001$, $r = 0.697$) positive correlation was also observed when MFCV was related to percentage fat of the thigh. This indicates that as the level of body fat increased the MFCV rate was increased. High levels of body fat have previously been shown to artificially elevate the MFCV signal (110). Therefore percentage fat of the thigh was added into the analysis as a covariant. Once the comparison was adjusted to take account of the effect of body fat no significant differences existed between groups for either the dominant or the non dominant leg. Therefore the difference apparent in the initial comparison seemed to be as a consequence of the participants' body fat rather than as a result of altered neuromuscular characteristics. However a difference in the response of the MFCV to the increase in contraction intensity was noted between the groups. While the MFCV of the control group continued to increase with each increment of intensity the MFCV of the patients group did not. Therefore additional variables are affecting the MFCV of the patient. This may be evidence of an increased proportion of type 2 fibres within the muscle fibres of MS patients. As the MFCV of the patients was elevated during the lower intensity contractions this suggests that the MS patients were recruiting type 2 fibres during the lower intensity contractions. This insinuates that MS patients may express more type 2 fibres within the muscle and hence these are the only fibres available for recruitment.

There was no significant difference in MFCV over the contraction intensities when comparing the dominant or the non dominant leg between the two groups. This therefore suggests that there was no alteration in neuromuscular response to increased torque production between the groups. However, when MFCV over the contraction was compared between legs within each group a significant difference was revealed within the control group but not in the patient group. Therefore differences in MFCV between

the contractions were detected in the control group but not the patient group. This may indicate a methodological problem with EMG data collection from the patient group. As mentioned previously findings from the present study show that patients have a higher percentage of fat around the thigh than control participants. It has been documented (110) that high level of subcutaneous fat can artificially elevated the MFCV reading thereby making the signal less sensitive to picking up any alteration of the signal. Previous research by Nielson et al (2007) (124) has also demonstrated that alteration in MFCV are less detectable the further away the electrode is situated from the innervation zone. In the present study the electrode was placed midway between the innervation zone and the distal tendon however in some cases it was necessary to move the electrode closer to the distal tendon in order to receive a signal. In doing so, according to Neilson et al (2007) we may have moved the electrode too far away from the innervation zone to detect any differences over contraction intensity. It is interesting that in the control group where the percentage fat of the thigh was lower than the patients, a difference in MFCV over the contraction was detected.

The difference observed over the contraction in the control group would indicate that as torque production increased MFCV increased. It is well accepted that when higher intensity contractions are required a greater proportion of type 2 muscle fibres are recruited (70; 113). It is also known that type 2 muscle fibres carry the electrical signal along the sarcolemma more quickly than type 1 fibres (5). Therefore an increase in MFCV in response to increased torque production indicates that more type 2 muscle fibres are being recruited.

There was no difference over the contraction in the patient group. This may be as a consequence of reduced sensitivity of the EMG technique because of the high level of fat

in the patient group. However it may also be a physiological response as research has shown (82) that MS patients exhibit a higher proportion of type 2 muscle fibres than control participants. Consequently, MS patients will inadvertently recruit type 2 fibres at lower contractions therefore MFCV will be higher at lower intensities. As contraction intensity increases more muscle fibre will be recruited, however the fibres that remain to be recruited will also be type 2 fibres therefore although more fibres are recruited there is no shift in recruitment from type 1 to type 2 hence the MFCV would not change as torque production increases.

In addition there is no significant difference between the dominant and non dominant leg within the patient or the control group. There was a similar neuromuscular response to the contraction intensities between the dominant and non dominant leg within each group. This would be expected in the control group however it is surprising in the patient group as MS tends to affect one side of the patient more than the other. This may, in part, be as a consequence of the elevated percentage fat of the thigh in the patient group. The layer of fat may have dampened the signal and as a result discrete differences within the muscle may have gone unnoticed.

Therefore, the MFCV results indicate that there were no differences in the neuromuscular characteristics of MS patients when compared to an age and sex matched control group. The MFCV rate changes in response to peripheral changes, such as change in distribution of muscle fibre type (26), muscle temperature (64), fatigue (148) and contraction intensity (168; 169); therefore unaltered MFCV between patients and controls indicates that no peripheral differences exist between the groups.

5.3.2. Amplitude – Root Mean Square (RMS)

The root mean square (RMS) is the measure of global motor unit recruitment and therefore is indicative of the neural activity received by the muscle from the central nervous system (110). There was no difference in RMS between the patient and the control group for the dominant and the non dominant leg throughout the contractions. These results indicate that there is no difference in neuromuscular recruitment strategies between the patient and the control group. As RMS is indicative of the neural activity received by the muscle from the CNS the results signify that there is no difference in the functional capacity of the CNS between the two groups.

There was no difference in RMS between the dominant and non dominant leg within each group. These results support the previous finding of the present study which shows that there is no difference in MFCV between the dominant and non dominant leg within each group.

In addition, RMS data was expressed relative to grams of fat within the thigh. This analysis was carried out as fat has a low pass filtering effect on the RMS data (110). The findings revealed no difference between the patient and the control group when multiplying the RMS by grams of fat in the thigh. This indicates that both groups were affected by the low pass filtering effect of fat to the same extent. However this analysis did show a significant difference between the contraction intensity as had previously been demonstrated by the percentage RMS data.

These findings add credence to the MFCV finding as the present RMS results indicate that similar amounts of neural activity is being received by both legs within each group and between the two groups. Therefore, it would be reasonable to expect similar

neuromuscular characteristics between the two groups as previous research has indicated that neural activity dictates phenotypic expression in mammalian muscle (133). The MFCV and RMS results from the present study support this contention. However, the RMS proves that the CNS is capable of delivering equivalent amounts of neural activity to the muscle for an equivalent relative intensity contraction. What the RMS cannot indicate is how much neural activity the muscle does receive or has received over time. The neural activity received by the muscle over time dictates the characteristic of the muscle (133). If the nature or amount of neural activity received by the muscle changes then the characteristics of the muscle will adapt in response altered innervation (133).

The greatest way to increase the amount of neural activity reaching a muscle is to increase the level of physical activity. The present study measured physical activity and showed that the level of habitual physical activity was reduced in the patient group. Therefore this suggests that less neural activity is being received by muscles of the MS groups than the control group. It has been shown that muscle responds to reduced innervation by reverting to type 2 muscle fibres (28; 101; 133; 165; 166). It has been shown that any innervation regardless of the nature of the innervations influences a shift in fibre type from type 2 to type 1 (133). Although the physical activity results show a difference in the activity levels between the groups the effect of the difference in physical activity levels were not apparent in the MFCV or the RMS findings. Perhaps the difference in physical activity was not enough. Even though the difference reached statistical significance it may not have reached physiological significance. Although a difference was detected between the groups both groups would be considered to be sedentary. Therefore, neither group was completely inactive nor neither group was highly active, therefore no extreme conditions had been placed on the muscle. This may explain why no differences in MFCV or RMS were detected.

There was a highly significant difference in the RMS between the contraction intensities in both groups however no interaction was identified between the groups. Both groups show similar RMS responses to the increase in contraction intensity, this indicates that the amount of neural activity leaving the CNS for each contraction is no different between the two groups. Therefore, patients with relatively mild MS (4-6 EDSS) maintain the ability to voluntarily generate torque throughout the full range of contraction intensities.

Patients exhibited the same amplitude response as the control group over all the contractions. This is interesting as it shows that patients are as capable at carrying out low level contraction as they are at high level contractions. In MS patients the lesions on the neurons in the CNS make it more difficult for the electrical signal to be transmitted past the point of the lesion (154). The bigger the lesion the more actions potentials are required at the point of the lesion to propagate the signal past the lesion (154). Therefore carrying out low level contractions potentially pose a problem for MS patients as there is less central drive required to carry out a low level contraction; potentially this could result in an insufficient amount of electrical activity achieved at the point of the lesion to propagate the signal past the lesion. However when high intensity contractions are required the central drive is heightened and the muscles are bombarded with electrical activity and hence more electrical activity will have reached the point of the lesion and hence maintained the signal along the neuron. The amplitude results in the present study show that there is no difference between the groups over all the contractions, therefore it would appear that the patients' low level contractions were not affected. It may be that the 20% contraction was high enough to ensure the signal was propagated. However it is intriguing when looking at the mean contraction intensity performed as patients carried out their 20% at an average of $28.24 \pm 8.32\%$ for the dominant leg and $30.03 \pm 14.24\%$ for

the non dominant leg; whereas controls carried out their 20% contraction much closer to their target intensity at $24.34 \pm 4.93\%$ for the dominant leg and $23.82 \pm 3.79\%$ for the non dominant leg. It is possible the patients carried the contraction out at the lowest intensity that they were able to. The central drive may have had to increase to ensure propagation along the neuron and past the lesions. However, once the central drive was elevated the neural activity reaching the muscle would be more than required to carry out the intended contraction hence the contraction was carried out at a higher intensity. The effect of neural propagation on spasticity of MS patients has been investigated. Smith and McDonald (1999)(154) outlines the effect of temperature on conduction block. Neural transmission of electrical impulse past the lesion is decreased as a result of a rise in body temperature. Schwid et al (2003) (146) investigated the effects of a single acute dose of cooling therapy on MS patients. They found that one high dose of cooling therapy produces significant improvements in neurological function.

Taking the MFCV and the RMS results together indicates that MS patients, with a relatively mild disability status (EDSS; 2.5-6), do not exhibit any adaptations with the CNS or to neuromuscular characteristics within the periphery. It may be that patients who exhibit a higher disability status do present with change in MFCV and RMS data; further research would need to be carried out to elucidate this. The present study recruited patients with mild disability with impaired gait in order to ensure the contraction protocol could be carried out.

5.3.3. Summary of Neuromuscular Characteristics

In summary, as no difference in MFCV was identified between the groups this suggests that no intra muscular adaptations have taken place within the vastus lateralis of MS

patients. Additionally, there was no difference in amplitude revealed between the two groups either, which indicates that no alterations in the neural activity received by the muscle exist in MS patients with relatively mild MS (EDSS 2.5-6). Therefore this study can conclude that in the relatively early stages of MS no alteration within the CNS and no peripheral changes have taken place when comparing MS patients to an age and sex matched control group.

5.4. Torque Production

5.4.1. Absolute Torque

Controls produced more absolute torque, whilst executing their MVC, than patients in the dominant and non dominant leg. This is to be expected and is in line with previous research (35) with similar sample size that shows that patients MVC was lower than control participants. In addition this study showed that patients were only able to generate $75\pm 22\%$ of their force generating capacity whereas controls were able to produce $94\pm 6\%$ of theirs. The present study did not investigate the percentage of force generating capacity.

There was no difference between the legs within the patient and the control group. This is surprising as all patients presented with a reported affected leg and less affected leg yet no difference was identified between the legs within the group.

5.4.2. Relative Torque

There was no difference in torque production per gram of muscle between the two groups for either the dominant and the non-dominant leg nor was there any difference between the two legs within either group.

Therefore, although the control group produced more absolute torque than the patient group; when the torque was expressed relative to lean thigh mass the difference was revoked. Torque per gram of muscle mass is indicative of muscle quality (46) and the results suggest that a gram of patients muscle is capable of producing the same torque as a gram of the controls muscle. As the torque generating capacity of the muscle is the same this suggests that the intra muscular mechanisms that generate force must also be the same. These include fibre type distribution (26), motor neuron activity (133), cross bridge cycling (60), Ca^{2+} ATPase activity (28) and energy metabolism (84). These results support the neuromuscular results generated from the present study, as it is well documented that the neural activity received through the motor neuron dictates the phenotype of the motor unit (133). The neuromuscular results from this study suggest the neural activity received by the patient and the control group were no different therefore it is not surprising that the force produced per gram of muscle mass also remained unchanged. The maintenance of the neural activity received by the vastus lateralis in the patients group has resulted in the maintenance of intra muscular mechanisms and hence resulted in the maintenance of torque generating capacity.

5.4.3. Absolute Lean Thigh Mass

When absolute LTM was analysed no difference was identified when solely comparing the dominant or the non dominant leg between the two groups. However; when both legs were considered together within each group a difference was identified between the two groups. Therefore, the patient group overall exhibited reduced muscle mass in comparison to the control group. However there was a difference in MVC between the groups for both the dominant and non dominant leg even though no difference in LTM was identified in either of the legs between the groups. Therefore controls were able to

produce a greater absolute amount of force than the patient group whilst exhibiting a similar size muscle mass as the patient group. This indicates that the patient group have a reduced muscle quality than the control group. However because there was a difference in LTM between the group when considering both legs; this shows that the control group exhibit a greater thigh muscle mass than the patient group. Therefore, there may not be a difference in muscle quality between the groups.

In addition, there was no difference in absolute LTM between the two legs within each group. This supports the previous finding that there was no significant difference in absolute force production between the dominant and non dominant leg within each group. As had been previously demonstrated muscle produces force relative to muscle cross sectional area. Maughan et al (1983) (104) found a significant positive correlation between strength and cross sectional area for both males and female. This demonstrated that as cross sectional area increased the maximum voluntary contraction was greater. Hence when no significant difference in MVC existed between the dominant and non dominant leg it follows that no difference existed in absolute LTM between the dominant and non dominant leg.

It has been established that no difference in MVC or LTM exists between the dominant and non dominant leg within each group therefore effectively there is no difference between the two legs within each group. This is surprising, especially within the patient group as most patients presented with a definite dominant leg. These results may in part be down to the protocol carried out as the contraction took place on the Bio Dex Systems 3 Isokinetic dynamometer. When the participant was set up on the chair, the contraction angle and direction was dictated thereby removing any requirement for co-ordinated movement. The contraction protocol also did not require alterations within the

contraction where the participant was required to react, again removing the need for co-ordinated movement. Therefore the protocol may have removed certain variables that affect MS patients' movement in everyday living, thereby making the differences between the limbs appear less significant.

Future research may be required to look more closely at the contribution that co-ordination has on MS patients' ability to carry out daily tasks. It may be that their muscles have the function capacity to carry the task out but the reduced precision of movement results in failure of the task.

5.4.4. Lean Thigh Mass Ratio

Lean Thigh Mass Ratio¹ (LTM Ratio) is used to express the lean mass of the thigh in comparison to the lean mass of the whole body. When lean thigh mass ratio was analysed no difference was identified when only comparing the dominant or the non dominant leg between each group. When both the dominant and the non-dominant leg were considered together within each group the difference was approaching significance ($P=0.077$). This is similar to the results obtained when including both legs into the comparison of absolute lean thigh mass between the groups. No difference had been identified when comparing only one leg but when both were considered a difference was revealed. In the case of the LTM ratio the finding did not reach significance when adding both legs into the comparison however the result was approaching significance.

Using LTM ratio is more robust than absolute LTM as the value is relative to the participants' lean body mass; however it is interesting that the results for the LTM ratio tend to follow the same propensity as the absolute LTM.

¹ Lean Thigh Mass (LTM) Ratio – $LTM / \text{Lean Body Mass (LBM)} * 100$

These results show that there is a tendency for the patients to exhibit muscle atrophy but only when both legs are considered in the comparison. As there is no significant difference in LTM or force production between the dominant and non dominant leg within each group including both merely increases the sample size.

This finding is interesting as when lean thigh mass increases this generally results from an increased use of the muscle through physical activity and this study shows that the control group was more active than the patient group. As a result of an increase in physical activity the muscle will increase in size (105). In addition to the increase in size the muscle should have exhibited neuromuscular adaptations to the increased neural activity received by the muscle. This would manifest itself as an increased expression of type 1 muscle fibres within the muscle (165; 166). Although a tendency for the size of the muscle to be greater in the control group was revealed in the present study no difference in neuromuscular characteristics were identified.

5.4.5. Correlation of MFCV to LTM Ratio

A negative correlation was revealed. As LTM increases MFCV decreases. This is what would be expected from a more trained muscle. As you would expect the bigger muscle to have received more neural innervation and hence present with more type 1 muscle fibre and therefore present a lower MFCV.

5.4.6. Summary of Torque Production

Controls produced more absolute force than patients however when the force was converted to force per gram of muscle mass no significant difference was revealed. Therefore the quality of the muscle remained unchanged between the patient and the

control group. Therefore; in MS patients with a disability status (EDSS) between 4 and 6, there was no detectable deterioration in muscle quality.

There was a tendency for patients to exhibit muscle atrophy as there was a difference between absolute LTM between the groups and the difference in LTM ratio between the groups was approaching significance. This tendency for MS patients to exhibit muscle atrophy is supported by the finding that MS patients had a reduced level of physical activity.

Therefore although there is a tendency for MS patients to have less functional muscle, the muscle that they do have exhibits no decline in muscle quality compared to the muscle of the age and sex matched control group.

5.5. Body Composition

Body composition in the present study was measured using Dual-energy X-ray Absorptiometry (DEXA) which is considered the reference method of body composition analysis (95). The precision, using DEXA, of the total body fat mass, lean mass and bone mineral content, expressed as %CV, was 2.0, 1.11 and 1.09%, respectively (85). Therefore the body composition measurements obtained in the present study are highly accurate and reliable (153; 157).

The radiation dose from a DEXA scan is very low and hence it is safe for sequential measurements to be made on volunteers (16; 19; 93)

5.5.1. Total Body Fat

Results from the present study found a difference in total percentage body fat between the patient and the control group. Controls had 30.6% (women 32.1, men 29.6%) body fat and patients had a higher level of body fat of 38.3% (women 40.5, men 35.8%). According to the National Institute of Health men with more than 25% percent body fat and women with more than 30% body fat are obese. On average, both the men and women in each of the groups have a higher level of body fat than is recommended for optimal health (59).

Percentage fat of the thigh, when considering both thighs, was different between the patient group and control group with patients exhibiting 44.13% and the control group exhibiting 34.27% fat. In addition, there was a difference in percentage body fat between the two groups when comparing the dominant and non-dominant leg. The percentage fat of the dominant thigh of the patient groups was 43.83% and the control group was 34.28%. The percentage fat of the non-dominant thigh for the patient group was 44.46% and the control group was 34.25%.

It is interesting that percentage fat of the thigh was greater than percentage fat of the whole body. This may explain why the MFCV values gathered in the present study are elevated in both groups. High levels of subcutaneous fat are known to artificially elevate the MFCV value when using surface EMG techniques (110; 111). The thigh appears to store a higher proportion of body fat than the body as a whole as patients had a whole percentage body fat of 38.27% and a combined thigh percentage fat of 44.13% and controls exhibited values of 30.16% and 34.27% respectively. If this is the case it may be more accurate to use a muscle that is situated where body fat does not accumulate as readily, such as the tibialis anterior which is situated to the front to the tibia. This muscle

is close to the skin and is less likely to be covered in high levels of body fat. MFCV values may have been lower than the present study which utilised the vastus lateralis to gather EMG data. Numerous studies (28; 80; 81; 83; 120; 121; 123; 148) which have investigated neuromuscular characteristics in health and disease have made use of the tibialis anterior muscle for data collection.

5.5.2. Correlation of MFCV to Total Percentage Body Fat

A positive correlation was identified between MFCV and percentage body fat; as body fat increased MFCV increased. This supports the contention that the surface EMG technique is affected by the subcutaneous layer of fat between the muscle fibre and the electrode. It is possible that this technique may more appropriate in populations where the percentage body fat remains within a healthy range or in an athletic population, where the percentage body fat tends to be relatively low (160).

5.5.3. Correlation of MFCV to Percentage Fat of the Thigh

A consistent positive correlation was evident between MFCV and percentage fat of the thigh for both groups independently and in both groups together. This indicates that MFCV increased with increasing levels of fat within the thigh. This is likely to be as a consequence of methodological limitations as fat is known to artificially increase the conduction velocity value obtained (110). However it is possible that the MFCV is in fact elevated in individuals with elevated levels of fat. If high levels of fat are indicative of lower levels of physical activity then it can be assumed that the muscles of individuals with high levels of body fat receive less neural activity and therefore the resulting muscle will exhibit more fast muscle fibres and therefore have an elevated MFCV (165; 166).

5.5.4. Bone Mineral Density

There was no difference in whole body or thigh BMD between the patient and the control groups. Neither was there a difference between the dominant and non dominant leg within each group. This is interesting as calcium homeostasis is closely linked to vitamin D metabolism. Vitamin D deficiency causes bone mineral reabsorption (97) and hence results in a reduced bone mineral density; therefore had vitamin D deficiency existed within the MS population it would have been likely to have resulted in a reduced bone mineral density. Results from the present study do not show any difference in Vitamin D concentration or BMD between the patient and the control group. However research has suggested that vitamin D plays a role in the pathogenesis of MS (8) and that higher sun exposure between the ages of 6-15 years was associated with a lower risk of MS (8). Therefore it is possible that at some point in time a vitamin D deficiency may have triggered the disease in individuals who were genetically susceptible to MS but was not significant enough to cause major bone mineral resorption.

Physical activity, especially weight bearing physical activity (48; 109), is known to increase BMD. BMD has been shown to remodel in response to impact and hence the more physically active a person is the more likely they are to have a high BMD, assuming vitamin D and calcium homeostasis is maintained (109). As the present study identified a significantly higher level of habitual activity in the control group, it would have been reasonable to expect the BMD to be higher too but this concept has not been supported by data from the present study. Although a difference in activity levels was identified between the groups, the overall activity levels of both groups remain low. Therefore, potentially the absolute difference in levels of physical activity was not great enough to manifest a difference in BMD.

5.6. Physical Activity

5.6.1. Habitual Physical Activity Levels

The results of the accelerometry data analysis show that patients are less habitually active than control participants as revealed by their mean and total counts per day. This is in agreement with Ng and Kent-Braun (1997) (122) who also identified a significant difference in physical activity levels, using three dimensional accelerometers for 7 days, between MS patients and controls from groups of similar size to the present study.

These physical activity findings support the absolute LTM and the LTM ratio results; the lower levels of physical activity apparent in the patient group may explain the tendency them to exhibit a lower LTM ratio than controls. Although the result of the comparison between the LTM ratios between groups did not reach significance the fact that the absolute values, when considering both legs, did reach significance supports this contention.

5.6.2. Effect of MS on Activity Levels

It is not surprising that MS patients have reduced levels of habitual physical activity; patients often exhibit impaired gait hence have reduced mobility (102; 109). It is also common for MS patients to experience fatigue (90; 119; 148) and therefore may rest or sleep throughout the day, resulting in a reduced level of daily physical activity. In addition many MS patients are unable to work and as a consequence do not incorporate any occupational associated physical activity into their day. Furthermore MS patients were previously advised by their physicians to avoid physical activity (11; 177) as the heat produced exacerbated their symptoms (11; 132; 175). It is now more clearly

understood that this is a transient change (11; 175) and that the patients symptoms return to normal after the heat load.

In addition, previous research suggests that MS patients have an increased energy cost of walking compared to control participants (125; 126), it is not surprising that the accelerometry data from the present study and from previous research (122) demonstrates patients as being less active. MS patients have an elevated energy cost for a given work load so even light workloads will challenge their cardiovascular system and energy metabolism as oxygen requirement can be up to 4 times the requirement of control participants. It is therefore reasonable that they tend not to raise their level of physical activity beyond relatively light work. This may also explain why MS patients in the present study have not demonstrated a difference in MFCV, which is indicative of a high proportion of type II fibre within the muscle. If patients are constantly requiring high levels of oxygen to carry out daily tasks they will be continually putting demands on their aerobic energy system. As the aerobic energy system is being stressed it will adapt to be more efficient so that energy demands can be made; such as the effect of endurance training (79). Therefore muscle fibres would adapt to become more aerobic in nature which is indicative of a slow twitch or a type I muscle fibre which transmits the action potential along the muscle fibre at a slower rate than the fast or type II muscle fibre (145). Contrary to this however previous research (82) has suggested that MS patients have a higher percentage of type 2 fibres than control participants which is consistent with bed rest (165; 166), spinal chord transaction (29) and long term paralysis (101). Therefore it may be the case that MS patients do not raise their level of physical activity to the point where they stress their aerobic capacity. At this point, they may experience discomfort and the sensation of fatigue and therefore cease physical activity. Therefore the muscles

will never experience enough stress to adapt to become more aerobic. Hence, MS patients exhibit more type II muscle fibres (82).

5.6.3. Benefits of Physical Activity

Exercise programmes can increase muscle mass therefore muscle strength may be increased and walking efficiency improved (163), thereby reducing the individuals' energy cost of walking, making daily living easier and allowing them to increase their habitual physical activity to levels where they would obtain health benefits.

Physical activity should be encouraged as the patients can gain from the health benefits from exercise. Numerous studies (65; 89; 119; 131; 159; 163; 176-178) have investigated the benefits of physical activity for MS patients. Gutierrez et al 2005 (65); Taylor et al (2006) (163); White et al (2004) (178) and White et al (2006) (176) studied the effect of resistance training among MS patients. Resistance training is good for MS patients as it is more targeted to specific muscle groups, it is slower and requires less coordination than some aerobic type exercise. It is also more controlled and reduces the risk of injury from falls during exercise. Gutierrez et al (2005) (65) showed that after 2 months of resistance training there was a significant increase ($P < 0.05$) in stride time and stride length and isometric strength also improved in 2 out of the 4 muscle groups tested. Taylor et al (2006) (163) demonstrated that the MS patients who completed a progressive resistance programme twice a week for 10 weeks exhibited a significant increase in the leg press 1 repetition maximum and in fast walking speed and a highly significant increase in leg press endurance and arm press 1 repetition maximum. This indicated that resistance training can be used to improve quality of life and maintain functional capacity in MS patients. In addition patients will gain from the health benefits of physical activity

which will reduce the likelihood of them developing cardiovascular disease (88), type 2 diabetes (147), the metabolic syndrome (128) and obesity (134). If MS patients can avoid contracting lifestyle associated diseases the management of their MS becomes less complex (116).

As well as the physical benefits, physical activity is known to improve psychological well being (87) and increase self image and mental well being (55) and therefore improving the patients' quality of life.

5.6.4. Correlation of MFCV to Total Physical Activity

A strong positive correlation was identified between MFCV of the patients' non dominant leg and patients' total physical activity. This indicates that MFCV increases as total physical activity is increased. This is contrary to what is believed to happen. Studies (4; 10; 133) have shown that muscle fibres that are innervated with an electrical impulse adapt to become more like type 1 muscle fibres regardless of the nature of the innervation. Whereas when innervation is removed, either from muscle preparations or in spinal chord transection the muscle revert to type 2 muscle fibres and hence would exhibit a faster MFCV (29; 101; 133; 165). Therefore in the present study a negative correlation was expected; where MFCV decreased as total physical activity increased. It may be that the participants in this study were not physically active enough and those changes would only be seen when greater amounts of physical activity were taking place.

The correlation of MFCV to total physical activity was not consistent throughout all the comparisons (see Table 4-20; in results section). A negative correlation was obtained for two correlations and no correlation existed for the other three comparisons. Therefore the highly significant correlation of the patients' non dominant leg to total physical activity is

not representative of all the correlations made between MFCV and total physical activity. As the result is greatly different from the other correlations the significant result may be down to chance and consequently not indicative of a physiological relationship.

5.6.5. Correlation of MFCV to Mean Physical Activity

A similar finding was revealed from the correlation of MFCV to mean physical activity. The outcome of the correlation was inconsistent throughout the comparisons. A highly significant correlation was identified within the patient non dominant leg however as the response throughout the comparisons are inconsistent it is unlikely that this highly significant finding is representative of a physiological relationship.

5.7. Blood Sample Analysis

5.7.1. Lipid Profile

No significant difference existed between the patients and the control group for either cholesterol, triglycerides, HDL or LDL cholesterol. This insinuates that the lifestyle variables which effect the blood lipid profile; such as diet (112; 130) and physical activity (51; 63; 94), are similar between the two groups.

It is interesting that physical activity is reduced and percentage body fat increased within the patient population yet they exhibit a similar blood profile. Physical activity is known to improve an individual's blood lipid profile (51; 63; 94) therefore a less favourable blood lipid profile would be expected within the patient population.

5.7.2. Vitamin D Concentration

A significant difference ($P < 0.05$) in Vitamin D concentration between the patient and the control group was detected. Vitamin D is one of the non-infectious agents that has been suggested to trigger MS in a genetically susceptible individual (8). Research by Soilu-Hanninen et al (2005) (155) demonstrated that MS patients exhibited a reduced level of Vitamin D than controls at the point of diagnosis. However Barnes et al (2007) revealed that no difference in Vitamin D levels existed between MS patients and controls (14). Vitamin D deficiency is widespread in the population in the absence of MS (69) therefore vitamin D deficiency is not the direct cause of MS although there is research to suggest an association with MS (7; 8).

In addition the vitamin D results are consistent with the BMD findings; no difference in BMD was found between the groups and therefore the fact that no difference in vitamin D was found was consistent.

The vitamin D analysis was carried out using only the values for the participants who had not been excluded from the trial due to insufficient EMG data. However a blood sample had been taken from these participants and was analysed. When statistical analysis was carried out on the vitamin D results from all the blood samples analysed the difference in vitamin D concentration between the patient and the control group was significant. The control group exhibited a higher concentration of Vitamin D than the patient group. This study could not identify the differences because a number of participants' data was excluded from previous analyses and therefore had to be excluded from this analysis. It is however important to look at this variable on its own because vitamin D is considered to be a risk factor for multiple sclerosis.

5.8. Methodological Implications

5.8.1. Surface Electromyography and Alternative Techniques

Surface electromyography (sEMG) is a non-invasive method used to measure the electric potential field evoked by active muscle fibres through intact skin (182). The electrode placed on the skin detects depolarisation of the sarcolemma. The validity of the measurement depends on a constant distance between the electrode and the sarcolemma; a thick subcutaneous fat layer can increase cross talk between motor units and introduce error into the data (156). In the present study, on average, both the groups are classified as obese therefore the level of body fat may have introduced error to the data collected and may account for the high MFCV values obtained.

The high level of fat in both groups may have also effected the orientation of the electrodes with respect to the muscle fibre direction. Electrodes were situated midway between the innervation zone and the tendon of the vastus lateralis; they were placed on the surface of the skin along the pennation angle of the muscle fibre. However when the contraction was carried out the layer of fat was displaced and hence resulted in the electrode being moved from its original position. In addition to the effect of cross talk as a result of high levels of fat (156) the displacement of the electrode may have confounded the issue.

In addition, the different intensity contraction may have resulted in the electrode being displaced to a different degree. For instance the electrode may be displaced less during the 20% contraction than during the higher intensity contractions. Therefore the EMG data from the 20% contraction may have been gathered from different muscle fibres than the higher intensity contractions. As the signal may have been gathered from different

muscle fibres for the different contraction intensities the signal may have been generated from different fibre types, which in itself would result in different conduction velocities being recorded.

In addition to the effect that high level subcutaneous fat has on the EMG recording fat distribution may also have an effect. The DEXA results highlighted that the percentage fat of the thigh was in fact higher than the percentage fat of the whole body. Therefore this shows that patients and controls store a disproportionately higher amount of fat around the thigh area. An observation from the DEXA scan results is that more fat was stored higher up the thigh than towards the knee. This means that there is a gradient in the distance between the sarcolemma and the skin surface for the whole length of the thigh. It appears that there may have been higher levels of fat beneath the first electrode and the subsequently less fat under each of the other electrodes. This has implication on EMG recordings as even slight differences in the volume of fat beneath each electrode may have a significant effect. However, whole body and percentage fat of the thigh was high within both groups so the effect of high levels of body fat were similar between the groups.

Intramuscular EMG techniques are also used to measure MFCV along muscle fibres; needle electrodes are inserted into the muscle at a set distance apart whereby measuring the impulse along the muscle fibre more directly (5; 182). This technique removes variables that affect the EMG such as level of subcutaneous fat between the muscle and skin. This may be a more viable technique when measuring electrical activity of muscle fibres in patient and sedentary populations where high levels of body fat may be prevalent.

5.8.2. Accelerometry

The GT1M accurately and concisely measures time varying acceleration ranging in magnitude from 0.05 to 2.0 G's (according to manufacturers technical details) in order to measure level of habitual physical activity. However the accelerometer detects movement from some activities better than others. For example the monitor is more likely to register activity counts from activity that required movement as the monitor gathers information through gravitational acceleration. Therefore, during activities such as walking, participants' hips are moving therefore the monitor will detect the movement. However, during activities such a cycling where the legs are working but the hips are not moving, the physical activity count for that activity may be underestimated. Also, the device fails to identify the alterations in gradient, therefore it could underestimate physical activity if they were climbing a set of stairs or going up a hill. In addition, the activity monitor cannot be worn in the water therefore if someone's main mode of physical activity was swimming accelerometry would greatly underestimate their habitual level of physical activity. Especially in a patient population who may rely on swimming, as it is a gentle form of exercise and specifically in MS as swimming can be carried out without an increase in body temperature (11) which is known to exacerbate their symptoms (86; 146; 154).

However, although these limitations reduces the likelihood of collecting accurate data, the same limitations exist for all participants and therefore the data collected for all participants are comparable. In addition, accelerometry is an objective method that is more accurate and reliable than subjective methods, such as recall questionnaires, which have several limitations (140).

5.8.3. Mode of Analysis of Accelerometry Data

Debate exists in the literature on the best way to analyse accelerometry data. It is common practice in accelerometry research to remove zeros from individual data sets if the zero has recurred continuously for 20 minutes or more (140). Various methods exist, however it is becoming more apparent that one method may not be correct for all populations. For instance, in a patient population it is not unusual for them to sit down for 20 minutes or sleep for a while during the day. If a string of zeros of up to 20 minutes was removed then the average counts per day will over estimate their level of habitual activity; however the total counts per day will remain unaffected. The intention of the accelerometry is to get an accurate value of actual physical activity and leaving the zeros in will do this more accurately than removing them. In the present study the method of removing a string of zeros of 60 minutes or more was used. This reduced the likelihood of removing zeros when the participant had in fact been inactive, thereby reflecting their mean level of physical activity more accurately.

Furthermore the same method of analysis may not be appropriate for both patients and controls. However it is possible that accelerometry data should be analysed differently to suit the population, or kept the same in order to make a valid comparison. In the present study the same method of removing zeros was used for both the patient and the control group.

Accelerometry data can also be used to indicate how long the participant spent in different intensities of activity such as; sedentary, light, moderate and vigorous activity. This method was not used in the present study primarily because physical activity is a secondary outcome and not the main finding. In addition the cut points which designated the different activity level were developed on a young healthy population and these cut

points may not be indicative of these activity levels in older and diseased populations (57). It has been shown that MS patients have an energy cost of walking up to four times greater than controls (125; 126). Therefore for any given value generated by the accelerometer the MS patients' energy cost will be much higher than if that value was generated from a control participant. The cut point would therefore not accurately describe the length of time that patients spent in each activity level; hence the decision was made to only use average and total counts for analysis of accelerometry data in the present study.

5.8.4. Sample Size

The original power calculation for this study indicated that 18 participants were required in each group to detect significant differences in MFCV. It was decided to aim to recruit 20 participants for each group to allow for drop out or spurious data. Every effort was made to recruit 20 participants into each group, however this proved not to be possible. Initially, 18 participants were recruited into the control, which in fact, met the power calculations minimum value. Only 16 patients were recruited, however this included almost all of the MS patients within the local MS societies who fitted the disability criteria and who were willing to participate. As a result of falling short of the power calculations values we may have missed some differences that do exist but were unable to identify. The sample size recruited was sufficient to detect a difference in MFCV between the groups in the dominant leg however had we obtained the larger sample size we may have been able to identify differences between the non-dominant legs as this was approaching significance.

However, in order to increase the patients sample size the area of recruitment would have to be greatly increased. This study, was advertised with MS societies within the central belt, however research suggests that there is a clustering of MS in Tayside in Scotland (44) and therefore if the recruitment area was increased to include Tayside the sample size may be adequate to identify any differences in MFCV.

5.9. Conclusion

MS patients exhibited faster MFCV with a similar number of motor units recruited than healthy ages and sex matched controls. An increased quantity of subcutaneous fat artificially elevates MFCV (110) and MS patients in this study had exhibited higher percentage fat than the controls. Therefore, when accounting for this confounding variable we then showed that there was in fact no difference in MFCV between the groups. However the patients MFCV responded differently to the increased contraction intensity than the controls which suggests altered neuromuscular processing within the patient group.

The MS group had reduced physical activity levels which caused a slight disuse atrophy of the lower limbs which did not compromise muscle quality. Therefore, similar quality of the muscle has resulted in unaltered neuromuscular recruitment in MS patients.

Overall, the results indicate that there was no difference in neuromuscular characteristics of MS patients compared to age and sex matched controls, even in the presence of altered body composition and physical activity levels.

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Appendix A

Ethical Approval



**UNIVERSITY OF
STIRLING**

DEPARTMENT OF
SPORTS STUDIES

Sport Studies Ethics Committee

Dr Angus Hunter
University of Stirling
Stirling FK9 4LA Scotland

Telephone: +44 (0) 1786 466497

Sasha Scott
Room 3A50
The University of Stirling
Department of Sport Studies
Stirling
FK9 4LA

11th July 07

Dear Sasha

Application no #161 Muscle Fibre Conduction Velocity of Multiple Sclerosis Patients during Isometric Contractions of the Knee Extensor

Thank you for your response from my letter of 26th June; I am happy that you have satisfied the conditions required; therefore you are now eligible to proceed with the study.

We wish you the best of luck with your study.

Yours sincerely,

Dr Angus Hunter
Chair of Sport Studies Ethics Committee

Appendix B

Recruitment Posters



**UNIVERSITY OF
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DEPARTMENT OF
SPORTS STUDIES

Health Research Participants Wanted



Are you:

Between 18 and 65 years of age?

Interested in assisting with health research!

Wish to gain feedback on your current state of health!

- **Leg Strength**
- **Cholesterol Level**
- **Accurate measure of body composition (fat and muscle)**
- **Physical activity level analysis**

If you are interested in taking part please contact:

Sasha Scott

0*****

s.m.scott@stir.ac.uk



**UNIVERSITY OF
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DEPARTMENT OF
SPORTS STUDIES

Multiple Sclerosis Research Participants Wanted



Are you: An MS Patient

Between 18 and 65 years of age?

Interested in assisting with research into multiple sclerosis

Have impaired mobility

Wish to gain feedback on your current state of health

- **Leg Strength**
- **Cholesterol Level**
- **Accurate measure of body composition (Fat and muscle)**
- **Physical activity level analysis**

If you are interested in taking part please contact:

Sasha Scott

0*****

s.m.scott@stir.ac.uk

Appendix C

Invitation Letter for Control Participants and Patients



**UNIVERSITY OF
STIRLING**

DEPARTMENT OF
SPORTS STUDIES

Sasha Scott
Dept. of Sport Studies
University of Stirling
Stirling
FK9 4LA

0*****
s.m.scott@stir.ac.uk

DATE: [DATE]

Dear [Participant]

PARTICIPANT INVITATION FOR STUDY OF MUSCLE FUNCTION IN MS PATIENTS

You are invited to take part in a study being carried out by the University of Stirling. In order to participate in the study you should fit the following description:

- Male or Female
- Mobile or mobile with an aid
- 18-65 years of age
- Free from cardiac complaints
- Free from MS

The study is comparing muscle function between MS patients and control participants. The information sheet explains what you will be asked to do if you agree to take part. Before you decide it is important for you to understand what is involved. Please take time to read the following information carefully and discuss it with others if you wish.

If there is anything that you are not clear about or if you would like more information on the study please contact Sasha Scott on: 0***** or s.m.scott@stir.ac.uk

Thank you for taking the time to consider participating in this study.

Yours sincerely

Sasha Scott
Postgraduate Research Student
Department of Sport Studies
University of Stirling

Dr Adrienne Hughes
Supervisor
adrienne.hughes@stir.ac.uk



**UNIVERSITY OF
STIRLING**

DEPARTMENT OF
SPORTS STUDIES

Sasha Scott
Dept. of Sport Studies
University of Stirling
Stirling
FK9 4LA

0*****
s.m.scott@stir.ac.uk

DATE: [DATE]

Dear [Patient]

PATIENT INVITATION FOR STUDY OF MUSCLE FUNCTION IN MS PATIENTS

You are invited to take part in a study being carried out by the University of Stirling. In order to participate in the study you should fit the following description:

- MS patient
- Male or Female
- Mobile or mobile with an aid
- 18-65 years of age
- Free from cardiac complaints

The study is comparing muscle function between MS patients and control participants. The information sheet explains what you will be asked to do if you agree to take part. Before you decide it is important for you to understand what is involved. Please take time to read the following information carefully and discuss it with others if you wish.

If there is anything that you are not clear about or if you would like more information on the study please contact Sasha Scott on: 0***** or s.m.scott@stir.ac.uk

Thank you for taking the time to consider participating in this study.

Yours sincerely

Sasha Scott
Postgraduate Research Student
Department of Sport Studies
University of Stirling

Dr Adrienne Hughes
Supervisor
adrienne.hughes@stir.ac.uk

Appendix D

Participant and Patient Information Sheet



**UNIVERSITY OF
STIRLING**

DEPARTMENT OF
SPORTS STUDIES

PARTICIPANT INFORMATION SHEET

MUSCLE FUNCTION IN MS PATIENTS



You are invited to take part in a research study that is being carried out in fulfilment of a Master of Philosophy (MPhil) degree at the University of Stirling. Before you decide whether or not to take part it is important for you to understand what is involved.

Please read this information sheet carefully.

1. What is the purpose of the study?

The purpose of this study is to determine if there is a difference in muscle fibre conduction velocity (MFCV)* in Multiple Sclerosis (MS) patients compared with age and sex matched control participants.

*Muscle Fibre Conduction Velocity is the speed at which an electrical impulse travels along a muscle fibre to trigger a muscular contraction.

2. Why have I been chosen?

You have been chosen as you are the same gender and are similar in age to one of our MS patients. The study aims to match each MS patient to an age and sex matched control participant in order for us to make a comparison.

We aim to recruit 20 MS patients and 20 age and sex matched controls.

3. Do I have to take part?

No. It is up to you to decide whether or not to take part. Participation is voluntary. Payment will not be given for participating in the study; however participants will be reimbursed for travel expenses up to the value of £10.

If you decide to take part you are free to withdraw from the study at any time and without giving a reason.

4. What will happen to me if I take part?

- Measurement of your physical activity habits using Accelerometry
- Diet log for 24 hours prior to testing
- 1 visit to the laboratory
- 1 Dual-energy X-ray Absorptiometry (DXA) scan to measure body composition

Accelerometry

We will ask you to wear an accelerometer, which is a little box that measures your level of physical activity by detecting movement. The accelerometer is attached to a belt which is worn around your waist and situated on your right hip. The accelerometer should be worn during all waking hours for 7 days. The accelerometer is small and can be worn under clothing without being noticed.

Laboratory Visit

The main part of the study involves one visit to the physiology laboratory at the University of Stirling. You will be asked to write down all the food and drink you consume for the 24 prior to your laboratory visit.

On arrival at the laboratory you will have your blood pressure measured and a blood sample* taken. Weight and height will be measured; we will also take a skin fold measurement of your thigh muscle.

* As we are taking a blood sample, you will be asked to fast for 12 prior to your laboratory visit. The blood sample will be taken first; you will then be offered tea or coffee, a roll and some fruit.

We will place electrodes on the skin surface over your thigh muscle to record the electrical impulse along the muscle. In order for the electrodes to pick up a clear signal we must first prepare the skin by shaving the area, then wiping it clean with an alcohol swab.

*Electrical impulses are naturally occurring in the muscle. You will not feel any difference between contracting your leg with or without the electrode on your skin. This is a completely non-invasive procedure.

The strength of your thigh muscle will be measured on a Bio-Dex isokinetic dynamometer (pictured on page 1). You will be strapped into the seat and your leg attached to a lever (same as the picture). You will be asked to push your leg away as

if you are trying to straighten it however the lever will not move causing a static contraction. While you are doing the static contraction the Bio-Dex measures the force that your thigh muscle is producing.

We will ask you to perform four sub-maximal contractions to familiarise yourself with the procedure. You will then perform two maximal contractions (i.e. push your leg away as hard as you can). You will then be asked to complete sub maximal contractions at 20, 40, 60 and 80% of your maximum. You will then be asked to complete sub maximal contractions at 20, 40, 60 and 80% of your maximum. Carrying out 3 contractions at each level; we will ask you to carry out each contraction for 7 seconds. You will be given 14 seconds rest in between each contraction.

Dual-energy X-ray Absorptiometry (DXA)

The DXA scan will be carried out at Yorkhill Children's Hospital in Glasgow. Transport will be provided if it is required. The DXA scan is a non-invasive procedure and is used to obtain an accurate measurement of fat mass, muscle mass and bone density. The DXA scanner gives a very small dose of radiation; much less than the amount of background radiation you would be exposed to from being outside for one day. Therefore this scan poses no health risk. You are required to lie still for about 10 minutes until the scan is complete.

5. Do I have to change anything if I take part?

You are not required to change any aspect of your lifestyle as a result of taking part in this study. Continue to take any medication that you have been prescribed and continue to follow your usual routine. We do however ask that you refrain from any form of strenuous exercise on the day before laboratory testing.

6. What is the procedure that is being utilised?

We are using a procedure called Surface Electromyography (sEMG) to obtain the main findings for this study. sEMG is a non invasive technique, where electrodes are placed onto prepared skin. The electrodes pick up electrical activity in the muscle below the surface of the skin.

7. What are the possible problems of taking part?

When asked to perform a maximal contraction, changes occur within the body to meet the demands of the effort. Heart rate, blood pressure and breathing rate can all increase. These are normal responses to a maximal effort. You will be given time to recover before you are asked to carry out the remaining contractions.

The DXA scan involves a small amount of radiation. We are all exposed to a small amount of radiation when we are outside. You get the same amount of radiation from the scan as you would from being outside for a few hours one day. Therefore this procedure poses no health risk.

One blood sample will be taken; you may experience a slight scratch as the needle punctures the skin. If you feel that the discomfort is too much the procedure will be stopped. The rest of the study can still continue as planned.

8. What are the possible benefits of taking part?

There are no intended clinical benefits to you from taking part in this study.

You may be related or have close association with someone who suffers from MS. Participating in this study will add to the body of knowledge and may improve the treatment for MS patients in the future.

You may be interested in taking part in the trial to find out information on your habitual level of physical activity (accelerometry), analysis of cholesterol levels and obtain accurate measurements of body composition (DXA Scan). These measurements give you a good indication of your current state of health.

9. What if something goes wrong?

If something goes wrong Stirling University carries Public Liability cover in respect of its legal liability for loss or damage to third party property and death or personal injury to any person not being an employee.

10. Will my taking part in this study be kept confidential?

The information collected about you in this study will be anonymised, i.e. linked to a special code that is stored separately on a password-protected computer file. Your identity will only be known to members of the research team. All information obtained in the study will be stored securely in the Dept of Sport Studies at the University of Stirling, and retained for a period of 6 after which it will be destroyed.'

With your permission, we will inform your GP (family doctor) of your participation in this study.

11. What will happen to the results of the research study?

The result of this study will be used to complete a thesis for a master's degree. In addition the results may be published in a medical journal.

You will not be identified in any report/publication.

In the event that any unusual observations occur, such as high blood pressure or raised cholesterol, we will, with your permission write to your GP and inform him/her of this observation.

12. What happens to my blood sample?

Your blood sample will be tested separately for Vitamin D content and Cholesterol levels.

The blood samples obtained in the study will be stored securely in the Department of Sport Studies Laboratory and retained for a period of 5 years. With your permission we would like to use the sample in future research concerning multiple sclerosis.

13. Who is organising and funding the research?

The University of Stirling, Department of Sport Studies is organising and funding this research project.

14. Who has reviewed the study?

This study has been approved by the University of Stirling, Department of Sport Studies Ethics Committee.

15. Contact for Further Information

Principal Researcher

Sasha Scott
Postgraduate Research Student
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FK9 4LA

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0*****

Thank you for reading this Information Sheet and considering taking part in this study.



**UNIVERSITY OF
STIRLING**

DEPARTMENT OF
SPORTS STUDIES

PATIENT INFORMATION SHEET

MUSCLE FUNCTION IN MS PATIENTS



You are invited to take part in a research study that is being carried out in fulfilment of a Master of Philosophy (MPhil) degree at the University of Stirling. Before you decide whether or not to take part it is important for you to understand what is involved.

Please read this information sheet carefully.

16. What is the purpose of the study?

The purpose of this study is to determine if there is a difference in muscle fibre conduction velocity (MFCV)* in Multiple Sclerosis (MS) patients compared with age and sex matched control participants.

*Muscle Fibre Conduction Velocity is the speed at which an electrical impulse travels along a muscle fibre to trigger a muscular contraction.

17. Why have I been chosen?

You have been chosen as you have been diagnosed with MS and you are mobile or mobile with an aid.

This study aims to recruit 20 MS patients who are mobile or mobile with an aid and are aged between 18 and 65 years of age.

18. Do I have to take part?

No. It is up to you to decide whether or not to take part. Participation is voluntary. Payment will not be given for participating in the study; however participants will be reimbursed for travel expenses up to the value of £10.

If you decide to take part you are free to withdraw from the study at any time and without giving a reason. A decision to withdraw or a decision not to take part, will not affect the standard of care you receive.

19. What will happen to me if I take part?

- Measurement of your physical activity habits using Accelerometry
- Diet log for 24 hours prior to testing
- 1 visit to the laboratory
- 1 Dual-energy X-ray Absorptiometry (DXA) scan to measure body composition

Accelerometry

We will ask you to wear an accelerometer, which is a little box that measures your level of physical activity by detecting movement. The accelerometer situated on your right hip and attached to a belt which is worn around your waist. The accelerometer should be worn during all waking hours for 7 days (with the exception of showering or swimming). The accelerometer is small and can be worn under clothing without being noticed.

Laboratory Visit

The main part of the study involves one visit to the physiology laboratory at the University of Stirling. You will be asked to write down all the food and drink you consume for the 24 prior to your laboratory visit.

On arrival at the laboratory you will have your blood pressure measured and a blood sample* taken. Weight and height will be measured; we will also take a skin fold measurement of your thigh muscle.

* As we are taking a blood sample, you will be asked to fast for 12 prior to your laboratory visit. The blood sample will be taken first; you will then be offered tea or coffee, a roll and some fruit.

We will place electrodes on the skin surface over your thigh muscle to record the electrical impulse along the muscle. In order for the electrodes to pick up a clear signal we must first prepare the skin by shaving the area, then wiping it clean with an alcohol swab.

*Electrical impulses are naturally occurring in the muscle. You will not feel any difference between moving your leg with or without the electrode on your skin. This is a completely non-invasive procedure.

The strength of your thigh muscle will be measured on a Bio-Dex isokinetic dynamometer (pictured on page 1). You will be strapped into the seat and your leg attached to a lever (same as the picture). You will be asked to push your leg away as if you are trying to straighten it however the lever will not move causing a static contraction. While you are doing the static contraction the Bio-Dex measures the force that your thigh muscle is producing.

We will ask you to perform four sub-maximal contractions to familiarise yourself with the procedure. You will then perform two maximal contractions (i.e. push your leg away as hard as you can). You will then be asked to complete sub maximal contractions at 20, 40, 60 and 80% of your maximum. Carrying out 3 contractions at each level; we will ask you to carry out each contraction for 7 seconds. You will be given 14 seconds rest in between each contraction.

Dual-energy X-ray Absorptiometry (DXA)

The DXA scan will be carried out at Yorkhill Children's Hospital in Glasgow. Transport will be provided if it is required. The DXA scan is a non-invasive procedure and is used to obtain an accurate measurement of fat mass, muscle mass and bone density. The DXA scanner gives a very small dose of radiation; much less than the amount of background radiation you would be exposed to from being outside for one day. Therefore this scan poses no health risk. You are required to lie still for about 10 minutes until the scan is complete.

20. Do I have to change anything if I take part?

You are not required to change any aspect of your lifestyle as a result of taking part in this study. Continue to take any medication that you have been prescribed and continue to follow your usual routine. We do however ask that you refrain from any form of strenuous exercise on the day before laboratory testing.

21. What is the procedure that is being utilised?

We are using a procedure called Surface Electromyography (sEMG) to obtain the main findings for this study. sEMG is a non invasive technique, where electrodes are placed onto prepared skin. The electrodes pick up electrical activity in the muscle below the surface of the skin.

22. Is there any danger or discomfort associated with this protocol?

When asked to perform a maximal contraction, changes occur within the body to meet the demands of the effort. Heart rate, blood pressure and breathing rate can all increase. These are normal responses to a maximal effort. You will be given time to recover before you are asked to carry out the remaining contractions.

The DXA scan involves a small amount of radiation. We are all exposed to a small amount of radiation when we are outside. You get the same amount of radiation from the scan as you would from being outside for a few hours one day. Therefore this procedure poses no health risk.

One blood sample will be taken; you may experience a slight scratch as the needle punctures the skin. If you feel that the discomfort is too much the procedure will be stopped. The rest of the study can still continue as planned.

23. What are the possible benefits of taking part?

There are no intended clinical benefits to you from taking part in this study.

As an MS patient you may wish to participate in a study which may contribute to the body of knowledge and therefore may benefit MS patients' treatment in the future.

You may be interested in taking part in the trial to find out information on your habitual level of physical activity (accelerometry), analysis of cholesterol levels and obtain accurate measurements of body composition (DXA Scan). These measurements give you a good indication of your current state of health.

24. What if something goes wrong?

If something goes wrong Stirling University carries Public Liability cover in respect of its legal liability for loss or damage to third party property and death or personal injury to any person not being an employee.

25. Will my taking part in this study be kept confidential?

The information collected about you in this study will be anonymised, i.e. linked to a special code that is stored separately on a password-protected computer file. Your identity will only be known to members of the research team. All information obtained in the study will be stored securely in the Department of Sport Studies at the University of Stirling, and retained for a period of 6 years after which it will be destroyed.

With your permission, we will inform your GP (family doctor) of your participation in this study.

26. What will happen to the results of the research study?

The result of this study will be used to complete a thesis for a master's degree. In addition the results may be published in a medical journal.

You will not be identified in any report/publication.

In the event that any unusual observations occur, such as high blood pressure or raised cholesterol, we will, with your permission write to your GP and inform them of this observation.

27. What happens to my blood sample?

Your blood sample will be tested separately for Vitamin D content and Cholesterol levels.

The blood samples obtained in the study will be stored securely in the Department of Sport Studies Laboratory and retained for a period of 5 years. With your permission we would like to use the sample in future research concerning multiple sclerosis.

28. Who is organising and funding the research?

The University of Stirling, Department of Sport Studies is organising and funding this research project.

29. Who has reviewed the study?

This study has been approved by the University of Stirling, Department of Sport Studies Ethics Committee.

30. Contact for Further Information

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Thank you for reading this Information Sheet and considering taking part in this study.

Appendix E

Pre-Participation Health Screen Questionnaire

Consent Form

Data Capture Sheet



Pre-Participation Health Screen Questionnaire

The purpose of the pre-participation health screen is:
 To optimise safety during exercise testing
 To identify medical risk factors which may contra-indicate exercise.

Name _____ Date of Birth _____

Address _____

Phone No. _____ Mobile _____

Gender _____ Work No _____

Doctor's Name _____ Phone No. _____

Surgery _____

Address _____

Physical Activity Index			
			Tick which one applies to you
Activity Level	Times per week	Risk level	
Inactive	0 occasional	Very High	
Semi-active	1	High	
Active	2-3	Moderate	
Very active	4 or more	Low	

SECTION A MEDICAL HISTORY

Have you ever been told that you have had or have any of the following conditions?

If yes, please mark with an X in the appropriate box:

CARDIAC (Heart Related Diseases)	
<input type="radio"/> Heart Attack	<input type="radio"/> High blood pressure
<input type="radio"/> Coronary thrombosis (blood clot)	<input type="radio"/> Rheumatic fever
<input type="radio"/> Narrowing of arteries	<input type="radio"/> Angina / Chest Pain
<input type="radio"/> High cholesterol	<input type="radio"/> Congenital Heart Disease
<input type="radio"/> Further / comments	

PULMONARY (Lung Diseases)	
<input type="radio"/> Asthma	<input type="radio"/> Exercise-induced asthma
<input type="radio"/> Chronic Bronchitis	<input type="radio"/> Emphysema
<input type="radio"/> T.B.	
<input type="radio"/> Other / comments	

OTHER	
<input type="radio"/> Type I Diabetes	<input type="radio"/> Type II Diabetes
<input type="radio"/> Anaemia (ion deficiency)	
<input type="radio"/> Kidney disease	
<input type="radio"/> Rheumatoid Arthritis	

ORTHOPAEDIC SURGERY (Musculoskeletal)	
<input type="radio"/> Neck	<input type="radio"/> Hip
<input type="radio"/> Back	<input type="radio"/> Knee
<input type="radio"/> Shoulder	<input type="radio"/> Ankle
<input type="radio"/> Arm	<input type="radio"/> Foot
<input type="radio"/> Other / comments	

INJURY	
Have you suffered any of the following injuries? If so, how long ago?	
<input type="radio"/> Neck vertebrae	<input type="radio"/> Back vertebrae
<input type="radio"/> Rotator cuff	<input type="radio"/> Impingement Syndrome (shoulder)
<input type="radio"/> Tennis elbow	<input type="radio"/> Runner's knee
<input type="radio"/> Iliotibial Band Syndrome	<input type="radio"/> Lower leg
<input type="radio"/> Achilles Tendonitis	<input type="radio"/> Plantar Fascitis
<input type="radio"/> Other / comments	

MEDICATION

Do you use medication at present for any of the following? (If yes, please state the drug)

- | | | | |
|-----------------------|------------------|-----------------------|---------------------|
| <input type="radio"/> | Heart rhythm | <input type="radio"/> | Blood pressure |
| <input type="radio"/> | Blood clotting. | <input type="radio"/> | Blood circulation |
| <input type="radio"/> | Asthma | <input type="radio"/> | Bronchitis |
| <input type="radio"/> | Emphysema | <input type="radio"/> | Flu |
| <input type="radio"/> | Diabetes | <input type="radio"/> | Thyroid dysfunction |
| <input type="radio"/> | Cholesterol | <input type="radio"/> | Anaemia |
| <input type="radio"/> | Kidney | <input type="radio"/> | Liver |
| <input type="radio"/> | Arthritis | <input type="radio"/> | Muscle injury |
| <input type="radio"/> | Other / comments | | |

SECTION B CARDIOVASCULAR DISEASE RISK INDEX

Please read the following questions carefully and answer each accurately. Mark your choice with an X.

History of heart attack or bypass surgery / angioplasty

- | | | | | | |
|---|-----------------------|------------------|---|-----------------------|-----------------|
| 0 | <input type="radio"/> | None | 5 | <input type="radio"/> | 1 – 2 years ago |
| 2 | <input type="radio"/> | Over 5 years ago | 8 | <input type="radio"/> | < 1 year ago |
| 4 | <input type="radio"/> | 3 – 5 years ago | | | |

Age/Gender Index

- | | | |
|---|-----------------------|-------------------------------------|
| 0 | <input type="radio"/> | Male / female under 30 years of age |
| 1 | <input type="radio"/> | 30 – 40 years of age |
| 2 | <input type="radio"/> | Female 40 - 50 years of age |
| 3 | <input type="radio"/> | Male 40 – 50 years of age |
| 3 | <input type="radio"/> | Female 50 – 60 years of age |
| 4 | <input type="radio"/> | Male 50 – 60 years of age |
| 4 | <input type="radio"/> | Male / female 60+ years of age |

Smoking status	
0	<input type="radio"/> None
1	<input type="radio"/> Pipe
2	<input type="radio"/> 1 – 10 cigarettes daily
3	<input type="radio"/> 11 – 20 cigarettes daily
4	<input type="radio"/> 21 – 30 cigarettes daily
5	<input type="radio"/> 31 – 40 cigarettes daily
6	<input type="radio"/> 41 – 60 cigarettes daily
8	<input type="radio"/> + 60 cigarettes daily
State how long you have smoked for:	
Years	_____ months _____

Diabetes	
0	<input type="radio"/> None
1	<input type="radio"/> Type 2 (non-insulin dependent)
2	<input type="radio"/> Type 1 (insulin dependent)

Occupational Activity level	
1	<input type="radio"/> Intense physical labour
2	<input type="radio"/> Moderate (walk often etc.)
3	<input type="radio"/> Sedentary

Work Stress Tension	
0	<input type="radio"/> No stress, very relaxed
1	<input type="radio"/> Moderate work stress and relaxed personality
2	<input type="radio"/> High work stress but cope well
3	<input type="radio"/> Very high work stress and tense personality
4	<input type="radio"/> Very high work stress, highly strung personality

Physical Activity Status (for a minimum of 30 minutes a session)	
1	<input type="radio"/> Exercise 4 or more times per week
2	<input type="radio"/> Exercise 2 – 3 times per week
3	<input type="radio"/> Recreational sport once a week
4	<input type="radio"/> Recreational sport occasionally or complete lack of exercise

SECTION C EXERCISE PARTICIPATION

Do you participate in any of the activities more than twice weekly?

(Please tick all relevant activities)

- | | |
|--|---|
| <input type="checkbox"/> Jogging more than 5 km | <input type="checkbox"/> Aerobic classes 45 min |
| <input type="checkbox"/> Cycling more than 45 min. | <input type="checkbox"/> Tennis 90 min |
| <input type="checkbox"/> Swimming more than 600 m | <input type="checkbox"/> Squash 45 min. |
| <input type="checkbox"/> Gym (Combined strength / aerobic) | <input type="checkbox"/> Team sport (outdoor) – rugby hockey, soccer |
| <input type="checkbox"/> Gym (weights only) | <input type="checkbox"/> Team sport (indoor) – basketball, netball, etc |
| <input type="checkbox"/> Gym (aerobic only) | <input type="checkbox"/> Canoeing / Rowing 45 min |

SECTION D

I have read, understood and completed this questionnaire to the best of my knowledge.

Signature: _____ Date _____



**UNIVERSITY OF
STIRLING**

DEPARTMENT OF
SPORTS STUDIES

Patient Identification Number for this trial:

CONSENT FORM

Title of Project: **MUSCLE FUNCTION IN MS PATIENTS**

Name of Researcher: **SASHA SCOTT**

Please initial box

1. I confirm that I have read and understood the information sheet dated 24 Sept 2007 (Version 2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without any medical care or legal rights being affected
3. I agree to my GP being informed of my participation in this study.
4. I agree to my GP being informed of the results of any of the tests that might have a bearing on my health or future care.
5. I agree to my left over blood sample being retained and used in future multiple sclerosis medical research
6. I agree to take part in the above study.

Name of Participant	Date	Signature
Name of person taking consent	Date	Signature

When complete, 1 for participant; 1 for researcher site file.


DATA CAPTURE SHEET

Participant Study Code		
Date		
Gender	Male <input type="checkbox"/>	Female <input type="checkbox"/>
Dominant Leg	Left <input type="checkbox"/>	Right <input type="checkbox"/>
Questionnaire	Completed <input type="checkbox"/>	Incomplete <input type="checkbox"/>
Blood Sample	Lipids <input type="checkbox"/>	Vitamin D <input type="checkbox"/>
Height (cm)	cm	
Weight (Kg)	Kg	
Weight as fat (Kg)	Kg	
% fat	%	
BMI		
Resting Heart Rate (bpm)		
Blood Pressure (mmHg)	Sys:	Dia:
Left Leg (nM) 100% MVC		
	20%:	40%:
	60%:	80%:
Right Leg (nM) 100% MVC		
	20%:	40%:
	60%:	80%:
Accelerometry		
Given Out - Date		
Date set to data collect		