Clinical Measures, Gait and Exercise in Mitochondrial Disease

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Abstract

Mitochondrial diseases are one of the most common forms of inherited neuromuscular disease. The presentations of these diseases are highly variable with both neurological and systemic involvement . Despite progress in identifying mitochondrial DNA mutations that result in disease, the natural history of mitochondrial diseases still remains unclear and no effective treatments are currently available (Pfeffer *et al.*, 2012). The use of numerous primary outcomes in studies has made comparisons between studies difficult. A recent Cochrane review recommended the use of measures that were more relevant to patients in studies.

This thesis aims to explore the use physiological measures alongside functional measures and gait in mitochondrial disease. The studies demonstrated that all functional outcome measures were able to discriminate between participants with mitochondrial disease and control subjects. However, gait characteristics were also able to discriminate between the two different mitochondrial genotypes. An aerobic exercise intervention resulted in an improvement in exercise capacity. However, disease severity, functional ability and gait measures remained unchanged.

The main findings from this thesis are that: Clinical functional measures and gait are relevant for use in the research and clinical management of mitochondrial disease to monitor disease burden. The improvement in exercise capacity following a cycling intervention was unable to be translated into an improvement in function or gait. Therefore further research into other types of interventions, which may improve activities relevant to patients, is required.

The outcome of any serious research can only be to make two questions grow where only one grew before.

Thorstein Veblen

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Author contribution

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Project proposals and design were written by the author, Professor Michael Trenell and Professor Lynn Rochester. The author applied for ethical approval for the projects with the assistance Geoff Bell and Professor Michael Trenell.

Participants were recruited and consented by the author and Dr Matthew Bates. The author oversaw the running of the studies and completed the majority of participant visits. Exercise testing was completed with the assistance of Dr Djordje Jakovljevic and Dr Matthew Bates. The author performed all gait assessments with the assistance of Dr Brook Galna. Data from gait assessments was retrieved by Dr Brook Galna.

Supervision of the exercise intervention was performed by the author.

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Publications and presentations

Publications

First name publications

Bates MG*, **Newman JH***, Jakovljevic DG, Hollingsworth KG, Alston CL, Zalewski P, Klawe JJ, Blamire AM, Macgowan GA, Keavney BD, Bourke JP, Schaefer A, McFarland R, Newton JL, Turnbull DM, Taylor RW, Trenell MI, Gorman GS. Defining cardiac adaptations and safety of endurance training in patients with m.3243A>G related mitochondrial disease. International Journal of Cardiology 2013, 168 (4), 3599-3608.

Galna B*, **Newman JH***, Jakovljevic DG, Bates MG, Schaefer AM, McFarland R, Turnbull DM, Trenell MI, Gorman GS, Rochester L. Discrete gait characteristics are associated with m.3243A > G and m.8344A > G variants of mitochondrial disease and its pathological consequences. Journal of Neurology 2014, 261 (1), 73-82.

Newman JH*, Galna B*, Jakovljevic DG, Bates MG, Schaefer AM, McFarland R, Turnbull DM, Trenell MI, Taylor RW, Rochester L, Gorman GS. Preliminary Evaluation of Clinician Rated Outcome Measures in Mitochondrial Disease. Journal of Neuromuscular Diseases 2015, (epub ahead of print)

(*Joint contributions)

Other publications

Spendiff S, Reza M, Murphy JL, Gorman G, Blakely EL, Taylor RW, Horvath R, Campbell G, **Newman J**, Lochmüller H, Turnbull DM. Mitochondrial DNA

deletions in muscle satellite cells: implications for therapies. Human Molecular Genetics 2013, 22 (23), 4739-4747.

Bates MG, Hollingsworth KG, **Newman J**, Jakovljevic DG, Blamire AM, MacGowan GA, Keavney BD, Chinnery PF, Turnbull DM, Taylor RW, Trenell MI, Gorman GS. Concentric hypertrophic remodeling and subendocardial dysfunction in mitochondrial DNA point mutation carriers. European Heart Journal - Cardiovascular Imaging 2013, 14, (7), 650-658.

Muscular Dystrophy Campaign, Exercise advice for adults with muscle wasting conditions. Adult Neuromuscular Physiotherapy Special Interest Group. Nov 2014

Gorman GS, Blakely EL, Hornig-Do H, Tuppen H, Greaves LC, Langping He, Baker A, Falkous G, **Newman J**, Trenell M, Lecky B, Petty R, Turnbull D, McFarland R, Taylor RW. Novel MTND1 mutations cause isolated exercise intolerance, complex I deficiency and increased assembly factor expression. Clin. Sci. (2015) 128 (895-904).

Presentations

Conference presentations

Newman J, Jakovljevic D, Galna B, Bates M, Turnbull D, Trenell M, Gorman G. Improving clinical trials evaluation: physiological and functional correlates in mitochondrial disease. Poster Presentation. Fifth UK Neuromuscular Translational Research Conference. March 2012.

Newman J, Galna B, Jakovljevic D, Bates M, Schaefer A, McFarland R, Turnbull D, Trenell M, Taylor R, Rochester L, Gorman G. Evaluation of functional outcome measures as a surrogate marker of disease severity in

patients with mitochondrial disease. Poster presentation. ABN, RCP joint meeting. Journal of neurology, neurosurgery, and psychiatry, 11/2013; 84(11).

Newman J, Galna B, Turnbull DM, Trenell MI, Gorman G, Rochester L. Can discrete gait characteristics serve as a surrogate marker of mitochondrial disease? Poster presentation. Third annual showcase meeting held by the cross-council Lifelong Health and Wellbeing (LLHW) programme. Nov 2013. Prize Winner.

Newman J, Galna B, Jakovljevic D, Bates M, Schaefer A, McFarland R, Turnbull D, Trenell M, Taylor R, Rochester L, Gorman G. Can aerobic exercise improve function in patients with mitochondrial disease? Poster presentation. UK Neuromuscular Translational Research Conference. March 2014. London. Neuromuscular Disorders 03/2014; 24:S13-S14.

Newman J, Galna B, Turnbull DM, Trenell MI, Rochester L, Gorman G. Gait analysis in patients with mitochondrial disease. UK Neuromuscular Translational Research Conference. March 2015.

Newman J, Galna B, Turnbull DM, Trenell MI, Rochester L, Gorman G. Gait analysis: Accuracy as a clinician reported measure in mitochondrial disease. UK Neuromuscular Translational Research Conference. March 2015.

Lay presentations

Newman, J. “Physiotherapy in Mitochondrial Disease”, Patient Information Day, SAGE, Gateshead. March 2011.

Newman, J. “Use it or Lose it”, Keeping Active Matters, Newcastle Biomedical Research Centre, May 2014.

Newman, J. “How to move and exercise safely?” NMD Patient conference, Centre for Life, March 2015.

Abbreviations

10MTW	10 metre timed walk
^{31}PMRS	^{31} Phosphorus Magnetic Resonance Spectroscopy
5XSTS	5 times sit to stand
6MWD	6 minute walk distance
a-vO_2 diff	Arterio venous oxygen difference
ACE-R	Addenbrooks Cognitive Examination –revised
ACSM	American College of Sports Medicine
ADP	Adenosine Diphosphate
AT	Anaerobic Threshold
ATP	Adenosine Triphosphate
AUC	Area under the curve
BMI	Body mass Index
BP	Blood Pressure
CO	Cardiac output
CO_2	Carbon Dioxide
CoA	Co enzyme A
COX	Cytochrome oxidase
CPOE	Chronic Progressive Opthalmoplegia
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
ETC	Electron transport chain
$FADH_2$	Flavin adenine dinucleotide
FOM	Functional Outcome Measure
GP	General Practitioner
H_2O	Water
HR	Heart Rate
IPAQ	International Physical Activity Questionnaire
MD	Mitochondrial Disease
MELAS	Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes
MERRF	Myoclonic epilepsy and ragged red fibres
MET	Metabolic equivalent
mtDNA	Mitochondrial DNA
NADH	Nicotinamide adenine dinucleotide

NMDAS	Newcastle Mitochondrial Disease Assessment Scale
O_2	Oxygen
OXPHOS	Oxidative Phosphorylation
PARQ	Physical Activity Readiness Questionnaire
PCr	Phosphocreatine
PGC1α	Peroxisome proliferator-activated receceptor gamma coactivator-1-alpha
RER	Respiratory exchange ratio
RNA	Ribonucleic acid
ROC	Receiver Operating Curve
Rpm	Revolutions per minute
SBP	Systolic Blood Pressure
SD	Standard deviation
SDH	Succinate dehydrogenase
SV	Stroke volume
TCA cycle	Citric Acid Cycle
TUG	Timed up and go
vCO_2	Carbon dioxide production
V_E	Minute Ventilation
vO_2	Oxygen consumption
vO_{2MAX}	Maximum exercise capacity
vO_{2PEAK}	Peak exercise capacity
W	Wattage
WR	Work rate

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Chapter 1 Introduction

1.1. Introduction

Mitochondrial diseases are one of the most common forms of inherited neuromuscular disease. The presentations of these diseases are highly variable with both neurological and systemic involvement (McFarland *et al.*, 2002). Muscle involvement in mitochondrial disease is common resulting in a variety of symptoms such as fatigue, exercise intolerance and progressive muscle weakness all of which can lead to significant disability. Despite progress in identifying mitochondrial DNA mutations that result in disease, the natural history of mitochondrial diseases still remains unclear and no effective treatments are currently available (Pfeffer *et al.*, 2012). The monitoring of disease progression and the evaluation of new treatments requires the use of accurate outcome measures; the use of multiple laboratory clinical measures in previous research studies has led to difficulty comparing studies and often the results have little meaning to patients (Pfeffer *et al.*, 2012).

Exercise therapy has proved beneficial in improving the exercise capacity of patients with mitochondrial disease (Taivassalo *et al.*, 2001; Cejudo *et al.*, 2005; Taivassalo *et al.*, 2006; Murphy *et al.*, 2008; Jeppesen *et al.*, 2009). Previous exercise studies have relied on extensive laboratory testing and invasive muscle biopsies. Such investigations are limited to a few research centres and are not practical for all patients. To review the effects of mitochondrial disease on patients and the effectiveness of interventions in a greater number of patients, simpler methods of measurement are required that can be translated into clinical practise.

The studies included in this thesis will investigate the measurement of various aspects of mitochondrial disease including impairments, activities and

participation (World Health Organization, 2001). The studies will assess exercise capacity, performance of functional tests and gait in a clinically affected group of patients. The aims of the studies are to demonstrate: 1) exercise is safe and beneficial in a discrete group of patients with mitochondrial disease and 2) simple functional outcome measures and gait analysis are valid tools in this population in both the clinical and research arenas.

This introductory chapter will review 1) the function of mitochondria, the diagnosis, manifestation and management of mitochondrial diseases and the treatments that are currently available; 2) literature regarding the effects of aerobic exercise on patients with mitochondrial disease compared with the general population and 3) the clinical measures used in this thesis (functional outcome measures, dynamometry and gait analysis) and their use in other populations.

Subsequent thesis chapters will discuss the individual studies contained within the thesis. Chapter 2 provides descriptions of all the methods contained within the four studies undertaken. Chapter 3 describes a cross-sectional study observing the use of physiological and functional measures in mitochondrial disease with chapter 4 describing a cross sectional study of gait characteristics in the two mitochondrial genotypes. Chapter 5 will provide a summary of the clinical measures contained in the two cross-sectional studies. Chapter 6 describes the effect of an exercise intervention on exercise capacity and functional outcome measures in a case controlled study with chapter 7 observing the effects of the same exercise intervention on gait characteristics. Finally, Chapter 8 will discuss the clinical implications of these studies and areas for future research.

1.2. Mitochondrial Physiology

Mitochondria are found in all nucleated cells within the body. There may be thousands of mitochondria within a cell, depending on its function. Their number, position, size and shape can adapt to the cells' requirements and function (Tiivel *et al.*, 2000; Taylor and Turnbull, 2005). Mitochondria are no longer seen as discrete organelles within the cell, but as dynamic units that are able to move throughout the cytosol, thus forming long moving chains with microtubules in the cytoskeleton of the cell (Chan, 2006b). Whilst moving they are constantly fusing and dividing (Margineantu *et al.*, 2002) which enable the mitochondria to exchange genetic materials and maintain their function (Chan, 2006a).

Knowledge of the function and structure of mitochondria is essential in the understanding of the consequences of mutations within the DNA of the mitochondria and therefore these are described below.

1.2.1 Mitochondrial Function

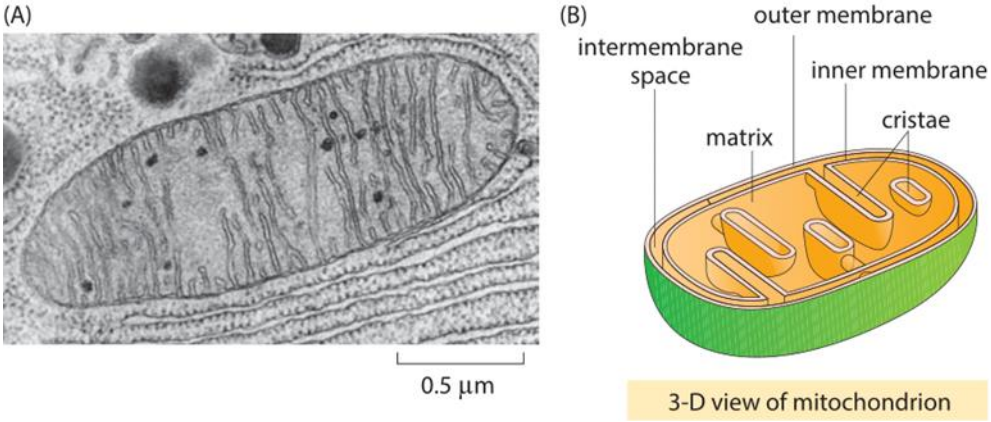
Mitochondria play many roles within the cell, though their main function is the production of Adenosine triphosphate (ATP). Mitochondria progressively reduce the products of other energy pathways to create an electron gradient, which is used to provide the energy to re-synthesise ATP from Adenosine diphosphate (ADP) and inorganic Phosphate (Pi).

Other key roles of the mitochondria are the regulation of calcium levels within the cell and apoptosis (Hengartner, 2000). As such, mitochondria play a central role in cellular function, through the provision of energy and the process of cell death.

1.2.2 Mitochondrial structure

Each mitochondrion has two highly specialised membranes; the two membranes create internal and external compartments within the mitochondria, known as matrices (Figure 1-1).

Figure 1-1: The mitochondrion.
(A) An electron microscope picture of the mitochondrion ***(Fawcett, 1994).*** *(B) A typical schematic representation of the mitochondria, adapted from Stryer Biochemistry 4th edition* ***(Stryer, 1995.)****.*



The outer membrane contains many transport proteins; these proteins form wide aqueous channels through the membrane and allow the membrane to be permeable to molecules of a certain size. The inner membrane is impermeable to the passage of ions and most small molecules, except where a path is provided by a transport membrane protein. The inner membrane of the mitochondrial membrane is highly convoluted forming cristae that project into the matrix. These cristae increase the surface area greatly, therefore providing a larger area for ATP production. The number of cristae varies according to the role of the cell and its energy requirements. The inner mitochondrial membrane contains proteins with three types of function; 1) proteins involved in the oxidation reactions of the electron transfer chain, 2) ATP synthase that makes ATP in the matrix, and 3) transport proteins that allow the passage of metabolites in and out of the matrix.

The matrix of the mitochondria contains enzymes that are involved in the breakdown of pyruvate and fatty acids (derivatives of glycolysis and fat beta oxidation). Each mitochondrion also contains within the matrix its own Deoxyribonucleic acid (DNA), Ribonucleic acid (RNA) and complete transcription and translation system allowing the manufacture of proteins used within the mitochondria.

The structure of mitochondria is specialised to its function; the presence of DNA within mitochondria is essential in the production of proteins used to generate energy within the mitochondria. Mitochondrial metabolism requires oxygen and two interrelated metabolic pathways to work together, the citric acid cycle (TCA cycle) and the electron transport chain (ETC).

Citric Acid Cycle

The first stage of the Citric Acid Cycle (TCA Cycle) involves the transportation of Pyruvate (formed from glycolysis) across the inner membrane of the mitochondria into the inner matrix. Within the inner matrix it is combined with coenzyme A to form Acetyl CoA. This is then fed into the citric acid cycle where it is progressively broken down, producing key substrates for the electron transport chain (Figure 1-2). The energy from the cycle is temporarily held in high-energy electrons of NADH and $FADH_2$. The TCA cycle in itself does not use oxygen, but will stall without its link to the ETC where oxygen is required as a terminal acceptor. The high-energy electrons produced (NADH and $FADH_2$) are then used in the second stage of mitochondrial respiration, the electron transport chain (ETC).

Figure 1-2: The citric acid cycle.
Acetyl CoA condenses with Oxaloactetate to form citrate. Then follow eight steps where electrons are removed and are donated to coenzymes NAD+ and FAD+ to form NADH, $FADH_2$ and CO_2. Each turn of the cycle generates three NADH, one FADH2, one ATP (from GTP) and two CO_2 molecules .One additional NADH and CO_2 are generated by the oxidation of pyruvate prior to entering the cycle (Alberts et al., 2004).

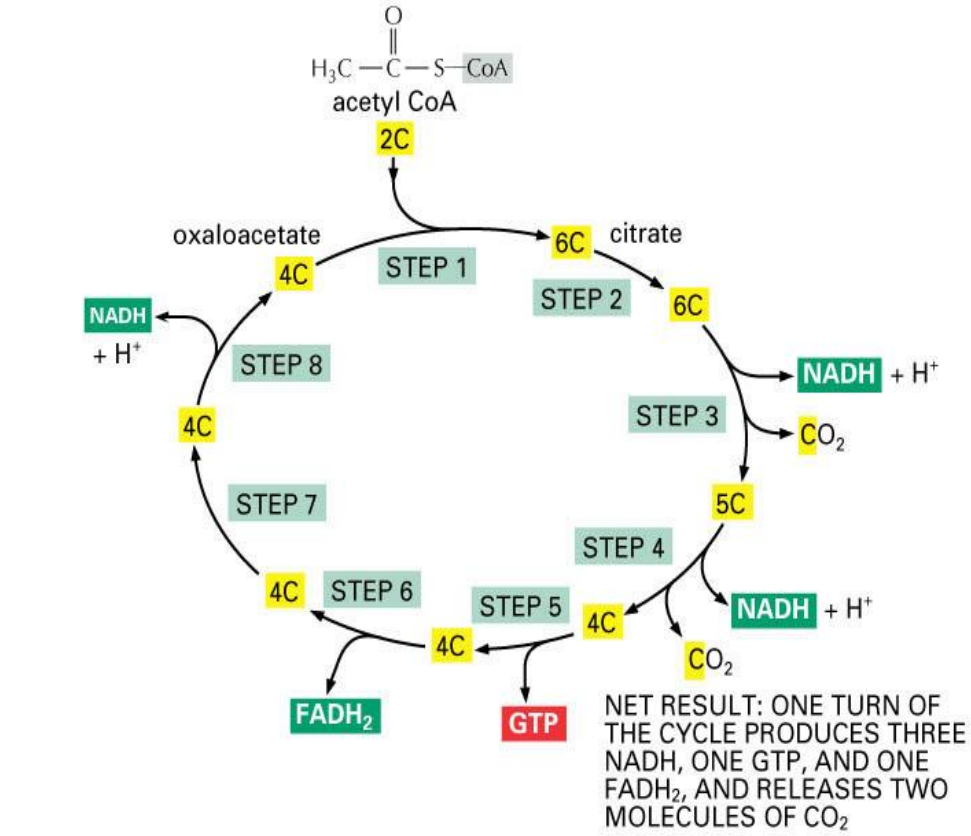


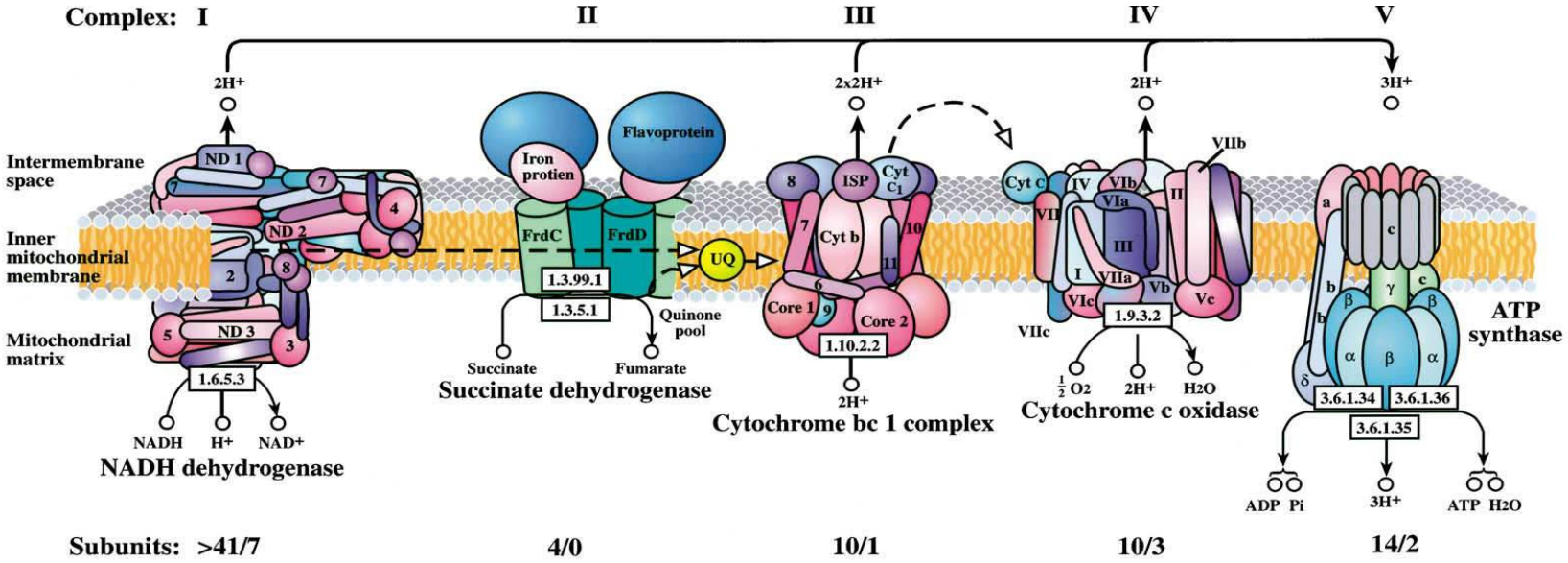
Figure 13-11 Essential Cell Biology, 2/e. (© 2004 Garland Science)

The electron transfer chain

The Electron transfer chain (ETC) is located along the inner membrane of the mitochondria and is present in many copies along the membrane.

The inner membrane of the mitochondria contains over 40 proteins of which 15 are directly involved in electron transport. Most of the proteins involved in the ETC are formed into five large respiratory enzyme complexes (Figure 1-3). High-energy electrons from NADH and $FADH_2$ (from the citric acid cycle) enter the ETC and become oxidized to NAD+ and FAD. The energy released by the flow of electrons through the ETC is used to pump protons out of the mitochondrial inner membrane through complexes I, III and IV and create an electrochemical gradient across the membrane. This gradient allows protons to flow back into the mitochondrial matrix via a proton channel in complex V (ATP synthase) and enables ATP to be synthesized from ADP and Pi (Figure 1-3). The process is known as oxidative phosphorylation and is capable of making 100’s of molecules of ATP a second.

Figure 1-3: A schematic representation of the 5 respiratory complexes of the eletron transport chain embedded in the mitochondrial inner membrane.
Electrons flow from NADH or succinate to complex I or II and then onto a ubiquinone pool. Electrons then flow from ubiquinnone through complexes III and IV.Simultaneously protons are moved across the membrane. The resulting proton gradient is used by complex V to generate ATP.Taken from (Mandavilli et al., 2002)



In summary, mitochondria play a key role in cellular function and longevity and are the primary site of ATP production from the breakdown of foodstuffs. The structure and position of mitochondria in the cell is very specific to the role of the cell. The consequences of impairments in mitochondrial function resulting in mitochondrial disease are discussed in the following section.

1.3. Mitochondrial Disease

Mitochondrial disease refers to a group of conditions where the mitochondria do not effectively break down metabolites to allow regeneration of ATP. As a result of the impaired ATP regeneration, mitochondrial disease normally presents in tissues requiring large amounts of energy, such as skeletal muscle, the heart and brain.

To understand how mutations in mtDNA result in disease a brief description of the mitochondrial genome is provided below.

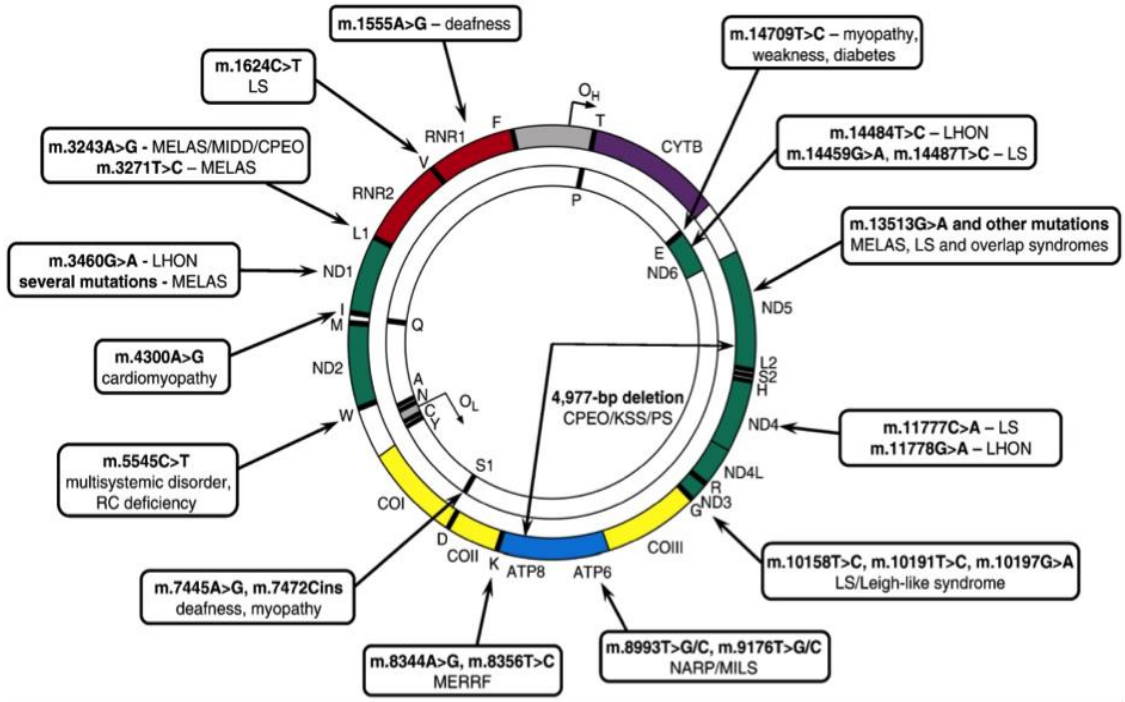
1.3.1 Mitochondrial Genome

The mtDNA molecule is much smaller than nucleic DNA with only 37 genes (Figure 1-4), which encode 13 polypeptides. All these polypeptides are units within the electron transfer chain and are found within the complexes I, III, IV, V of the electron transfer chain (Figure 1-3). The remaining protein subunits within the electron transfer chain are nuclear encoded with complex II being completely coded by nuclear genes. Mitochondrial diseases can be caused by mutations within both the mitochondrial or nuclear genome but this thesis will limit itself to the description of mutations of mitochondrial DNA.

Unlike nuclear DNA, mtDNA is strictly maternally inherited. It is thought that the higher mutation rates seen within the mitochondrial genome are due to oxidative damage caused by the genomes proximity to the respiratory chain in the inner mitochondrial membrane (Mandavilli *et al.*, 2002). Within each cell the mtDNA is arranged in stable protein complexes termed nucleoids, which are exchanged between mitochondria during mitochondrial division. Division of mitochondria is independent of the cell cycle and is driven by the energy demand of the cell and the need to produce ATP (Bogenhagen and Clayton, 1977). Although the exact mechanism of mitochondrial division is still a matter for debate, the process by which mitochondria divide is thought to affect the transmission and progression of mitochondrial diseases (Chan, 2006a; Chen and Chan, 2010; Picard *et al.*, 2011).

Each mitochondrion contains multiple copies of mtDNA. When the copies of mtDNA within a cell are identical it is known as a homoplasmy. The presence of more than one type of mtDNA within a cell is known as heteroplasmy. Heteroplasmy can occur when a mutation has occurred within the mtDNA, which is then replicated as mitochondria divide to produce a mixture of wild and mutant DNA within a cell (Larsson and Clayton, 1995). The percentage of mutant mtDNA can vary from cell to cell and tissue to tissue. The division of mitochondria and the ratio of wild to mutant mitochondria are important in understanding the transmission, manifestation and progression of mitochondrial diseases.

Figure 1-4: A schematic diagram of the mitochondrial genome
The circular, double stranded human mitochondrial genome is shown with common mutations and associated clinical presentations highlighted. (CPEO-chronic progressive external opthalmoplegia, LHON-Leber hereditary optic neuropathy, LS-Leigh syndrome, MELAS–mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes, MERRF-myoclonic epilepsy and ragged red fibres, MILS-maternally inherited Leigh syndrome, NARP-neurogenic weakness, ataxia and retinitis pigmentosa, PS-Pearsons syndrome). (Tuppen et al., 2010).



1.3.2 Mitochondrial Disease

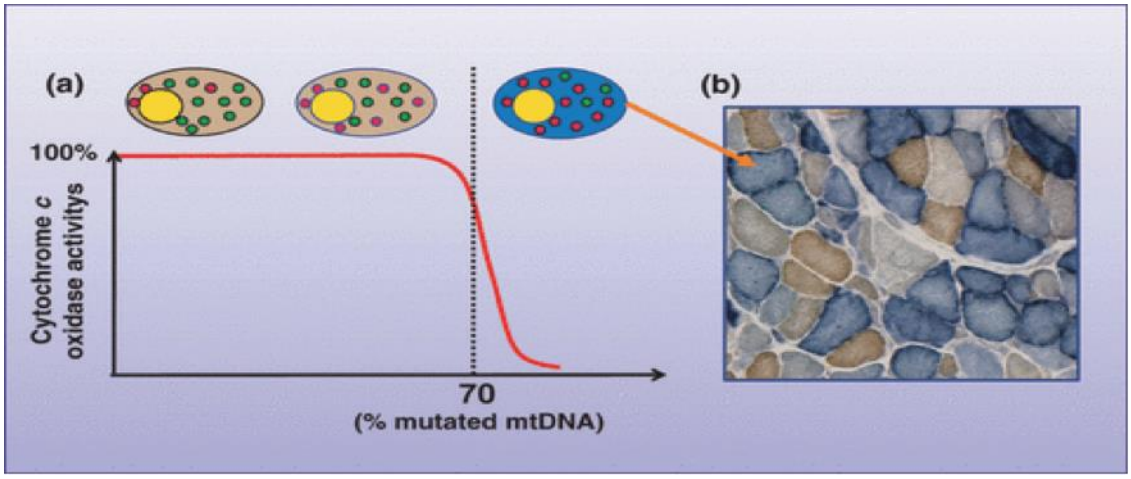
The first pathogenic mutation in mtDNA was identified in 1988 (Holt *et al.*, 1988), since then over 250 others have been reported. Mitochondrial diseases are due to a wide variety of mutations and present with different phenotypes, and with different ages of onset (MITOMAP, 2009). The reported prevalence of these diseases has changed as the diagnosis of mitochondrial disease has improved and was recently estimated to be around 1 in 10,000 in the North East of England with a potential of a further 1 in 6,000 at risk (Schaefer *et al.*, 2008; Gorman *et al.*, 2015) and pathogenic mutations being reported in greater than 1/200 live births (Elliott *et al.*, 2008). The manifestation of disease may be dictated by number of factors. The threshold effect will be discussed below as it

relates to concerns voiced in previous exercise trials in patients with mitochondrial disease.

Threshold effect

Within cells there is usually a mixture of wild and mutant types of mitochondrial DNA. Symptoms of mitochondrial disease only become apparent when the ratio of mutant and wild type mitochondria reaches a certain level. Once this threshold is reached a biochemical deficit can occur and the cell will cease to function correctly. The threshold required to produce clinical features varies from tissue to tissue but is typically 60-90%, although may be lower in tissues with high-energy requirements (Figure 1-5). It should be noted however, that this theory does not explain the whole presentation as an exact correlation between clinical severity of symptoms and proportion of mutant mtDNA is not always apparent (Wong, 2007).

Figure 1-5: A diagram of mtDNA heteroplasmy and the threshold effect.
Graph (a) shows the effect of increasing mtDNA mutation load on mitochondrial function (wild type mitochondria are shown as green circles, mutated mitochondria as red circles). When the level of mutated mtDNA reaches a critical threshold (70% in this example) biochemical activity is severely reduced leading to mitochondria dysfunction. This is illustrated in the transversely-orientated section of skeletal muscle (b) which has been reacted for cytochrome c oxidase cytochemical activity (McFarland and Turnbull, 2009).



The progress of a mutation to reach a threshold is thought to be affected by mitochondrial replication and a number of potential theories have been proposed but are beyond the remit of this thesis.

So far this section has described the mitochondrial genome and how mutations within mtDNA can replicate and result in increasing levels of mutated mtDNA. This results in reduced cell function and progresses to disease manifestation. As well as levels of mutation, the variety of symptoms reported in mitochondrial disease can also be dictated by the position and type of mutation.

1.3.3 Mitochondrial Mutations

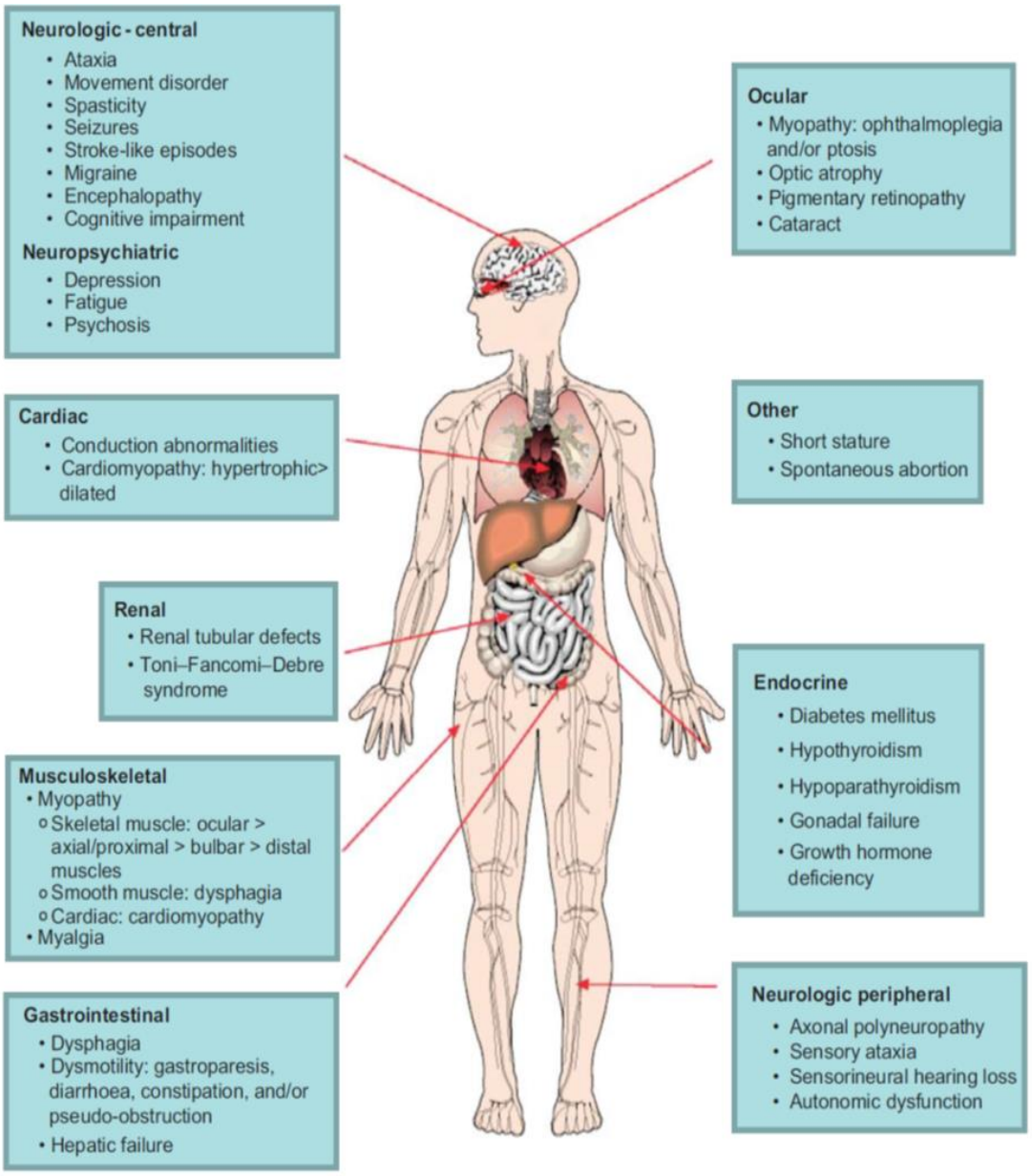
Mutations of mtDNA can occur in a variety of forms and locations as shown in Figure 1-4. There are two types of mitochondrial DNA mutation: 1) Point mutations are usually maternally inherited and mostly heteroplasmic and 2) rearrangements, which can be single or multiple. Single deletions usually occur sporadically and early in development and due to their size can span several genes. Multiple deletions vary in length, with the number and tissue distribution of the mtDNA with deletions being important rather than the size and location of the deletion (Zeviani *et al.*, 1988; Moraes *et al.*, 1995). Hence the manifestation of mitochondrial disease is highly unpredictable and dependent on a number of factors. These include the mutation position and type, mitochondrial segregation and replication, as well as the type of tissue the mutation occurs within and the energy requirement of that tissue.

1.3.4 Clinical presentations of pathogenic mitochondrial mutations

As mitochondria are present in all nucleated cells, mutations within the mitochondrial genome can produce a great variety of symptoms affecting a

number of the body's systems, which are shown in Figure 1-6. Some of these symptoms have been classified into different clinical syndromes and phenotypes. A number of these syndromes have very specific features and are easy to diagnose whereas others are non-specific and may have multiple differential diagnoses.

Figure 1-6: Clinical features of mitochondrial disease, by organ system. (Pfeffer and Chinnery, 2013).



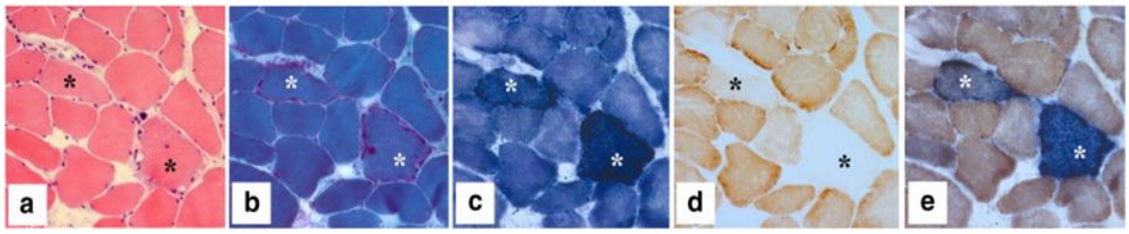
1.3.5 Diagnosis of mitochondrial disease

The variety of presentations of mitochondrial disease makes diagnosis difficult. Therefore diagnosis is a multidisciplinary approach and requires clinical assessment alongside histochemical and biochemical testing using diagnostic algorithms (McFarland and Turnbull, 2009).

Although some mitochondrial disorders can be diagnosed by molecular genetic screening of blood DNA, due to the tissue specific presentation of mitochondrial disease, the investigation of muscle biopsy tissue is often required to provide a correct and accurate diagnosis for the majority of patients (McFarland *et al.*, 2002) and remains the gold standard for detection of mitochondrial disease (Figure 1-7) (McFarland and Turnbull, 2009). The presence of ragged red fibres is a unique muscle biopsy finding in mitochondrial disease (Figure 1-7b). The application of various enzyme reactions enables identification of fibres with impaired mitochondrial function. Application of the succinate dehydrogenase (SDH) assay identifies disorders within complex II of the mitochondrial respiratory chain and is unaffected by abnormalities of the mtDNA (Figure 1-7c). This staining is used alongside cytochrome c oxidase (COX) staining which produces a brown fibre. The variety of COX activity between the different fibres is apparent in mitochondrial disorders and gives the typical mosaic pattern of COX activity that is indicative of a heteroplasmic mtDNA mutation (Figure 1-7d). The extremely pale fibres seen in COX histochemistry are easily observed when SDH staining is applied and are stained vivid blue when the fibre is COX deficient but retains SDH activity (Figure 1-7e). These muscle biopsy findings can also be useful in providing information concerning muscle function;

therefore muscle biopsies have been used in clinical trials concerning exercise capacity as well as for diagnosis (Murphy *et al.*, 2012).

Figure 1-7: Histochemical changes seen in a muscle biopsy section linked with Mitochondrial Disease.
Two muscle fibres are highlighted with an asterisk and are shown in a series of transverse muscle sections; a) general muscle morphology; b) classical ragged red muscle fibres; c) SDH staining; d) COX histochemistry showing COX-deficient fibres within a population of normal fibres and a typical "mosaic" picture; e) SDH/COX histochemistry (Tuppen et al., 2010)



In summary mitochondrial diseases remain difficult to diagnose due to the variety and the differing severity of symptoms. To attempt to reduce the variability of presentations seen in the studies in this thesis, the studies only included two genotypes: m.3243A>G and m.8344A>G. The following section will discuss these two genotypes in more detail.

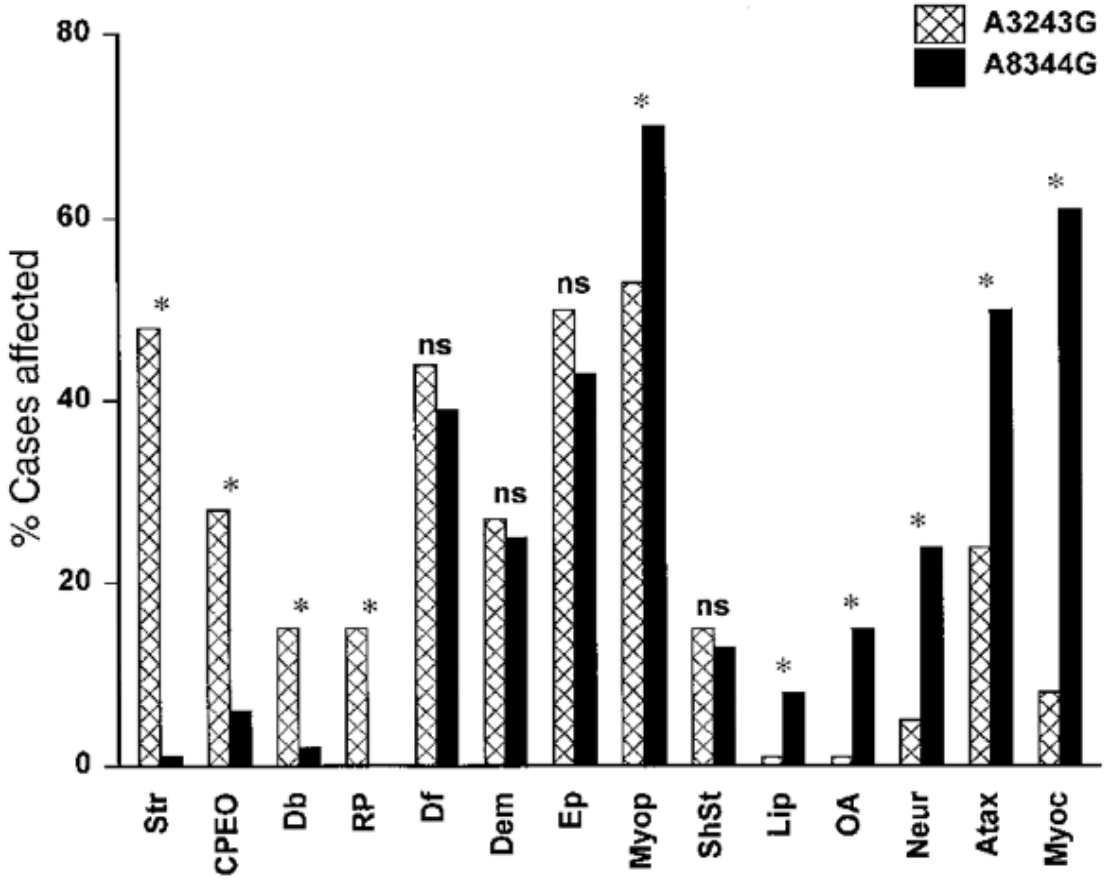
1.3.6 Disease manifestation of the m.3243A>G and m.8344A>G mutations

The two genotypes (m.3243A>G and m.8344A>G) included in this thesis can result in a great variety of symptoms varying in severity (Figure 1-8). A common clinical feature to both genotypes is myopathy, present in over 50% of both genotypes (Chinnery *et al.*, 1997).

The m.3243A>G mutation is the most common mitochondrial point mutation, identified in 1990 in association with Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis and Stroke-like Episodes (MELAS) (Goto *et al.*, 1990; Wallace, 1992) and is reported to occur in 80% of MELAS cases (Hammans *et al.*, 1995). People with MELAS typically present with stroke-like episodes that do not

usually follow a recognised vascular distribution and are not the result of ischaemic lesions. Seizures are also a common symptom, although other symptoms such as migraine like headaches, encephalopathy, ataxia, and cognitive impairment are often present. The m.3243A>G mutation makes up 23% of the Mitochondrial Diseases Patient Cohort Study; which is the largest cohort of living patients with confirmed mitochondrial disease, although only 10 % of patients exhibited the classical MELAS phenotype (Nesbitt *et al.*, 2013). Other phenotypes exhibited in the Newcastle cohort include maternally inherited deafness and diabetes (MIDD), progressive ophthalmoplegia (PEO), Leigh syndrome and isolated myopathy with another 28% of carriers not exhibiting symptoms consistent with any reported syndromes. Also some carriers of the m.3243A>G mutation do not present with clinically severe disease (Manwaring *et al.*, 2007) whilst others demonstrate features consistent with the Myoclonic epilepsy with ragged red fibres (MERRF) phenotype which is typically caused by the m.8344A>G mutation (Hammans *et al.*, 1993; Hammans *et al.*, 1995).

Figure 1-8: Percentage frequency of clinical features associated with the 3243A>G and 8344A>G mitochondrial mutations.
*(Str-recurrent stroke like episodes; CPEO-chronic progressive external opthalmoplegia; Db-Diabetes; RP-pigmentary retinopathy; Df-Deafness; Dem-dementia; Ep-Epilepsy; Myop-myopathy; ShSt-Short stature; Lip-Lipomata; OA-Optic Atrophy; Neuro-neuropathy; Atax-ataxia; Myoc-myoclonnus. *p<0.05, ns-not statistically significant (Chinnery et al., 1997).*



Myoclonic epilepsy with ragged red fibres (MERRF) was originally described as progressive myoclonic epilepsy, ataxia and myopathy (Silvestri G *et al.*, 1993) and is usually due to the m.8344A>G mutation (Hammans *et al.*, 1993). MERRF and the m.8344A>G mutation are rare, representing 4% of all pathogenic mtDNA mutations (Remes *et al.*, 2003; Schaefer *et al.*, 2008). This condition often results in neuro-degeneration, which presents in late childhood or early adulthood following a normal childhood development. The presenting symptom is usually one of myoclonus, which then progresses to include proximal myopathy, epilepsy, cerebellar ataxia, optic atrophy and deafness. Non-

neurological manifestations include cardiac symptoms such as Wolff-Parkinsons-White syndrome and multiple lipomas.

Clinical features in both genotypes are highly variable in terms of age of onset, severity of symptoms and organs involved (Graf *et al.*, 1993). Although clear differences in frequency of clinical symptoms are usually reported between the two genotypes, deafness, dementia, short stature and myopathy are common to both mutations. The variability of clinical presentations is partially dependant on heteroplasmy levels, with clear relationships evident between clinical features for both m.3243A>G and m.8344A>G genotypes and the level of mutation within muscle tissue (Chinnery *et al.*, 1997).

1.3.7 Treatment of Mitochondrial Disease

Although research into the mitochondrial genome and discovery of pathogenic mutations continues at a pace, treatment of mitochondrial disease remains essentially supportive in nature (Chinnery *et al.*, 2006; Pfeffer *et al.*, 2012). Management of mitochondrial conditions currently involves regular monitoring of symptoms by an extensive multi-disciplinary team. Despite no cure being available for mitochondrial disease, management of symptoms can improve quality of life and reduce morbidity (Rahman and Hanna, 2009). Numerous treatments are currently being used in mitochondrial disease and have been discussed in detail in recent reviews (Horvath *et al.*, 2008; Pfeffer *et al.*, 2012; Pfeffer and Chinnery, 2013). Full descriptions of all current treatments are beyond the scope of this thesis; the treatment chosen for this thesis is aerobic exercise and will be discussed in the following sections.

1.4. The effects of exercise on the body

Exercise is known to improve exercise capacity, muscle strength and the performance of activities in daily life alongside providing some protection from chronic diseases (Stump *et al.*, 2006; Wolfe, 2006; Ruiz *et al.*, 2008). Exercise capacity has also been shown to be a prognostic indicator for cardio-vascular disease and more recently linked with all-cause mortality (Haskell *et al.*, 2007; Kodama *et al.*, 2009; Lee *et al.*, 2012).

This section will review the physiological and metabolic adaptations of acute and chronic exercise in healthy individuals. These changes will then be discussed in relation to the effects of exercise in people with mitochondrial disease. Exercise or physical activities take on many forms; aerobic exercise is the activity undertaken in this thesis and therefore discussion will concentrate on this type of activity.

Exercise has a dose response relationship, with responses dependent on the type, duration, length and number of repetitions performed during exercise (Wenger and Bell, 1986). Aerobic exercise is low in intensity, performed over a long duration and depends primarily on the aerobic energy system; examples of aerobic exercise are activities such as walking, jogging and cycling (Powell *et al.*, 2011).

To aid the description of the body's response to aerobic exercise the effects of exercise on three different body systems are described separately: Cardiovascular, respiratory and muscular systems.

When moving from rest to exertion the energy requirements of exercising muscles are increased. ATP stores within the muscle are depleted within

seconds and therefore are required to be re-synthesized to provide continuous energy to the cell (ACSM, 2006; Mcardle *et al.*, 2007). The increased energy requirement is met by an increase in the metabolic rate of the cell. The increased metabolic rate requires the increased delivery of oxygen and nutrients and the removal of waste by-products. To maintain cells equilibrium during exercise adaptation by the respiratory and cardiovascular systems is required. To ensure adequate O_2 to the tissues changes occur in heart rate, stroke volume, cardiac output, blood flow, blood pressure, arteriovenous oxygen difference and pulmonary ventilation (ACSM, 2006; Mcardle *et al.*, 2007).

1.4.1 Cardiovascular response to acute exercise

Cardiovascular changes in response to exercise are reflected in an increase in heart rate with work rate (WR) and O_2 uptake. The maximum heart rate achievable is dependent on age, body position, fitness, disease and medication (ACSM, 2006). Neural control centres receive impulses from the motor cortex as it recruits the muscles required for the physical activity. This feed-forward mechanism coordinates a rapid increase of heart rate and altered blood vessel flow around the body to optimise tissue perfusion and maintain central blood pressure (Innes *et al.*, 1992; Nóbrega *et al.*, 1994; Stewart *et al.*, 1998; Stockton *et al.*, 2011). This therefore allows the heart to transition from beating at 75-80 beats min^{-1} during ambulation to exceed 200 beats min^{-1} during maximal exercise (Bjørnstad *et al.*, 1994). Alongside increased heart rate the stroke volume increases with work rate; at rest stroke volume is 60-100ml.beat $^{-1}$ but during exercise it can raise to 100-120ml.beat $^{-1}$. Cardiac Output is a product of heart rate and stroke volume. In healthy adults this also increases linearly with

work rate (WR). At rest it is 5L.min^{-1} and can increase to a maximum of around 20L.min^{-1}.

Control of blood pressure (BP) is achieved by adjusting peripheral resistance. This can be achieved by various mechanisms; neural control directly on the arterioles, locally released substances called endothelial-derived relaxing factors (nitric oxide) and local changes in chemical concentrations within active skeletal muscle. During exercise systolic blood pressure (SBP) increases linearly with an 8-12 mm.Hg increase per metabolic equivalent (MET) of activity. As BP directly relates to Cardiac Output (CO) and peripheral resistance measurement of BP provides a non-invasive method of monitoring the pumping of the heart during exercise and is used during exercise testing.

Arteriovenous Oxygen difference (a-vO_2 diff) is the difference in O_2 content of arterial and venous blood and therefore shows the level of extraction of oxygen by the tissues. This can go from 5ml.dl^{-1} to 15ml.dl^{-1}during the transition from rest to maximum exercise (ACSM, 2006). This increase can be due to a number of factors such as an increase in the number of capillaries used surrounding each muscle fibre and enhanced activity of enzymes within the mitochondria.

In summary a variety of cardiovascular changes occur in response to an acute bout of aerobic exercise; these can be both centrally and locally mediated (Ferretti, 2014). The integration of these responses allows tissues to receive the nutrients required during periods of increased energy demand, such as during exercise. The magnitude of response is governed by the type and duration of the exercise undertaken and the measurement of these responses can give an insight into the exercise capacity of the individual. If exercise is repeated, long-term changes of the cardiovascular system occur and are described below.

Stroke Volume (SV) is increased with chronic exercise due to cardiac hypertrophy resulting in more forceful contractions. The increased SV allows the person to exercise at the same work rate but with a reduced HR and a reduced myocardial demand (C G Blomqvist and Bengt, 1983). Improvement in a-vO_2 difference occurs due to increased capillary density and improved mitochondrial activity (Holloszy and Coyle, 1984; Flück, 2006; Daussin *et al.*, 2008). This adaptation may be relevant in mitochondrial disorders and will be discussed in greater depth in the section explaining the changes that occur in muscle as a result of exercise.

1.4.2 Response of ventilation system to exercise

In addition to cardiac adaptation to exercise, our breathing rate is adjusted by a number of mechanisms to meet the metabolic requirements of cells. A response occurs when neural circuits relay information from higher centres within the brain, lungs and throughout the body to coordinate a response in ventilation.

Ventilation increases exponentially to reach a steady state dependant on the exercise intensity and once a steady state has been reached, fine-tuning of ventilation occurs dependant on peripheral sensory feedback mechanisms. These responses result in the following changes in ventilation: Minute Ventilation (V_E) is increased by 15-25 fold during maximum exercise from approximately 6L.min^{-1} at rest. This increase in V_E is directly proportional to the increase in the consumption of O_2 (vO_2) and level of CO_2 produced (vCO_2). At a critical intensity V_E increases disproportionately relative to vO_2. This coincides with increase in lactate and vCO_2 (Davis *et al.*, 1976). The disproportionate increase is due to the need to remove CO_2 and is the result of increased buffering of lactate that has accumulated due to anaerobic glycolysis. The

increased CO_2 exhaled causes the respiratory exchange ratio (RER=vCO_2/vO_2) to exceed 1.00. The rise in V_E and rise of RER above 1 is thought to indicate that demands for energy have exceeded that which can be provided by mitochondrial respiration alone and has resulted in additional energy provision by anaerobic means. It should however be noted that confirmation of a link between these ventilatory changes and glycolytic events has proved difficult (Davis *et al.*, 1976; Svedahl and MacIntosh, 2003).

This threshold in practice is known as anaerobic, ventilatory or lactic threshold and has been used to provide a measure of fatigue resistance (Morris *et al.*, 2008). Exercise training has the potential to reduce blood lactate concentrations during sub-maximal exercise as a result of raising the workload intensity at which the threshold occurs, thus showing an improved exercise performance. Instead of an invasive method of measuring lactate, it is common to use the surrogate measure of ventilatory threshold, which can be inferred from the point at which vCO_2 exhaled increases in relation to vO_2 inhaled. This threshold occurs at 60-80% of vO_{2MAX} but with training can increase by 10-25% (ACSM, 2006). Changes in ventilation easily can be measured and give an indication of changes in physical performance and are used in this thesis as measurements of exercise performance.

As ventilation appears to be increased due to the need to remove excess CO_2 rather than the need for O_2, it is not thought that O_2 delivery limits reaching peak exercise capacity in the healthy population (ACSM, 2006). As the pulmonary system has a fast acting response to exercise and does not limit maximum exercise capacity its requirement to adapt to continued training is less pronounced than the cardiovascular system.

1.4.3 Peripheral response to exercise

Peripheral adaptations in muscle tissue occur alongside the central adaptations described above. Skeletal muscle is very malleable and has the ability to alter its structure according to the demands put upon it. This plasticity can be seen in the changes in muscle force, endurance and contractile velocity (Graham *et al.*, 2008). The plasticity of muscle is specific to the stimulus and is demonstrated by high repetition, low load training resulting in a differentiation of muscle fibres toward a fatigue resistant phenotype, whereas conversely training with high loads results in fibre hypertrophy (Pette and Staron, 2001).

Prior to the 1960's it was thought that central adaptations to exercise were solely responsible for the increased capacity to provide O_2 to exercising muscle. Holloszy was the first to show that changes in mitochondrial content within the skeletal muscles of rats occurred with endurance exercise training (Holloszy, 1967). Changes in mitochondrial content occur by a complicated pathway involving gene transcription and protein synthesis in response to acute and repeated exercise. Chronic endurance training results in high mitochondrial volume (Hood *et al.*, 2006; Fritz and Lusardi, 2009), increased capillary density (Saltin and Gollnick, 1983) and a shift of character of the muscle fibre toward slow fibre type (Flück, 2006). Together these changes enable maximum levels of substrates to be delivered and efficiently used by the cell.

Increased mitochondrial content in a cell is a result of increased mitochondrial biogenesis. Mitochondrial biogenesis occurs when signalling reactions activate proteins to increase gene transcription, these signals are dependent on the intensity and duration of contractile activity and fibre type (Favier *et al.*, 2008). As a result protein synthesis is accelerated, and proteins are imported into the

mitochondria from the cytosol. Once in the mitochondria the proteins act as either metabolic enzymes forming part of the electron transfer chain complexes, or become transcription factors for mtDNA. Therefore, mitochondrial biogenesis results in an expansion of the mitochondrial network leading to an increase in the capacity for the cell to produce energy.

Chronic exercise leads to an increased mitochondrial content within muscle, which in turn results in improved muscular endurance and reduced fatigability in muscle during normal activities. However the converse is also true, maintenance of muscle mass and oxidative capacity of a muscle is reliant on contractile stimuli. Without stimulation, muscle function deteriorates and results in reductions in mitochondrial content and respiratory chain enzyme activity (Taivassalo and Haller, 2004). This de-conditioning of muscle can be seen during periods of inactivity, such as bed rest or sedentary behaviour. As sedentary behaviour is common within people with mitochondrial disease the effects of deconditioning may also have a part to play in determining the exercise capacity in the mitochondrial disease population and is discussed in the section describing exercise in mitochondrial disease.

In conclusion, adaptations of the body to exercise take place both centrally and peripherally and are likely to improve some of the symptoms of mitochondrial disease. Adaptations are dependent on the type, intensity and duration of exercise.

1.5. Exercise and mitochondrial disease

Various types of exercise have shown promise in mitochondrial disease although it remains debatable whether benefits are due to changes in the

primary biochemical defect or the reversal of low levels of activity (Taivassalo and Haller, 2004; Taivassalo *et al.*, 2006; Tarnopolsky, 2014). The following section will discuss the results of previous exercise studies in neuromuscular conditions and in particular mitochondrial disease. The results of these studies lay the foundation for the intervention included in this thesis.

1.5.1 Aerobic training

Aerobic training in a disease free population as previously reported provides benefits to the cardiovascular and muscular systems. Large numbers of patients (>20%) with a mitochondrial disorder report exercise intolerance as a symptom (Mancuso *et al.*, 2012). Exercise intolerance in the mitochondrial population may be a result of a primary biochemical defect but is likely exacerbated by a sedentary lifestyle (Taivassalo and Haller, 2004). Activity levels have been shown to be decreased in people with a variety of Neuromuscular Diseases (Tudor-Locke *et al.*, 2009) and the same is true in mitochondrial disease, where it was recently demonstrated that 78% of participants performed less than the recommended 10, 000 daily steps (Apabhai *et al.*, 2011). The causes of a sedentary lifestyle in patients with mitochondrial disease are likely to be multifactorial and may include: excessive fatigue at low levels of activity; avoidance of activities that exacerbate symptoms of muscle weakness along with pain and historical advice to conserve energy (Tarnopolsky, 2014).

Due to the rarity of the majority of neuromuscular conditions, initial research on the effects of exercise tended to group all neuromuscular conditions together (Wright *et al.*, 1996; Taivassalo *et al.*, 1999a; Kilmer, 2002b; Kilmer, 2002a). The results of these studies were often flawed due to the varied responses to exercise of different conditions, with some conditions responding negatively to

exercise (Kilmer DD, 2002). As the responses of neuromuscular conditions to exercise were diverse and dependent on the type of exercise undertaken and the condition being investigated, subsequent research studies were performed on individual neuromuscular diseases.

One of the first exercise studies involving patients with mitochondrial disease included a patient with a cytochrome c oxidase deficiency. This patient after 8 weeks of aerobic training (supervised cycling 3-4 times a week, at 60-80% of Heart rate reserve) demonstrated an improvement in exercise capacity (70% from baseline) despite a basic metabolic defect. It was therefore surmised that aerobic training was able reduce the effects of chronic deconditioning, partially improve exercise capacity and therefore had the potential to improve the patient's condition (Taivassalo *et al.*, 1996). However this was only in one patient so further research was required.

The same research group progressed to investigate10 patients with a variety of mitochondrial disorders due to both mitochondrial and nuclear DNA mutations. This study included directly supervised treadmill training (with the participant attending the facility to exercise initially) 3-4 times per week at 60-80% of heart rate reserve (HHR = maximal heart rate–resting heart rate, maximal heart rate=220-age) for 20 -30 minutes .It was noted that not all patients were able to increase to or maintain heart rate within the ranges set without muscle pain and therefore some participants performed interval type training with rests until 20 minutes of training had been completed. Exercise was then progressed at rates tailored to the individual, with exercise being extended towards a 30 minute continuous exercise session. Exercise intensity was then increased with either an increased treadmill speed or elevation to maintain exercise in the set heart

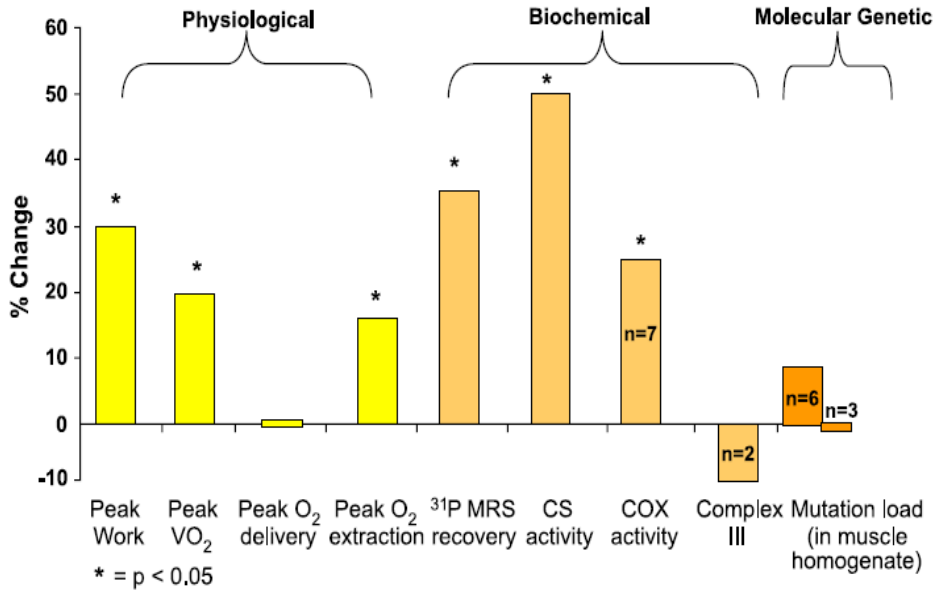
rate range. Alongside measures of exercise capacity, this study measured ADP recovery time using Magnetic Resonance Spectroscopy and quality of life using the SF36 quality of life questionnaire. This program of exercise led to participants performing very different levels of absolute training and may in part explain the very varied responses to the exercise intervention. No adverse responses were detected and improvements were seen in both central and peripheral adaptation, with a reduction in heart rate at rest, during sub-maximal (15%) and peak exercise (10%), improvement in ADP recovery, reduced lactate levels at rest and post exercise testing and no increase in Creatine Kinase (a marker of muscle damage). From this study it was hypothesised that peripheral limitations in mitochondrial patients were improved by training due to the large response reported in the ADP recovery time post exercise (Taivassalo *et al.*, 1998). These results were reported as different to the responses previously seen in healthy populations, where increased capacity to exercise was explained by an increase in cardiac output with peripheral adaptation being smaller.

Despite the improvements in exercise capacity and oxygen utilisation inferring an improvement in mitochondrial function, concern remained over potential preferential proliferation of mutant mitochondrial DNA alongside wild type and the long-term effects of such proliferation (Taivassalo *et al.*, 1998). To address these concerns further trials were necessary to identify the mechanisms for the improved exercise capacity reported in these studies, hence the previous study was repeated with the addition of analysis of muscle biopsies pre and post intervention (Taivassalo *et al.*, 2001). In this study again 10 patients with heterogeneous clinical, biochemical and molecular presentations of

mitochondrial disease were recruited. Exercise in this study was performed on a stationary bike with heart rate and duration of exercise monitored using a POLAR watch. Intensity was at 70-80% of maximum heart rate (220-age), duration was 30 minutes for the first 7 weeks (3-4 times/ week), then 40 minutes for the remaining 7 weeks (3-4 times per week), equating to 50 sessions. Peak exercise testing was performed twice and either the performance averaged or the better performance was presented, alongside the results of submaximal testing. Exercise testing post exercise again showed a significant increase in work capacity (30%) along with an increased capacity to extract oxygen (20%) and a normalisation of the hyperkinetic (exaggerated heart rate) response to exercise previously reported with a reduction in the ratio between cardiac output and oxygen uptake (Haller *et al.*, 1989; Tarnopolsky, 2004), with Magnetic Resonance Spectroscopy reporting an increased oxidative capacity in muscle. In addition muscle biopsy results demonstrated an increase in citrate synthase (CS) (49%), Succinate Dehydrogenase (SDH) (43%) and Cytochrome complex (COX) activity alongside mitochondrial proliferation, although the percentage of mutant mtDNA was variable. The basis for the improvement in work rate was shown to be enhanced skeletal muscle oxidation with increased oxygen uptake and increased respiratory chain enzyme activity. Unfortunately the increase in mitochondrial volume reported was greater than the increase in enzyme activity; therefore this disproportionate increase was thought to be due to a greater proliferation of mutant DNA. It was also noted that the variable response to exercise was dependant on the type of mutation, with a negative response seen in patients with mutations affecting complex III. A summary of these results is shown in Figure 1-9. The response to exercise was again varied which in part may be due variety of clinical presentations and genotypes reported in the study.

No record of variations in exercise delivery were reported and exercise was tailored to maximal heart (220–age) not performance at baseline, which will have delivered similar levels absolute exercise by all participants. Despite performing similar levels of absolute exercise versus relative reductions in the variability of responses of individuals was not seen.

Figure 1-9: Changes in physiological, biochemical and molecular genetic variables in 10 patients with mtDNA defects following 14 weeks of endurance exercise training
(Taivassalo and Haller, 2004).



The increase in mitochondrial volume thought to be due to preferential proliferation of mutant mtDNA continued to raise concerns over the long term effects of exercise, as disease progression was thought to be linked to the accumulation of mutant mtDNA leading to increases in the number of respiratory incompetent fibres (Chinnery *et al.*, 2003). Further work therefore was required to examine the effects of long-term exercise and whether proliferation of the mutant DNA varied in specific areas along a single muscle fibre or between fibres.

Whilst studies continued to evaluate the effect of exercise in mitochondrial disease, Taviassalo *et al* (2003) also reported great variation in exercise capacity in mitochondrial patients. The exercise capacity of 40 patients with a variety of mitochondrial disorders was compared to healthy volunteers. Of the 40 patients 23% had a level of mutation load reported. This work confirmed that the peak work capacities of patients with mitochondrial disease were significantly lower than that reported in healthy controls. Although a third were equivalent to healthy sedentary controls and one was comparable to a healthy control. The range of exercise performance was very broad, with two thirds of the participants unable to perform activity above 5 METS, where a MET (metabolic equivalent) is the physiological measure indicating the energy cost of the physical activity in relation to 1 MET= 3.5 ml.O_2.kg.min. This level of impairment is important to note as performance below this level would lead to impairment in daily activities such as moderate speed walking (3 METS) and climbing of stairs (3-6 METS). This study also confirmed relationships between exercise capacity and varying levels of oxidative impairment and that exercise capacity was directly proportional to the ability to extract and utilise oxygen (a-vO_2 difference) not cardiac output (Taivassalo *et al.*, 2003). As the level of variability in exercise capacity reported in this study was broad, the study recommended that future work in this area should attempt to reduce the heterogeneity of participant groups by limiting the genotypes involved in future studies.

A common criticism and limitation of the exercise studies reported thus far is the lack of blinding or randomisation performed. This has meant that only one exercise study has been included in the Cochrane review of treatments in

mitochondrial disease (Pfeffer *et al.*, 2012). The blinding of participants in exercise studies is difficult, if not impossible in such a sedentary population, where even the performance of a “sham” exercise intervention is likely to have a response (Lee *et al.*, 2008). Although not blinded, one exercise trial did randomise participants to an exercise intervention (Cejudo *et al.*, 2005). This trial confirmed the beneficial effect of aerobic training reported in previous studies alongside an improvement in an exercise field test; the shuttle walking test (Singh *et al.*, 1992). Changes reported were similar to previous studies; vO_{2MAX} increased by 28 %, peak workload by 16%, although no information was provided concerning termination of exercise test (e.g. maximum heart rate, RPE or symptom limited). The increase in peak heart rate on retesting could imply that participants may have worked harder on retesting. This is unlikely as improvement was also seen in the anaerobic threshold (improved by 22%). As in previous studies, this study included patients (n = 20) with a variety of mitochondrial mutations, with participants performing a cycling exercise (70% of baseline work rate, 12 weeks, 3 sessions / week) along with some upper limb activities, therefore sessions lasted longer than previous studies (1 hour) and also were directly supervised. Unfortunately the study did not have the capacity to investigate mutation loads and the histological and biochemical changes in response to exercise, so was unable to further inform the mechanisms of improved exercise capacity and allay fears of preferential proliferation in mutated mtDNA. Another study by Trenell *et al* also used a field test for exercise capacity to investigate the effects of aerobic exercise in mitochondrial disease. This study included 10 patients with mixture of presentations and 10 disease free participants matched for age, gender and physical activity. Exercise was again cycling; at 70-80% age predicted maximal heart rate for 30

minutes. However it did report that exercise was tailored to the individual and supervised with participants completing 95% of sessions. Alongside exercise testing and phosphorus magnetic resonance spectroscopy this study used the 6-minute walk distance (6MWD). This study demonstrated an improvement in muscle mass, cross-sectional muscle area, along with improved rates of oxidation and phosphocreatine (PCr) recovery. The improvements reported from the imaging data were replicated in the distance walked over 6 minutes, oxygen extraction and peak work rate during a maximal exercise test (Trenell *et al.*, 2006). Although this study was able to show the potential of novel non-invasive methods in monitoring changes in mitochondrial function it was unable to shed further light on the proliferation of mutant mitochondrial DNA.

To allay fears and allow the recommendation of exercise as a potential therapy in mitochondrial disease long-term exercise studies were still required. This was achieved in two studies with the effects of long-term exercise being investigated in two different populations of patients.

Jeppeson *et al.* (2006) compared a group of 20 patients with 4 different mutations. These patients included eight relatives of the index case with m.3243A>G, 4 of which were unaffected, against 13 sedentary control participants (Jeppesen *et al.*, 2006b). This study included patients with low levels of mitochondrial mutation to ensure that any changes in mutation load would not be impaired by a ceiling effect. Following a 12-week home based exercise program monitored by a weekly phone call. The exercise again was cycling; 30 minutes, heart rate corresponding to 65-75% of baseline vO_{2MAX}, to complete 50 sessions. Thirteen participants stopped exercising and were re-tested after 8 weeks of deconditioning. Participant's performance following an

exercise intervention replicated the findings of previous studies with an increase in maximum exercise capacity (26%) and workload post training (29%), and self-reported physical activity, which post deconditioning returned to pre exercise levels. The percentage increase in exercise performance reported in the participants with mitochondrial disease was higher than in the healthy controls and reassuringly the mutation load remained the same. The mitochondrial DNA quantity increased in the mitochondrial group, but returned to pre training levels post deconditioning, and showed no relationship to pre training levels or maximum exercise capacity. Levels of respiratory chain enzymes were similar in both healthy and mitochondrial disease groups, increased with training and returned to pre training levels post deconditioning, however the increase was not related to training response (change in vO_2). As no changes were reported in mutation load, Creatine Kinase levels, muscle regeneration and apotosis, the study provided the necessary reassurance for exercise to be considered safe in the management of mitochondrial disease. This study was the first study to report the number of participants that were unable to complete the study (n=10), eight of these were due to failure to complete 50 % of the exercise sessions.

At the same time another study in a different group of patients also addressed the concern of preferential proliferation of the mutant mtDNA. This study investigated the hypothesis that habitual inactivity in patients with mitochondrial disease led to further reduction of mitochondrial volume and therefore further restricted the oxidative capacity in patients (Taivassalo *et al.*, 2006). The study chose eight patients with a large-scale deletion of their mtDNA with exercise intolerance ranging from mild to severe. All participants completed a 14 week

exercise program (home based cycling); Sessions were monitored by heart rate (POLAR watch), 30 minutes, 3 times a week for the first 7 weeks and increased to 40 minutes for the last 7 weeks at 70 -80% of heart rate maximum. Four participants then continued for a prolonged intervention of a further 14 weeks whilst the other four discontinued training. At three different time points (week 0, 14 and 28) exercise testing was recorded along with evaluation of proportion, distribution and copy number of mutant and wild type mtDNA in muscle. As in other trials, increases were noted in work capacity (26%), oxygen uptake (11%) and utilization (11%) after 14 weeks training. Continued training maintained these and further improvement was reported in a-vO_2 difference. Patients who stopped training exhibited the opposite response with exercise parameters returning to baseline levels. Histo-chemical analysis showed no change in COX negative fibres after 14 or 28 weeks. Biochemical analysis demonstrated only a trend toward increased activity in respiratory chain complexes with training due to considerable variation and small numbers, which was compatible with previous studies and was thought to represent an increase in mitochondrial activity. No change was reported in percentage mutation loads in all arms of the study, with the use of PCR (Polymerase chain reaction) and Southern blot also reporting no change in the amount of deleted mtDNA with training or detraining. This study also reported one injury, although this was in the detraining group.

The findings of these two studies provided reassurance concerning the use of exercise as a therapy in the genotypes studied. The reduction in mtDNA copy number in COX deficient fibres with detraining supported the theory that COX deficiency is associated with the amount of wild type not mutated mitochondria

(Durham *et al.*, 2005; Murphy *et al.*, 2012) and emphasised the detrimental effect of reduced levels of activity in mitochondrial disorders.

As exercise is likely to be a continued intervention throughout the lifetime of a patient, further trials have been performed to assess the effects of lengthier training protocols. This was attempted in a study of four patients with different mutations trained over a period of two years with analysis of muscle biopsies and exercise testing (Jeppesen *et al.*, 2009). Training was performed for 3 months (5 times per week) then stopped for 3-12 months then training restarted for 12 months. All training was performed on a stationary bike at an intensity of 70% of baseline vO_{2MAX} as previous trials by this group and supervised by phone. The three patients who completed all the training periods reported increased exercise capacity and workload, whilst deconditioning again resulted in a return to baseline values. The mutation load was unchanged over the two-year period along with the number of COX negative, apoptotic or necrotic fibres. Citrate synthase increased after the first three months then plateaued whereas the capillary density did not increase until after 12 months as compared to 3 months in the healthy normal population. All these parameters indicated that endurance training was safe in patients when performed for up to 12 months. Although these results were promising for the long-term use of exercise, the numbers were very small, with recruitment impaired by repeated muscle biopsies and motivation to continue with training. These problems also have also been seen in long-term exercise studies attempted in Newcastle (unpublished).

The above trials repeatedly report improvement in exercise performance following an exercise intervention, albeit the reporting is difficult to compare due

to the wide range of outcomes used. The variability of response is likely due to the small numbers involved and the great variety of clinical presentations and mitochondrial mutations included in studies. This said the variability could also be due to the type and intensity of training and the method of exercise testing. The studies reviewed here set exercise intensity from variables such as: age predicted heart rate, percentage of exercise capacity at baseline or % of heart rate reserve all of which may result in different levels of exercise intensity being performed. Studies also reported different methods of testing exercise capacity (maximal, sub-maximal), with and without familiarisation, following different protocols with different criteria for ceasing exercise testing. Consensus between groups involved in exercise testing of patients with mitochondrial disease may result in less variability and allow comparisons to be made between studies (Tarnopolsky, 2014).

The limiting of types of mutations used in exercise studies has been also been recommended to reduce the variability of response to interventions. This may not be successful due to the variety of presentations even within a genotype and therefore limiting the types of phenotypes included or stratifying participants by levels of disease severity may be more successful. This would require greater adoption of the NMDAS and recording of mutation loads than has been done in the studies discussed here.

In summary, exercise can play an important role in accelerating mitochondrial biogenesis and may have the potential to reduce the effects of mitochondrial dysfunction seen with age, disuse and muscle disease (Hood, 2009). The use of exercise in mitochondrial disease has been shown to be beneficial, improving: exercise capacity, muscle strength and quality of life. The adaptations to

exercise in mitochondrial patients appear to be peripheral, with improved oxygen utilisation due to increased mitochondrial copy number and respiratory enzyme activity. Exercise does not appear to affect mutation load as no adverse events have been reported and therefore exercise has been deemed safe in the majority of patients with a point mutation or single deletion. However the safety of exercise as a therapy in all mitochondrial patients has yet to be fully proven. Despite this lack of proof increased physical activity is being recommended in clinical practice for all patients as reduced activity has been shown to be detrimental to mitochondrial function.

1.6. Dynamometry

In addition to the peak exercise testing performed in previous exercise studies the studies in this thesis measure muscle strength with isokinetic dynamometry. A common symptom of mitochondrial disease in the two genotypes investigated is proximal myopathy and this is ameliorated with exercise therapy (Cejudo *et al.*, 2005; Murphy *et al.*, 2008). Measurement of muscle strength by isokinetic dynamometry has been shown to be reliable (Claiborne *et al.*, 2009); reproducible (Sapega, 1990) and relate to function. Muscle strength is also assessed in the studies by manual muscle testing as part of the Newcastle Mitochondrial Disease Assessment Scale (NMDAS).

All methods of muscle testing have their strengths and weaknesses but isokinetic dynamometry provides an optimal loading of muscles through a dynamic movement at a selected speed (Baltzopoulos and Brodie, 1989). These features allow the safe and accurate measurement of a great variety of conditions including the extremely weak patient (Tiffreau *et al.*, 2007). Good stabilisation and placement of body parts, consistent instruction and

familiarisation with equipment allow isokinetic dynamometers to accurately measure torque and have good retest reliability with speeds between 30-60 degrees per second being effective in revealing muscle deficits (Sapega, 1990). Isokinetic dynamometers due to their size and cost will have a limited use in the clinical world, but their capacity to measure a wide range of abilities from very weak neuromuscular participants to healthy controls (Burnett *et al.*, 1990; Julia *et al.*, 2010; Lerario *et al.*, 2012; El Mhandi and Bethoux, 2013) make their use within neuromuscular research fundamental.

Alongside measures of exercise performance relatively few exercise studies have measured function and quality of life. The studies in this thesis evaluate the use of such measures in mitochondrial diseases alongside established measures of exercise capacity. The following section will discuss the current use of functional measures in clinical and research settings in populations with and without mitochondrial disease, with specific reference to the measures used in this thesis.

1.7. Functional outcome measures in clinical and research settings.

Functional outcome measures are quick and inexpensive to deliver and are commonly used in clinical practise by therapists to either describe status, document change, explain performance or to predict outcome (Bohannon, 1989). As stated earlier, previous studies into mitochondrial disease have utilised numerous measures of impairment including: respiratory enzyme activity, mitochondrial copy number, exercise capacity and muscle strength. Unfortunately a limited number have used recognised measures that record the activity or participation of patients (World Health Organization, 2001). A recent Cochrane review of interventional studies in mitochondrial disease noted that

the numerous primary outcomes used made comparisons between studies difficult and that researchers need to include outcomes that matter to patients as well as outcomes that are of interest to the researcher (Pfeffer *et al.*, 2012).

Functional outcome measures became popular in the 1990's when they were introduced to evaluate the effects of therapy. Functional measures can be described as disease specific (Loewen and Anderson, 1988; Bérard *et al.*, 2005) or they can be non-specific. Non-specific measures include the many timed tests used in clinical practice and allow comparisons between different disease groups.

Numerous outcome measures are available for use in clinical practise and research, with the choice of measure used dependent upon the population being measured, their abilities and the intervention being performed. The choice of measure is very important, as a common complaint with these types of measures are their "floor and ceiling effects". These effects mean that often more than one measure is required to capture the abilities of the majority of patients (Guralnik *et al.*, 2000).

The outcome measures chosen for this thesis relate to the symptoms often present in mitochondrial disease such as proximal myopathy, reduced exercise tolerance and fatigue. To measure the effects of these impairments on functional tasks the following measures were chosen: Timed up and Go (TUG), five times sit to stand (5XSTS), 10-metre timed walk (10MTW) and the 6-minute walk distance (6MWD). Of these measures the 6-minute walk distance is the only one to have previously been used in this population.

The 10m-timed walk (10MTW) is commonly used in clinical practice and measures the time taken to walk, steps taken and cadence over 10 metres. This test provides a measure of gait speed and steps taken (Holland *et al.*, 2006). Gait speed has been shown to correlate to a number of other measures and health outcomes such as; chair rise (Whitney *et al.*, 2005) community walking in the elderly (Busse *et al.*, 2006), hospitalisation (Rabadi and Blau, 2005), falls (De Rekeneire *et al.*, 2003), dementia (Atkinson *et al.*, 2007; Bohannon and Williams Andrews, 2011) and mortality (Ostir *et al.*, 2007; Studenski *et al.*, 2011). The test is a simple and robust measure and has been regularly used in research, in fact it has been described as an almost perfect measure (Wade, 1992). However it does have its limitations, as it only gives a picture of walking over 10 meters in one direction in a controlled environment and therefore may not reflect the difficulties patients have when walking in a community setting.

Sit to stand measures have been reported to be potential surrogate measures for lower limb strength and therefore may have the potential to measure the common symptom of proximal myopathy in mitochondrial disease (Gross *et al.*, 1998; Inkster *et al.*, 2003). Although dispute exists in the literature over whether the measurement of sit to stand does correlates to muscle power alone and which muscles are important in the movement of sit to stand. Bohannon *et al* have demonstrated correlations between knee extensors strength and the ability to sit to stand (Bohannon, 1998), whilst other researchers feel that other muscle groups such as those around the hip to be important (Gross *et al.*, 1998). Despite the debate over which muscles are most important in this movement, sit to stand remains an important indicator of daily function and therefore is a valuable and simple function to measure (Bohannon *et al.*, 2007).

The timed up and go (TUG) measure includes the movement from sitting to standing, along with walking with a change of direction and therefore may more accurately reflect patients day to day activity (Bohannon *et al.*, 2007). This test has the added benefit over other measures of sit to stand as it allows use of upper limbs, therefore patients with severe proximal myopathy are able to perform it. The TUG is well documented as a good predictor of functional mobility and risk of falling in the elderly (Podsiadlo D *et al.*, 1991) and has more recently been used in other diverse populations such as muscular dystrophies and cancer (Scholtes *et al.*, 2008; Willis *et al.*, 2011).

Exercise intolerance and fatigue are common symptoms of mitochondrial disease (Mancuso *et al.*, 2012) and can be measured in the clinical setting by measuring the distance walked over six minutes. The six minute walk (6MWD) is reported to measure the integrated response of all systems involved during exercise, but does not enable analysis of which organ or system is impaired (2002). The measure was initially used to measure sub maximal exercise capacity in patients with heart and lung related diseases. More recently this measure has been used in other populations including neuromuscular conditions such as Duchene Muscular Dystrophy and Spinal muscular atrophy as a surrogate marker of disease severity (Takeuchi *et al.*, 2008; McDonald *et al.*, 2010). One previous study within a population of patients with mitochondrial disease has used the 6MWD to demonstrate an improvement following an exercise intervention (Trenell *et al.*, 2006). The possible relationship between exercise capacity and function in this population meant it was an ideal measure to include in this thesis.

The number of outcome measures available for use in clinical and research settings is vast and cover a wide range of activities. The measures chosen for this thesis relate to the common manifestations of mitochondrial disease. Some of the measures overlap to allow the measurement of patients with a wide variety of abilities. The investigation of functional measures is imperative in this disease group to enable easy measurement of functional capabilities. Measurement of function will provide valuable data concerning disease progression and help evaluate the effects of interventions in a way that is meaningful to patients. The studies in this thesis will for the first time evaluate the use of functional measures in mitochondrial disease.

1.8. The use of gait analysis in clinical and research settings.

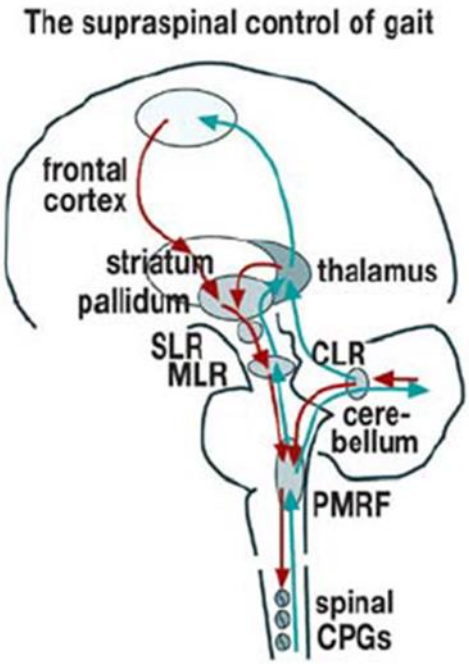
Functional outcome measures provide quick and simple measures of function but do not specifically identify the mechanisms for any change in function. More precise analysis of gait can provide improved discrimination concerning the cause of walking impairments and better identify possible interventions. The inclusion of gait analysis in the studies in this thesis will provide preliminary information concerning the walking impairments that are a result of mitochondrial disease and therefore provide guidance for future interventions.

Locomotion is critical to the independent day-to-day functioning of people, with walking still being the primary method of moving from place to place. Walking in healthy people is generally automatic with integration occurring between motor, sensory and cognitive systems (Jahn *et al.*, 2010) with impairments in walking resulting in reduced levels of activity and participation in the community (Schmid *et al.*, 2007; Fritz and Lusardi, 2009).

Gait speed is a key measure in clinical and rehabilitative settings and has been shown to relate to functional ability (Studenski *et al.*, 2003), community ambulation (Busse *et al.*, 2006), independence and falls in both elderly and neurological populations (Verghese *et al.*, 2009). Changes in gait speed represent a common endpoint for alterations in multiple gait characteristics, but remain a crude measure with little indication to the causes of change (Lord and Rochester, 2005). Measurement of components of the gait cycle can provide an understanding of the causes for this loss of walking speed and have been shown to be sensitive markers of ageing (Jahn *et al.*, 2010), pathology and disease progression (Lord *et al.*, 2011; Mirelman *et al.*, 2011; Galna *et al.*, 2013b; Galna *et al.*, 2014; Rochester *et al.*, 2014b).

The rhythmic patterns of gait are generated in centres within the spinal cord, which interact with the sensory systems and are under the control of the locomotor areas of the brain. It appears that gait is controlled primarily by the pre motor and motor areas of the frontal cortex, with connections to the basal ganglia, locomotor areas of the brain stem and cerebellum which link with the spinal generators. These connections allow us to remain still, commence walking and change speed or direction in relation to our environment (Figure 1-10). Many of these areas of the central nervous system can be affected in mitochondrial disease and therefore patients are very likely to exhibit difficulties with walking (Turnbull *et al.*, 2010; Galna *et al.*, 2013b).

Figure 1-10: The current concept of the supraspinal control of gait. Impulses from the motor and pre motor areas of the frontal cortex disinhibit brainstem locomotion areas via the basal ganglia. To initiate or altar a pattern of movement signals travels from the midbrain to the spinal generators. The cerebellum modulates the rhythm and speed of gait. Afferent signals from the limbs (blue) are able to alter the gait pattern through feedback loops by way of spinocerebellothalamic connections. (CLR-cerebellar locomotor region; CPG-central pattern generators; MLR-midbrain locomotor region; PMRF-pontomedullary reticular formation; SLR-subthalamic locomotor region)(Jahn et al., 2010).



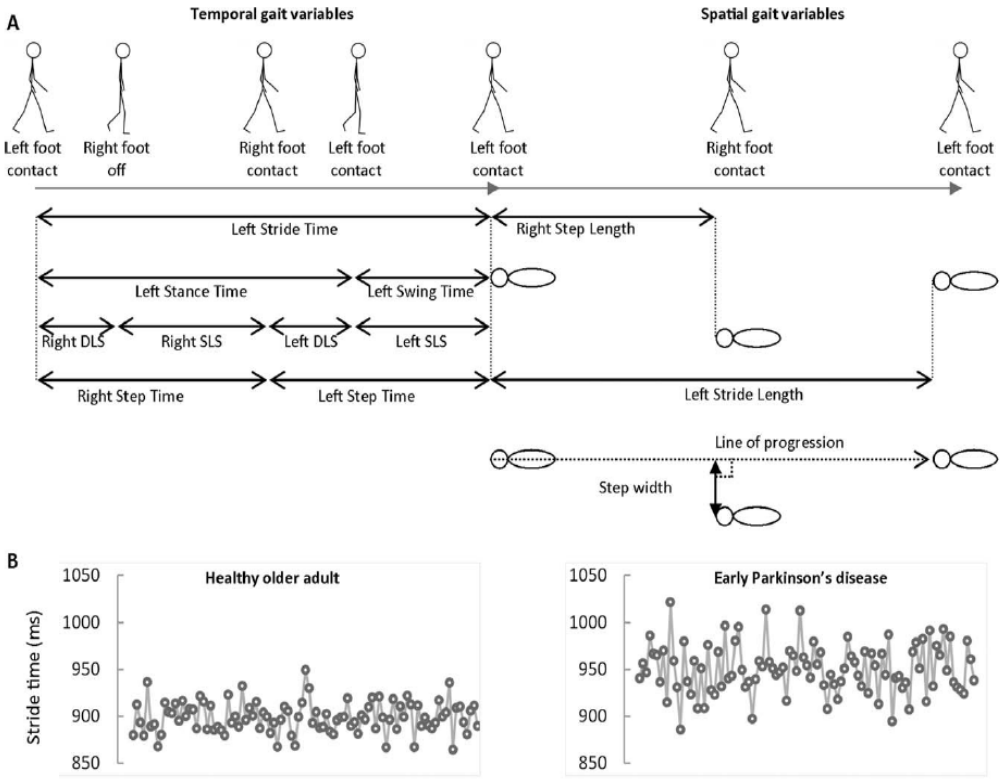
Measurement of gait can be performed in a variety of ways and has evolved over time from walking along paper recording footprints to electronic walkways and the use of 3 dimensional motion analyses. Despite this progress, gait analysis has had limited uptake in clinical practise due to the costs and expertise required to perform (Toro *et al.*, 2003). The section below will explain the various parameters measured using gait analysis in this thesis and how changes in these parameters can provide insights into the mechanisms resulting in gait impairment.

1.8.1 Models of gait analysis

Gait measurement can be grouped into two areas: Spatiotemporal measures that record a typical gait cycle (Figure 1-11A) and dynamic features of gait (Figure 1-11B), which show the variations of the spatiotemporal measures between steps (Lord *et al.*, 2013a). Spatiotemporal measures of gait have been shown to be reliable and valid (Bohannon, 1997; Lim *et al.*, 2005), but the reliability of dynamic features of gait has yet to be established despite demonstrating validity (Lord *et al.*, 2011). Also gait analysis can be performed alongside measurement of O_2 consumption to provide additional information concerning the efficiency of walking (Newman *et al.*, 2007; Wert *et al.*, 2010). Although this involves repeated walking over electronic walkways and treadmill testing with cumbersome equipment and harnesses and as such may not reflect normal community ambulation (Dawes *et al.*, 2004).

Finally measurement of gait has also been used to assess non-motor attributes of disease with changes in gait being reported alongside impaired executive function, attention and general cognitive decline (Verghese *et al.*, 2008; Yogev-Seligmann *et al.*, 2008) and directly linked with pathology (Rochester *et al.*, 2012). These findings have led to gait being recognised as a surrogate marker of cognitive function (Lord *et al.*, 2013b).

Figure 1-11: An illustration of spatiotemporal gait parameters.
***A**-The most common temporal measures are shown on the left, and spatial variables on the right (SLS-single limb support time; DLS-double limb support time).*
***B** Stride time variability is illustrated for 100 consecutive strides for a healthy example and an individual with Parkinson's disease (Lord et al., 2013a).*



Multiple protocols have been outlined in gait studies, with testing including different distances dependent on gait characteristics and conditions investigated (Lord *et al.*, 2013b), with walking being performed either continuously or intermittently and with or without a dual task. The dual tasks used are often cognitive but can include activities such as: changing pace or direction and talking whilst walking. The value of the addition of a secondary (dual) task activity is that it places further stress on the locomotor systems whilst walking and is likely to be more representative of walking in the community. In some conditions it has been noted that secondary tasks lead to a reduction in cognitive compensation and result in latent motor deficits being revealed. This has been reported in Parkinson's disease, where a slowing of gait with

increased variability was reported with the addition of a dual task (Hausdorff *et al.*, 2003; Rochester *et al.*, 2004; Yogev *et al.*, 2005; Lord *et al.*, 2011; Kelly *et al.*, 2012b). Although it should be noted that these changes have also been reported as normal features of ageing (Rochester *et al.*, 2014a).

Unfortunately the use of different protocols and different dual tasks makes direct comparisons between trials difficult (Al-Yahya *et al.*, 2011). To enable better interpretation of patterns of gait impairment attempts have been made to place characteristics into conceptual models. The model used in this thesis is based upon a model devised by Lord *et al* (2013b). This model represented 84.6 % of the variance of gait in Parkinson's disease patients and controls and resulted in 16 variables being defined within the five domains of: pace; rhythm; variability; asymmetry and postural control. The model uses spatial, temporal and dynamic aspects of gait assessment, so capturing all possible gait impairments that may result from mitochondrial disease.

1.8.2 Gait analysis in neurological conditions

Involvement of the central nervous system in mitochondrial disease is common (McFarland *et al.*, 2002; Turnbull *et al.*, 2010). Gait analysis has not previously been performed in this disease group, but use of gait analysis in other neurological conditions provides insights into its potential use in mitochondrial disease. Due to the selective nature of gait impairments particular neurological conditions and symptoms appear to demonstrate specific changes in gait characteristics. Patients with Parkinson's disease have been reported to demonstrate reduced stride length and speed, plus increased variability of stride length (Hausdorff *et al.*, 1998; Morris *et al.*, 1998; Stolze *et al.*, 2001; Galna *et al.*, 2014), patients with ataxia show an increase in gait variability even in a so

called pre–clinical state (Ilg *et al.*, 2007; Mirelman *et al.*, 2011; Rochester *et al.*, 2014b) and gait asymmetry is common following stroke (Schmid *et al.*, 2007).

The reduced levels of activity seen in patients with mitochondrial disease were previously thought to be due to muscle symptoms resulting in reduced exercise tolerance and excessive fatigue. However it should not be ruled out that limitations in activity might also be due to primary neurological pathology or secondary compensatory mechanisms leading to inefficient and impaired gait. Therefore the measurement of gait in patients with mitochondrial disease may be of value as impairments may be detectable before they are detectable by the naked eye. As no previous studies have been performed on this population it is not possible to predict what gait impairments may be prevalent, but due to the multisystem nature of mitochondrial disease it is likely that gait will be affected in this population.

To summarise, gait speed can be seen to deteriorate with pathology and age but provides little indication of the mechanisms that lead to this decline. The measurement of individual gait characteristics is a more sensitive and selective method of interpreting gait impairments and is capable of identifying gait impairments not seen by observation of walking alone. Early detection of gait impairments may allow earlier intervention before compensations have been entrenched and are potentially more amenable to treatment. If these attributes are demonstrable within a mitochondrial disease population the use of gait analysis may be a valid tool for use in future clinical trials.

1.8.3 Executive summary

This review of mitochondrial disease, exercise and relevant methodology has reiterated the difficulty in researching this highly variable disease involving multiple bodily systems. The variability of genotype and phenotype expression, along with variability of symptoms and rate of disease progression, makes the evaluation of treatments in mitochondrial disease problematic. Currently no cure is available for mitochondrial disease, although on-going management of symptoms is critical to prevent unnecessary complications of disease, deterioration of function and to maintain quality of life.

Exercise therapy has been shown to be beneficial in improving the exercise capacity and quality of life of patients with mitochondrial disease and the initial concerns over its safety appear to have been allayed. The multitude of genotypes involved and the numerous primary outcomes used in exercise studies in mitochondrial patients have made comparisons between studies difficult. Previous research into the effects of exercise has involved lengthy, expensive and invasive techniques. The high participant burden of these exercise trials has led to difficulties in recruitment and retention to studies. The use of more simple and patient relevant measures may enable a greater insight into the day-to-day consequences of mitochondrial disease on our patients.

In light of this information the studies that make up this thesis aim to answer the following research questions

1. What are the effects of an aerobic exercise intervention on the exercise capacity, disease burden, quality of life and performance of functional tasks in a discreet group of mitochondrial patients?

2. Are measures of disease burden, exercise capacity and functional ability associated in mitochondrial disease?
3. Can gait characteristics discriminate between patients with mitochondrial disease, a sedentary control group and between mitochondrial genotypes?
4. Can improvements in exercise capacity be translated to improvements in gait?
5. Can evaluation of different clinical measures provide clinicians and researchers with a limited battery of measures suitable for use in research and the clinical management of mitochondrial disease?

Chapter 2 Methods and materials

2.1 Introduction

This chapter will describe the methods and materials used in the four studies included in this thesis. An outline of the method and materials used in each individual study will be included in the relevant study chapters.

The studies included in this thesis form part of a larger body of work investigating the cardiac adaptations to exercise in patients with mitochondrial disease. Participants were required to perform further investigations as part of this larger body of work, which will not be described here but did affect the inclusion criteria to the studies contained in this thesis (Bates *et al.*, 2013).

2.2 Recruitment

Participants were recruited from the MRC Centre for Neuromuscular Diseases Mitochondrial Disease patient Cohort Study (RES/0211/7552). Eligible participants had: a biopsy proven mitochondrial disease; age 18-60 (years); BMI 20-35 kg/m^2 and did not take part in regular exercise. Sixty patients were identified, sent a patient information sheet outlining the trial and invited to make contact if they wished to participate.

Healthy controls were recruited from advertisements posted throughout Newcastle University and on the University home page. Participants were asked to contact if they wished to participate in an exercise trial and were not already performing formal exercise or considered themselves sedentary.

2.3 Consent and Ethical Process

Potential recruits were given the relevant patient or participant information sheet (Appendix A) prior to attending for their initial visit. This gave participants time to

read the information and to formulate any questions to ask at their initial appointment.

During the initial visit, details of the study were explained, and any potential risks discussed. The researcher also answered any questions regarding participation in the study at this visit. If the volunteer was happy to proceed with taking part in the study and met the inclusion criteria, they were asked to sign a consent form (Appendix B). At this point, participants were made aware that their GP would be informed about their participation in the study and also, that they may withdraw at any point without affecting their future healthcare.

Institutional ethical approval (Ref 09/H0904/58) was obtained and the study complied with the Declaration of Helsinki.

2.3.1 Inclusion criteria

All eligible patients were verbally invited to participate based on the following clinical inclusion criteria: (i) clinical stability for >6 months; (ii) ability to use an upright stationary bicycle ergometer; (iii) no current participation in regular physical activity (≤1 weekly session); (iv) reported exercise intolerance and fatigue.

Sedentary controls participants (performed less than 10,000 steps or performed no formal exercise) required a normal ECG and no history of cardiovascular or metabolic disease. Control participants were matched to mitochondrial group by age, gender.

2.3.2 Exclusion criteria

Participants were excluded if they had a known cardiac involvement, co morbidities precluding exercise training, and contra-indications to Magnetic Resonance Imaging.

2.3.3 Trial design

All participants attended an initial visit over two days. This initial assessment formed the two cross-sectional studies included in this thesis (chapter 3 and 4). Following the initial visit, 12 patients with mitochondrial disease and the 11 sedentary volunteers went forward to complete a case controlled intervention (exercise) study (chapter 6 and 7).

2.4 **Initial Visit**

All assessments for the initial visit took place between 2 centres: The Clinical Research Facility, Royal Victoria Infirmary, Newcastle and the Clinical Ageing Research Unit, Newcastle University.

Both initial and final visits included numerous assessments over 2 days. The following section will describe the assessments performed as part of both the cross-sectional and case controlled exercise studies reported in this thesis.

2.4.1 Physical Examination

All volunteers underwent a physical examination comprising: auscultation of the heart and lungs, evaluation of the abdomen for any abnormalities, inspection of the lower extremities for oedema and arterial pulses, inspection of the skin and assessment of reflexes.

Disease severity in patients with mitochondrial disease was assessed and disease burden established using the Newcastle Mitochondrial Disease Adult Scale (Schaefer *et al.*, 2006) and mitochondrial mutation load was derived from urinary epithelial cells (Shanske *et al.*, 2004; Whittaker *et al.*, 2009).

2.4.2 Physical Activity Readiness Questionnaire (PARQ)

The PARQ, endorsed by the American College of Sports Medicine (ACSM), includes medical history, medication, current activity levels, and identifies whether the volunteer feels there are any barriers that may prevent them from exercising.

Participants completed the PARQ, then were stratified based on medical history and current “fitness” levels into one of three risk categories: high, moderate, and low. The PARQ identified volunteers for who exercise testing required medical supervision and where intervention was inappropriate (Appendix C).

Anyone found to have contraindications to exercise or exercise testing was excluded at this point and a letter written to their GP containing the relevant findings (see *Risk Definition and Standard Operating Procedures for Exercise Testing* for further details) based on ACSM guidelines.

2.4.3 Blood samples

Fasting blood samples were analysed in a Clinical Pathology Accredited laboratory (Newcastle Upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry) for: liver enzymes (ALT, AST, GGT), lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerols, haematology), HbA1c, creatine kinase. Serum samples were collected in silica clot activator

polymer gel containing vacutainers (BD Diagnostics, Plymouth, England) - total cholesterol, triglycerols, and liver enzymes were measured using a Roche Modular P and test kits (Roche Diagnostics Ltd, Burgess Hill, UK). HbA1c was measured using a TOSOH HLC-723G7 (Tosoh Corporation, Tokyo, Japan). These results are reported as part of a thesis by Dr. M. Bates to investigate the cardiac adaptions in mitochondrial disease.

2.4.4 Cognitive Testing

Cognitive function was tested using the revised Addenbrookes Cognitive Examination (ACE-R). The ACE-R takes between 12 and 20 min to administer and score in a clinical setting. It contains 5 sub-scores, each one representing one cognitive domain: attention/orientation, memory, fluency, language and visuospatial ability. The ACE-R maximum score is 100, calculated by the addition of the all domains. A score below 88 was indicative of mild cognitive impairment (Eneida *et al.*, 2006).

2.4.5 Anthropometry

Height was measured without shoes and using a standard rigid stadiometer to the nearest 0.1cm. Weight was measured to the nearest 0.2kg on calibrated scales without shoes (Seca medical 769 column scale, USA). Body mass index was calculated (weight divided by the square of the participant's height).

2.4.6 Whole Body Composition

Whole body composition was determined using air displacement plethysmography using a BodPod (Life Measurement Inc., Concord, CA, USA) (Figure 2-1). This was calibrated before each measurement using the known

calibration standard. The technique has been validated against the reference standard of hydrostatic weighing, dual-energy X-ray absorptiometry and bioelectrical impedance in healthy and overweight/obese adults (Sardinha *et al.*, 1998; Biaggi *et al.*, 1999; Fields DA *et al.*, 2005). All patients had their body composition measured whilst fasted, wearing tight fitting underwear and the provided lycra cap. This minimised the effects of hydration/recent food intake and air trapping respectively. The manufacturers indicate that the general error range of the Bodpod is 1-2% (the same as hydrostatic weighing).

Figure 2-1: Image of Bodpod used to measure body composition

2.4.7 Measurement of resting substrate metabolism

Following an overnight fast (≥ 8 hours with no food or beverages) resting substrate oxidation was determined by expired gas analysis (CORTEX Biophysik, Leipzig, Germany) using a Hans Rudolf breathing mask while participants lay supine for 30 minutes in a quiet room, without speaking or sleeping and with minimal movement. The calorimeter gas analysers were calibrated before every measurement for gas volume and composition, and

ambient air pressure. Twenty minutes of recording were used to calculate substrate oxidation from oxygen consumption and carbon dioxide production values using stoichiometric equations

VO_2 (l/min) = 0.746c + 2.03f + 6.04n

VCO_2 (l/min) = 0.746c + 1.43f + 4.89n (Frayn, 1983).

Where c is grams of carbohydrate oxidised per minute, f grams of fat per minute and n is grams of urinary nitrogen excreted per minute (Frayn, 1983).

2.4.8 Maximal Aerobic Capacity

The gas analysis system (Metalyzer 3B; Cortex, Leipzig, Germany) was allowed to warm up for a minimum of 2 hours before the test; calibration (volume and gas) was undertaken on the day of each test and documented.

A progressive exercise test was undertaken to assess peak oxygen consumption and work rate, anaerobic threshold and oxygen extraction, using an electronically braked recumbent cycle ergometer (Corival Lode BV, Groningen, The Netherlands).

Volunteers underwent a resting 12-lead electrocardiogram (ECG; Custo med GmbH, Ottobrunn, Germany) and blood pressure monitoring (Suntech Tango+, Suntech Medical Ltd, Oxford, UK) in a recumbent seated position, to measure normal cardiac function prior to, and throughout, the exercise test. An ECG was used to continuously monitor heart rhythm, and blood pressure was measured every 2 minutes during the exercise test. Both ECG and BP were monitored for at least 5 minutes after the exercise test had been terminated.

Exercise protocols used differed for control participants and patients with mitochondrial disease to allow all participants to achieve a VO_{2PEAK} within ≈ 15 minutes of the onset of the exercise test.

Control protocol

After a 5 minute warm up at 25W, resistance was increased by 1W every 8 seconds until the participant could no longer maintain a cadence of 60 rpm, chose to stop, or continuing was contraindicated (*Risk Definition and Standard Operating Procedures for Exercise Testing* for further details).

Mitochondrial protocols

Patients with MD performed one of three exercise protocols (Table 2-1). The protocol performed was dependent upon subjective assessment of the participant's physical function. Three protocols were necessary due to the variability in the abilities of subjects with mitochondrial disease. These protocols were based on protocols used in a previous exercise study; Exercise training in patients with mitochondrial disease: Assessing the benefits (unpublished).

The same protocol was repeated in all progressive exercise tests performed by that individual. The test was terminated under the same circumstances as the control subjects. A member of the MRC Muscle Performance and Exercise Training Laboratory conducted exercise testing. All staff members were certified to undertake exercise tests and Cardiopulmonary Resuscitation.

Table 2-1: Progressive exercise test protocols for participants with mitochondrial disease.

Time (minutes)	Work Rate (Wattage)		
	Low	Medium	High
0	0	40	90
1	5	45	95
2	10	50	100
3	15	55	105
4	20	60	110
5	25	65	115
6	30	70	120
7	35	75	125
8	40	80	130
9	45	85	135
10	50	90	140
11	55	95	145
12	65	100	150
13	70	105	155
14	75	110	160
15	80	115	165
16	85	120	170
17	90	125	175
18	95	130	180
19	100	135	185
20	105	140	190

2.4.9 Cardiac output estimates

Cardiac output was measured at rest and during exercise testing continuously using bio-reactance techniques (NICOM, Cheetah Medical, Deleware, USA). Four dual-surface electrodes were used to establish electrical contact with the body; two were applied over the trapezius muscle on either side of the upper torso and two on the lower posterior torso lateral to the margin of the latissimus dorsi muscle (Jakovljevic *et al.*, 2012, ; Jones *et al.*, 2015). Electrode

connections were secured with medical tape and all connecting wires supported to ensure minimal movement artefact.

Cardiac output was then calculated as the product of stroke volume and heart rate. Arterial-venous difference was calculated theoretically and cardiac output was determined for a given oxygen uptake using the equation previously suggested by Stringer and colleagues (Stringer *et al.*, 1997).

2.4.10 Physical Activity

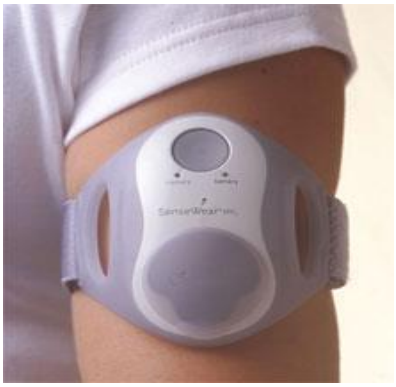
Physical activity and energy expenditure were assessed objectively using a validated multi-sensor array (SenseWear Pro$_3$, Bodymedia Inc, Pennsylvania, USA 2.5) (St-Onge *et al.*, 2007; Moore *et al.*, 2012). Participants were asked to wear the armband on their right upper arm (at the mid-humerus point on the triceps) for seven days (Figure 2-2). All subjects were instructed to remove the armband only for bathing/showering purposes or any water activity.

The armband uses a 2-axis accelerometer, a heat flux sensor, a galvanic skin response sensor, and a near-body ambient temperature sensor to capture data. The participants' date of birth, height, body weight, sex, hand dominance and smoking status (smoker or non-smoker) were also used by the device to calculate energy expenditure.

The armband produced the following data as units per day: total energy expenditure; active energy expenditure; average metabolic equivalents (METs); duration of sedentary time (≤ 2.9 MET); duration of physical activity (> 3.0 MET); duration of moderate physical activity (3.0-5.9 MET); duration of vigorous activity (6.0-9.0 MET); duration of very vigorous activity (≥ 9.0 MET); number of

steps; sleep duration; and duration armband worn. This data was then averaged over the seven days worn to provide daily activity levels.

Figure 2-2: An image of a Sensewear activity monitor.



Participants also completed the International Physical Activity Questionnaire (IPAQ) for the same seven days the SenseWear armband was worn. The IPAQ asks participants to recall their physical activity over the previous seven days. The IPAQ includes four activity domains: work-related physical activity, transportation, household, recreation and leisure time activity and also reports time spent sitting. The IPAQ was scored using guidelines produced by the IPAQ Group (www.ipaq.ki.se/scoring.pdf). The IPAQ has been demonstrated to have comparable levels of validity to other physical activity self-report measures (Craig CL *et al.*, 2003) (Appendix D).

2.4.11 Muscle strength testing

Hip flexor and extensor strength was measured using an isokinetic dynamometer (CSMI HUMAC®/NORM testing and rehabilitation system). Calibration of the CSMI Norm system was done as stated in the user manual; 100lbs of weight were dropped with the arm at a pre-set length to verify the accuracy of torque measures. Calibration was done preceding each test and recorded in a calibration log.

Testing was performed in a supine position with the participant stabilized with a waist and torso strap. The axis of rotation was aligned with the participant's greater trochanter of the femur of the test leg. The lever arm was positioned on the lower third of the quadriceps just superior to the patella. The starting position was recorded, noting position of the bed along with the position and length of the dynamometer arm. This starting position was repeated on any subsequent visits (Julia *et al.*, 2010).

A concentric hip flexor/extensor pattern was chosen at a velocity of 60 degree/sec. The range of motion of hip movement was set for each individual participant. Participants underwent 6 sub-maximal contractions to familiarize themselves with the range of movement followed by a minute's rest. To prevent the participant from sliding up the bed, the tester provided further stabilization at the participant's shoulders. The participant then performed six maximal repetitions with consistent verbal encouragement. Both left and right sides were tested with a minutes rest between each test. Peak torque measurements were recorded for hip flexion and hip extension movements at the end of the test.

No dynamometry testing was performed within a few hours of the maximal cycle test and preferably testing was performed on a separate day. Due to the extensive testing included in visits, dynamometry was not always performed at the same time of day (Guette *et al.*, 2005).

2.4.12 Functional Outcome Measures

All functional outcomes and gait measurements were performed at the same time in the gait laboratory in the Clinical Aging Research Unit. The short "10m walks" (single and dual task conditions); "Timed up and go" and "Sit to stand"

tests were completed in a counterbalanced order (Appendix E). The 6-minute walk always occurred last in the testing session to avoid fatigue affecting other measures.

Five times sit to stand (5XSTS)

Using a standard chair (height 43 – 46 cm) the participant was timed (seconds) completing the task of sitting to standing 5 times.

The procedure was explained to the patient prior to testing. Participants were asked to: cross both arms across their chest, begin in a seated position, stand up (knees fully extended) and sit down (full weight through chair). Instructions to the participant were: *"I want you to stand up and sit down 5 times as quickly as you can when I say 'Go'"* (Lord *et al.*, 2002; Whitney *et al.*, 2005). Participants were informed to begin and the timer was started, the timer was stopped on the return to the seated position following the fifth sit to stand movement.

Timed up and Go Test (TUG)

The timed up and go (TUG) test measures, in seconds, the time taken by an individual to stand up from a standard armed chair (approximate seat height 43–46cm), walk at a comfortable speed a distance of 3 meters, turn, walk back to the chair and sit down again (Podsiadlo D *et al.*, 1991).

Instructions to the participant *"When I say 'go' I want you to stand up and walk to the line, turn, walk back to the chair and sit down again. Walk at your normal pace."*

Participants were able to use the arms of the chair to assist them when standing. Regular footwear was used and they were able to use their customary walking aid. However no physical assistance was given. The patient walked through the

test once before being timed in order to familiarise themselves with the procedure.

The time taken for the patient to complete the test (i.e. from the word ‘go’ until the patient was seated back in the chair) is recorded in seconds using a stopwatch. The test was completed three times, whereby the mean of the three performances was recorded.

10 meter timed walk (10MTW)

Participants were asked to walk a 10m distance; walking speed was tested at the subject’s comfortable speed and three repetitions were carried out. The participant was able to walk without assistance of another person, although use of a walking aid was permitted if necessary (Wade, 1992).

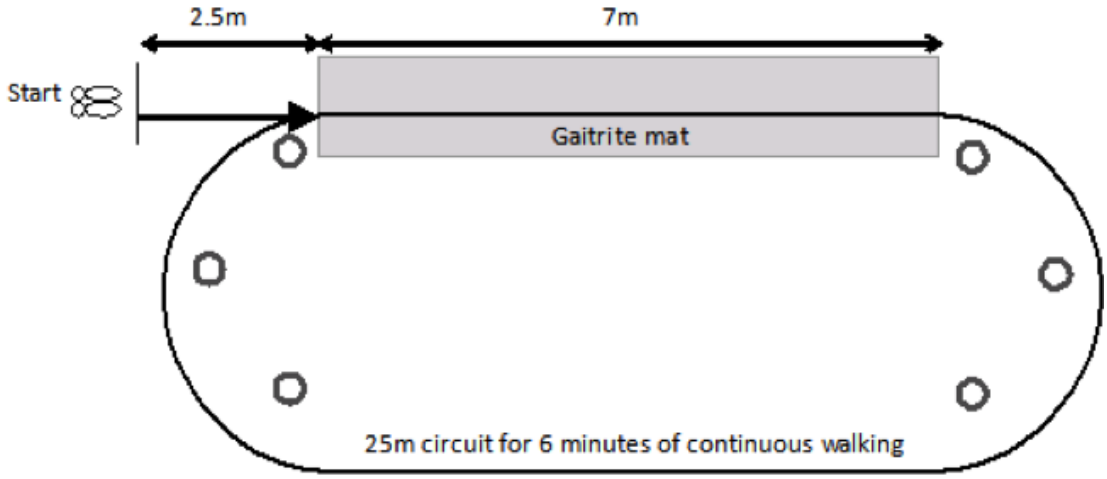
Six Minute Walk Distance (6MWD)

The patient sat in a chair located near the start position for at least 10 minutes before starting the test to allow for recovery from previous testing.

Participants were instructed as follows: *“The object of this test is to walk as far as possible for 6 minutes. You will walk around the course marked* (Figure 2-3). *Six minutes is a long time to walk so you will be exerting yourself. You may get out of breath or become exhausted. You are permitted to slow down, to stop and to rest as necessary. You may lean against the wall while resting or sit down on the chair, but resume walking as soon as you are able.*

You will be walking back and forth around the cones; you should not hesitate as you walk around the cones. Now I am going to show you”.

Figure 2-3: Gait laboratory set up for 6-minute walk.



A lap of the walking course was demonstrated. This was followed by: *"Are you ready to do that? I am going to keep track of the number of laps you complete. I will tick a box each time you pass this starting line. Remember that the object is to walk AS FAR AS POSSIBLE for 6 minutes, but don't run or jog. Start now or whenever you are ready".* This test was based upon "American Thoracic Society Statement: Guidelines for the Six-Minute Walk Test." (2002).

The therapist notified the participant when each minute had elapsed**.** When six minutes had elapsed on the stopwatch the participant was requested to stop and the distance walked was recorded.

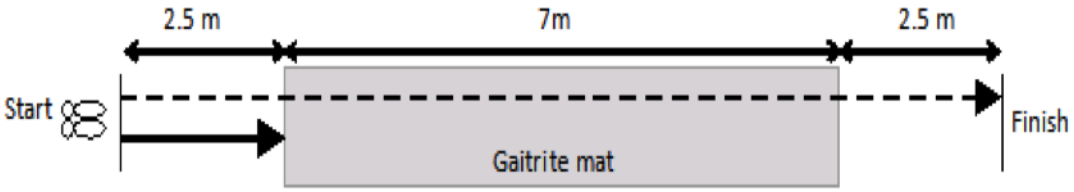
2.4.13 Assessment of Gait

Gait was assessed using a 7m long × 0.6m wide instrumented mat (Platinum model Gaitrite, software version 4.5, CIR systems, USA). Previous studies have verified the Gaitrite mat to be a valid and reliable method for measuring mean gait characteristics in young and old adults with and without pathology (Menz *et*

al., 2004; Kuys *et al.*, 2011). Data for individual steps for each condition was extracted from the Gaitrite database using Microsoft Access 2007.

Participants were asked to perform 3 different walking tasks (intermittent walks ± dual task and a continuous walk over 6 minutes). During the intermittent walks participants were instructed to perform three 12m walks at their "comfortable pace". The mat was placed in the centre of the 12 m walkway to ensure that steady gait speed was measured (Figure 2-4).

Figure 2-4: Gait laboratory set up for testing single and dual walks.



The concurrent cognitive task for the dual task condition consisted of a digit span task. The participant listened to a string of digits and repeated them back whilst walking. The strings of digits consisted of a range of pre-recorded random strings of digits spoken at a cadence of one digit per second. The length of the strings of digits played during walking was assessed prior to testing with the participant in a seated position. In sitting strings of an increasing number of digits were played (starting from strings of 2 digits) until the participant failed to correctly recall two strings of the same length.

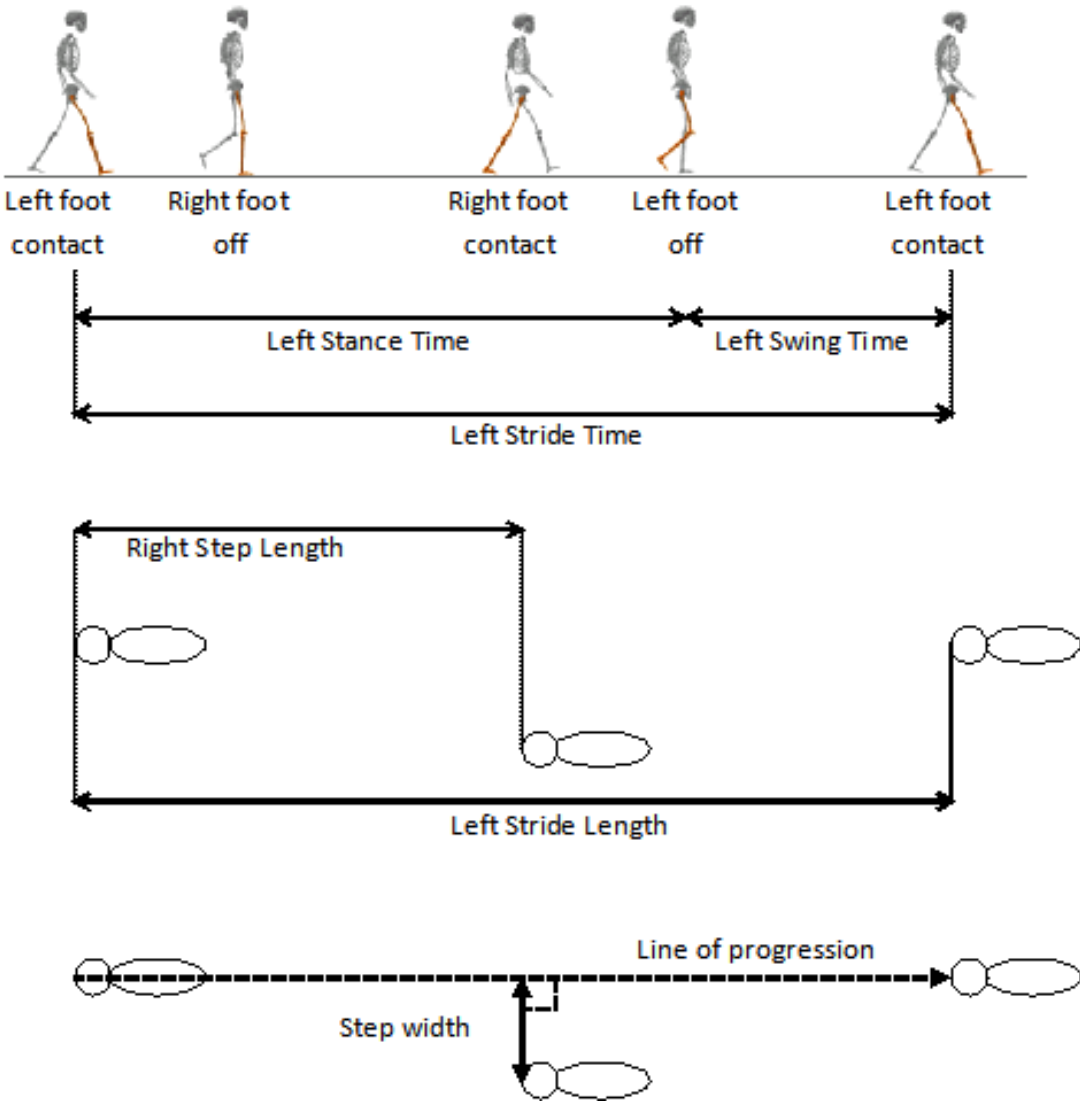
The continuous walks involved participants walking continuously around a 25m oval circuit, instructions provided were as stated above for the 6 minute timed walk, where participants were encouraged to walk as far as possible for 6 minutes and were provided with prompts every minute (Society, 2002). Gait

characteristics were repeatedly recorded as they walked over a mat placed on one side of the circuit (Figure 2-3).

Gait was quantified for all three walking tasks (single task, dual task and continuous walks) using a predefined model with five domains hypothesized to reflect independent features of neural control and characterise the features of gait associated with mitochondrial disease and its genotypes (Lord *et al.*, 2013b). This model included: pace (step velocity and step length); rhythm (cadence); variability (step time and step length variability); asymmetry (step time asymmetry); and postural control (step width, step width variability, step length asymmetry) (Figure 2-5).

Gait variability, otherwise known as gait dynamics, was measured to characterise the step-to-step fluctuations over the walking task. Gait variability was calculated for step length, step time, step width (Galna *et al.*, 2013a).

Figure 2-5: Illustration of step length; step width and step timing characteristics measured in this study.
Step velocity (walking speed) was also measured (calculated as step length divided by step time).



To avoid confounding variability with gait asymmetry variability was calculated as the combined within-persons variance of the left and the right steps as in equation 1 (Galna *et al.*, 2013a). Hence the Standard Deviation of Left & Right steps was the combined within-person standard deviation calculated from the left steps and the right steps. The variance of left steps and right steps was calculated separately for each person.

Equation 1 $$SD_{\text{Left \& Right}} = \sqrt{\frac{(Variance_{Left\ Steps} + Variance_{Right\ Steps})}{2}}$$

Asymmetry was calculated as the absolute difference between the left and right step length and step time.

2.5 **Aerobic training intervention**

Upon completion of baseline tests, 24 participants undertook 16 weeks of aerobic exercise therapy (13 patients with MD and 12 sedentary controls). Exercise sessions were conducted at a local gym or on a recumbent bike at home. Each session consisted of working towards 45 minutes recumbent cycling at a heart rate corresponding to ≈ 60-70% VO_{2PEAK} and were performed 3 times a week (48 sessions in total). The intensity of exercise sessions were tailored to the individual and increased progressively over the 16 weeks. Stretches were performed prior to exercising and gentle upper body exercises using resistance bands were included as part of the training schedule. During each session a heart rate monitor was worn (RS400, Polar, Finland) and the exercise was recorded in an exercise diary.

2.6 **Interim Visit**

At 8 weeks a maximal progressive exercise test was repeated using the same protocol as the initial test. The exercising heart rate range was reassessed and altered as required (to remain at 70% of VO_{2PEAK}). Individual exercise goals were reset and the importance of adherence to the program emphasised.

Data from the Polar heart rate monitors was downloaded and matched against reported exercise sessions recorded in an exercise diary. 80% exercise session adherence was required. Monitored via diary and downloaded data from POLAR watch and weekly telephone calls.

Participants with mitochondrial disease provided another blood sample to check the Creatine Kinase (CK) level.

2.7 **Final Visit**

All the assessments performed at the initial visit were repeated at the final visit except assessment of physical activity (Sensewear, IPAQ). Every attempt was made to repeat the timings of the initial visit. Exercise diary and POLAR watch were retrieved from participants.

Chapter 3 The use of physiological and functional measures in mitochondrial disease.

3.1 **Introduction**

Mitochondrial diseases are a group of neuromuscular disorders, which affect up to 8,000 adults in the UK (Schaefer *et al.*, 2008). Recording the natural history and evaluating interventions in this group of diseases has proved problematic due to multi-system involvement and the lack of correlation between mitochondrial genotype and phenotype. A recent Cochrane review (Pfeffer *et al.*, 2012) commented on the multitude of primary outcomes used in studies of mitochondrial disease and how this has made comparison between studies difficult. The review advised that future studies should investigate sub-groups of mitochondrial diseases and include the use of outcomes that have clinical meaning for patients and their quality of life.

The purpose of this study is to evaluate the relationships between biological, clinical and functional measures by assessing disease burden, exercise capacity and functional ability within two specific mitochondrial genotypes. Currently disease severity can be determined by, i) levels of mitochondrial DNA mutation found in tissues such as muscle (Chinnery *et al.*, 1997) or urine (McDonnell *et al.*, 2004; Shanske *et al.*, 2004; Whittaker *et al.*, 2009) and ii) clinically by using the Newcastle Mitochondrial Disease Adult Scale (NMDAS) (Schaefer *et al.*, 2006). These investigations can be invasive, time consuming and costly. Previously the exercise capacity of patients with mitochondrial disease has been used as a proxy measure of mitochondrial function (Jeppesen *et al.*, 2003; Taivassalo *et al.*, 2003; Tarnopolsky, 2004). Functional outcome measures are quick and inexpensive to deliver and are commonly used in clinical practice by therapists (Yoward *et al.*, 2008). More recently functional outcome measures have been adopted by the research community to measure many aspects of disease such as; disease severity, natural history, prediction of

future levels of disability and the effects of interventions in neurological and neuromuscular populations (Takeuchi *et al.*, 2008; Ries *et al.*, 2009; McDonald *et al.*, 2010).

Demonstration of relationships between these three types of assessment (disease severity, exercise capacity and functional ability) may enable professionals to use simple functional outcome measures in the clinical setting as potential proxy measures of disease burden. The wider use of outcome measures in clinical practice will provide more detailed information concerning the natural history of mitochondrial diseases and reflect how our patients' daily lives are affected by mitochondrial disease.

3.2 **Study aim and hypothesis**

Aim

The aim of this study is to assess the usefulness of physiological and functional measures in mitochondrial disease.

Primary hypothesis

Physiological and functional measures will be able to discriminate between mitochondrial patients and a sedentary control group.

Secondary hypotheses

Exercise capacity will be impaired within a discreet group of mitochondrial patients (two genotypes m.3243A>G, m.8344A>G) when compared to a sedentary control group.

Measures of exercise capacity and functional ability will demonstrate relationships to disease severity in mitochondrial disease.

Dynamometry will be able to detect proximal muscle weakness in a group of mitochondrial patients.

3.3 **Subjects and Methods**

Full descriptions of the subjects and methods used in this study are contained within Chapter 2. A brief outline of methods and subjects included in this cross-sectional study are described below.

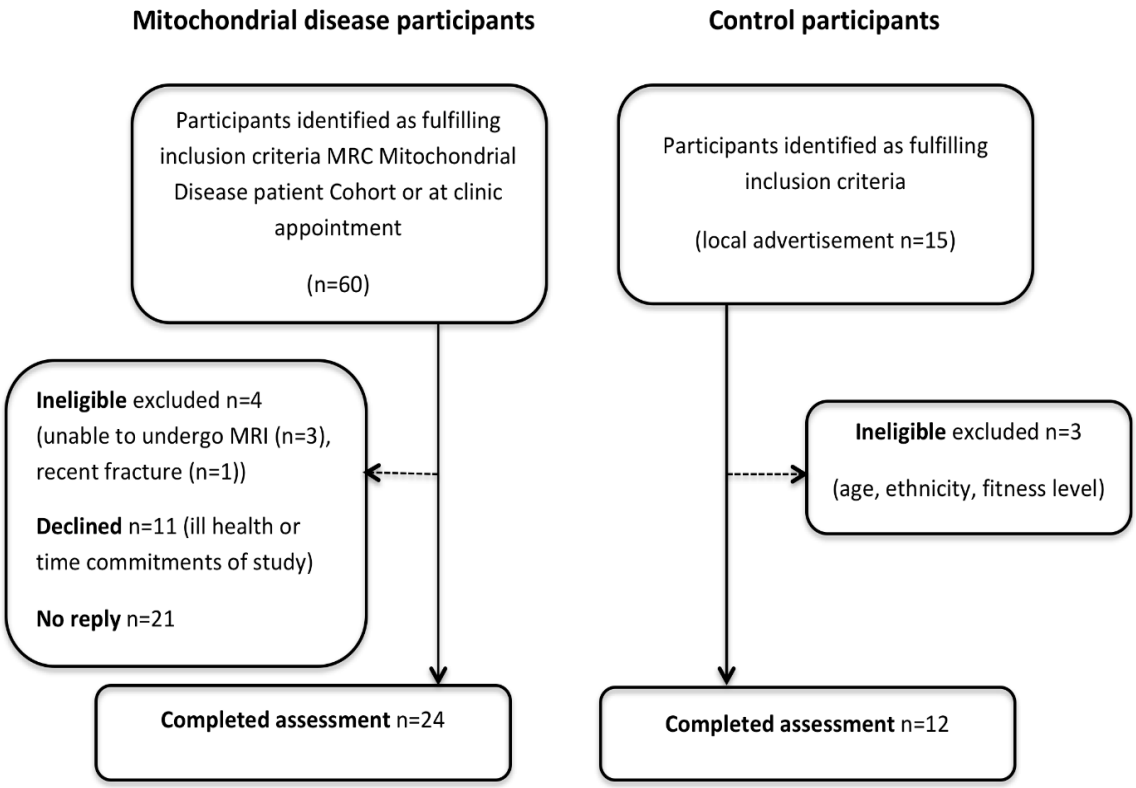
Twenty-four patients with mitochondrial disease (MD) clinically identified and laboratory confirmed as due to the m.3243A>G or m.8344A>G mitochondrial DNA mutation were recruited from the Newcastle Mitochondrial Disease Cohort. Twelve untrained healthy controls with normal ECG and no history of cardiovascular or metabolic disease were recruited through local advertisement (Figure 3-1).

Institutional ethical approval (Ref 09/H0904/58) and written informed consent were obtained and the study complied with the Declaration of Helsinki.

All assessments were undertaken between the two centres listed in the methods section over a period of two days. This was necessary due to the distance required to travel to the centres by some of the participants with mitochondrial disease. Over the two days both disease and control groups underwent assessment of resting metabolism, peak exercise capacity, proximal muscle strength (hip flexors, hip extensors) and lower limb function and were provided with a Sensewear activity monitor to assess physical activity.

In addition the disease burden and cognition of participants with mitochondrial disease was assessed, whilst sedentary control volunteers underwent a review of their medical history with a physical examination and completed the Physical Activity Readiness Questionnaire prior to exercise testing (Appendix C).

Figure 3-1: Consort diagram for the study of physiological and functional measures in mitochondrial disease.



3.3.1 Disease Burden

All Subjects underwent a physical examination by an experienced clinician, disease severity was assessed and disease burden was established using the Newcastle Mitochondrial Disease Adult Scale (Schaefer *et al.*, 2006). Mutation load, an established marker of clinical outcome was derived from urinary epithelial cells (McDonnell *et al.*, 2004; Shanske *et al.*, 2004; Whittaker *et al.*, 2009).

3.3.2 Physical Activity Levels

Physical activity and energy expenditure were assessed objectively for seven days using a validated multi-sensor array (SenseWear Pro_3, Bodymedia Inc, Pennsylvania, USA 2.5) (St-Onge *et al.*, 2007).

Participants also completed the International Physical Activity Questionnaire (IPAQ) (Hagströmer *et al.*, 2006) (Appendix D) for the same seven days that the SenseWear armband was worn.

3.3.3 Anthropometry

Body weight (kg) and standing height (m) were measured. Whole body composition was determined using air displacement plethysmography using a BodPod (Life Measurement Inc., Concord, CA, USA).

3.3.4 Resting Substrate metabolism

Following an overnight fast, resting substrate oxidation was determined by expired gas analysis (CORTEX Biophysik, Leipzig, Germany) using a Hans Rudolf breathing mask while participants lay supine for 30 minutes in a quiet room.

3.3.5 Progressive Exercise testing

A peak exercise test was performed; a number of parameters including peak exercise capacity (VO_{2PEAK}), anaerobic threshold, peak workload and systemic arteriovenous oxygen difference (a-vO_2 diff) were measured.

3.3.6 Muscle Strength

Proximal muscle power was measured using isokinetic dynamometry of the hip flexor and extensor muscles. Average peak torque was calculated for each muscle group (Julia *et al.*, 2010).

3.3.7 Functional outcome measures

Four functional outcome measures were performed to assess functional ability: Six-minute walk distance (6MWD) (American Thoracic Society Statement, 2002); 10 metre timed walk (10MTW) (Wade, 1992), Timed up and go (TUG) (Podsiadlo D *et al.*, 1991) and the 5 times sit to stand tests (5XSTS) (Lord *et al.*, 2002).

3.3.8 Cognitive Ability

Cognitive function was tested using the revised Addenbrookes Cognitive Examination (ACE-R) (Appendix F). A score below 88 was used as an indication of mild cognitive impairment (Eneida *et al.*, 2006).

3.3.9 Quality of Life

Quality of life was assessed using the short form (SF12) health survey, which already forms part of the Newcastle Mitochondrial Disease Adult Scale (Schaefer *et al.*, 2006).

3.4 **Statistical Analysis**

Differences between groups were calculated by an unpaired student t test or the Mann-Whitney U test as deemed appropriate. Significant statistical differences were assumed for *p* values < .05 and are labelled *, *p* values < .01 are labelled**. Associations between parameters were analysed by Spearman or Pearson's correlation dependant on assessment for normality. To allow for multiple comparisons statistical significance was assumed for *p values* <. 01 and are labelled**. Data is presented as means ± SD for continuous data and as numbers or percentages for categorical data unless otherwise stated.

All statistical analysis was carried out using SPSS version 20 (SPSS Inc. Chicago, IL, USA).

3.5 **Results**

All participants attended for all assessments. Three mitochondrial patients were unable to complete dynamometry testing due to ill health at time of testing (diarrhoea, migraine and a negative response to an exercise test earlier in day).

The following sections will report the clinical characteristics of the mitochondrial group followed by the physical activity levels, exercise capacity and functional ability of both mitochondrial and control groups. Finally relationships between disease severity, mutation load and the physiological and functional measures used in the study are reported.

3.5.1 Characteristics of participants

No significant differences are seen between the groups for age (years) (Mitochondrial Disease (MD) group 41 ± 13, Control group 37 ± 12, *p*= .407), height (cm) (MD group 169 ± 10, Control group 172 ± 10, *p*= .392) and gender (MD group 14 male, 10 female, Control group 7 male, 5 female). Body mass (kg) and BMI (kg/m^2) were significantly lower in participants with mitochondrial disease than controls (MD 63.9 ±15, Control 80.2±15, *p*= *.005***; MD 22 ± 5, Control 27 ± 5, *p*= *.009***).Mitochondrial participant's clinical features, NMDAS scores and mutation load are also shown in Table 3-1.

Participants with the m.3243A<G mutation had a lower disease burden than the m.8344A<G group (NMDAS 16 ± 8 vs. 39 ± 17, *p*= *.023**; mutation load (%) 65 ± 18 vs. 88 ± 9, *p*= *.001***). Clinical presentations within the mitochondrial disease group varied considerably with a wide range of symptoms being reported. Six mitochondrial patients showed cognitive impairment when measured using the ACE-R (scored <88).

Table 3-1: Demographic and clinical details of participants with mitochondrial disease.

Age	Sex	Height (cm)	Mass (kg)	BMI (kg/m^2)	Heteroplasmy (%)	FVC (l)	Cognitive score (ACE-R)	Principle clinical features
m.3243A>G								
58	F	173	52.1	17.4	59	2.6	98	Hearing loss, ataxia, constipation, underweight, myopathy, myalgia, exercise intolerance
39	M	172	74.6	25.2	80	5.0	93	Hearing loss, diabetes, migraine, fatigue, hypothyroidism, ataxia, constipation, exercise intolerance
37	F	164	50.7	18.9	48	2.8	91	Hearing loss, diabetes, exercise intolerance, ataxia, dysarthria, asthma
42	M	176	83.7	27.0	82	4.0	90	Hearing loss, diabetes, migraine, ataxia, depression, exercise intolerance
47	M	182	64.0	19.3	63	3.1	94	Hearing loss, diabetes, fatigue, ataxia, myalgia, depression, myopathy, neuropathy, ptosis, PEO
38	F	164	54.2	20.1	53	2.6	97	Hearing loss, migraine, constipation, myopathy, fatigue, exercise intolerance, asthma
22	M	183	59.2	17.7	89	4.2	94	Hearing loss, migraine, epilepsy, ataxia, constipation, underweight, exercise intolerance, fatigue
36	M	179	72.4	22.6	80	4.8	95	Migraine, exercise intolerance
50	M	162	64.6	24.6	87	3.3	66	Hearing Loss, exercise intolerance, ataxia, myopathy, fatigue, depression, retinopathy, epilepsy, encephalopathy, cognitive decline, stroke-like episodes
55	F	153	45.8	19.5	68	2.5	97	Hearing loss, diabetes, myopathy, exercise intolerance, ataxia, constipation, depression, retinopathy, PEO, ptosis, short stature, mild dysphagia, hypertension
42	F	154	49.0	20.7	43	2.6	90	Hearing loss, diabetes, ataxia, constipation, depression, exercise intolerance, short stature
50	F	169	63.4	22.2	34	4.0	96	Migraine, fatigue, hypothyroidism, myalgia, constipation, hypertension, dyslipidemia, coeliac disease

58	M	186	89.8	25.9	66	2.6	87	Hearing loss, diabetes, ataxia, retinopathy, constipation, hypertension, dysarthria, myopathy, neuropathy, exercise intolerance
53	F	164	59.1	22.0	22	3.6	92	Hearing loss, ataxia, retinopathy, constipation, myopathy, exercise intolerance
25	M	174	49.8	16.5	90	3.4	89	Hearing loss, migraine, ataxia, retinopathy, constipation, depression, dysarthria, myopathy, exercise intolerance, short stature, asthma
18	F	154	39.5	16.7	59	2.6	86	Hearing loss, retinopathy, underweight, myopathy, exercise intolerance, short stature
24	F	155	64.0	26.7	72	3.3	89	Hearing loss, migraine, ataxia, retinopathy, constipation, depression, dysarthria, myopathy, exercise intolerance, short stature
55	M	179	104.5	32.6	76	2.5	97	Hearing loss, diabetes, depression
m.8344A>G								
28	M	173	51.7	17.3	95	4.2	71	Hearing loss, fatigue, epilepsy, ataxia, retinopathy, constipation, dysarthria, myopathy, myoclonus, neuropathy, exercise intolerance, underweight
25	M	176	68.0	22.0	94	4.6	n/a	Epilepsy, ataxia, retinopathy, depression, dysarthria, exercise intolerance, myopathy, myoclonus
46	M	163	70.9	26.7	77	3.6	88	Mild concentric LVH, lipomata, exercise intolerance, dysphagia, myoclonic jerks, ataxia, seizures, neuropathy, fasciculation's, constipation, hearing loss, myopathy, parenchymal lung disease
59	F	162	69.8	26.6	75	3.0	98	Myoclonus, deaf, diabetes, lipomata, myopathy, mild concentric LVH
28	M	180	56.2	17.3	93	4.9	85	Hearing loss, migraine, epilepsy, ataxia, constipation, underweight, depression, dysarthria, myopathy, neuropathy, exercise intolerance
38	M	160	76.5	29.9	94	1.6	67	Lipomata, hearing loss, migraine, epilepsy, ataxia, retinopathy, depression, dysarthria, myopathy, myoclonus, neuropathy, exercise intolerance, short stature

FVC -Forced vital capacity

Quality of life scores were lower in the mitochondrial sample compared with the control group when measured using the SF12 questionnaire, physical composite score (PCS) (MD group 38 ± 5 vs. control 55 ± 12, $p< .001^{**}$), mental composite score (MCS) (MD group 42 ± 12 vs. control 47 ± 12, $p= .186$)

Activity levels were significantly different between the groups when measured using Sensewear activity monitors despite attempts to match activity levels, participants with mitochondrial disease expending less energy and performing less steps daily. Whereas this difference was not replicated using the self-report physical activity questionnaire (IPAQ), participants with mitochondrial disease reporting that they performed a similar level of activity when compared to the sedentary control group, but both being classed as minimally active Table 3-2.

*Table 3-2: Activity levels of control and mitochondrial disease participants. Data is reported as median (quartiles), statistically significant results are shown in bold. ** p<.01*

Variable	**Control Group**	**Mitochondrial Group**	***p* value**
Energy expenditure (calories)	2584 (2194, 2948)	2007 (1770, 2183)	**.001****
Activity level (steps per day)	9296 (7455, 12123)	5924 (2979, 7533)	**.005****
International Physical Activity Questionnaire (Metabolic equivalents/min/week)	2504 (901, 4933)	1822 (969, 10413)	.880

3.5.2 Resting metabolism

Resting substrate oxidation showed no significant difference between the two groups (Table 3-3).

Table 3-3: Resting substrate oxidation of both participants with mitochondrial disease and controls.

Variable	MD group	Control group	*p* value
Ventilation (l)	7.7 (2.4)	7.5 (1.7)	.771
Oxygen consumption ($\dot{V}O_2$) (l.min)	0.27 (0.07)	0.28 (0.06)	.286
Carbon Dioxide consumption ($\dot{V}CO_2$) (l.min)	0.23 (0.07)	0.26 (0.05)	.438
Carbohydrate oxidation (mg.kg.min)	2.8 (1.5)	2.9 (0.9)	.875
Lipid oxidation (mg.kg.min)	1.1 (0.6)	0.8 (0.3)	.057
Respiratory Quotient	0.87 (0.08)	0.86 (0.19)	.745

3.5.3 Exercise capacity

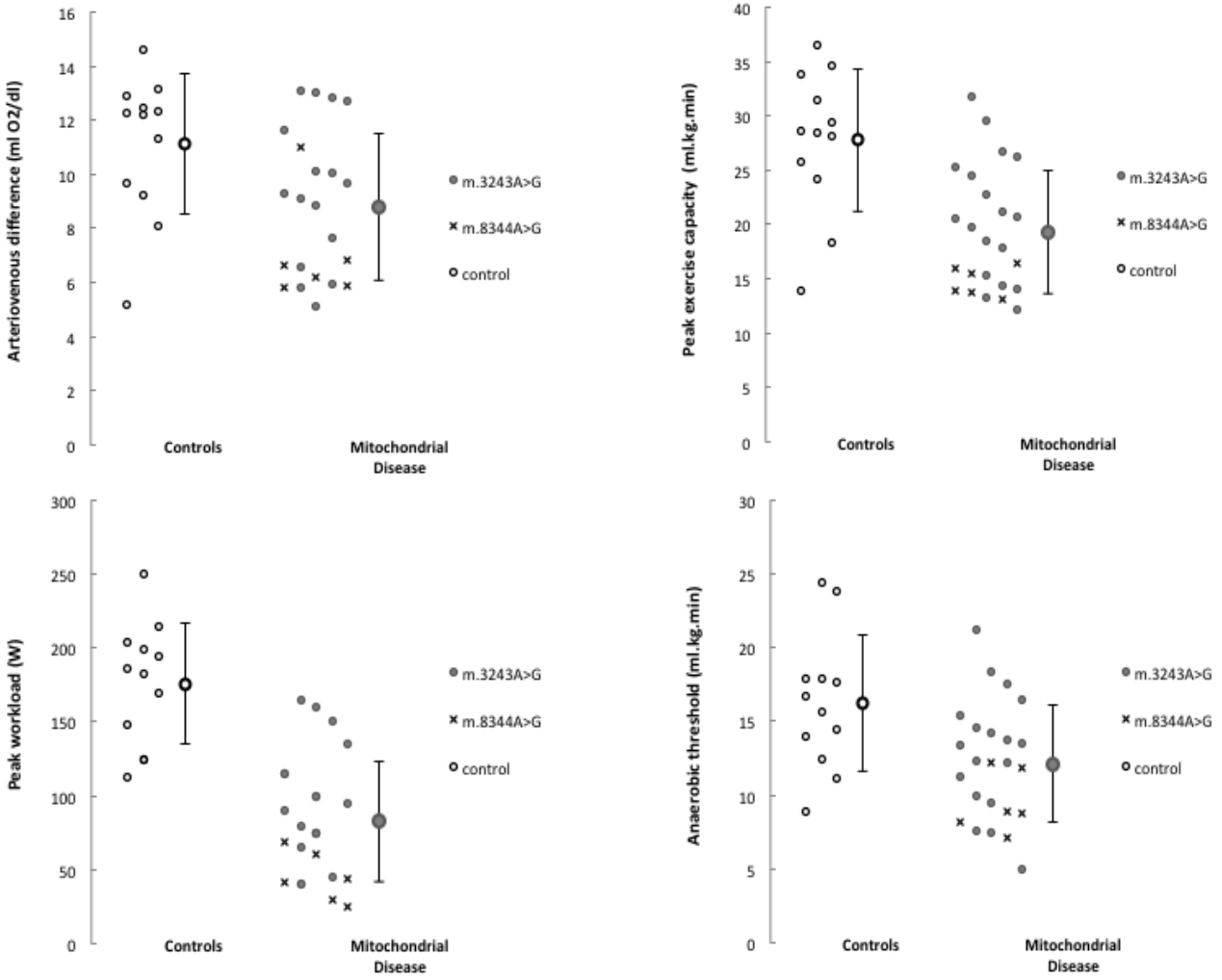
Exercise parameters measured during a peak exercise test for both groups are shown in Table 3-4 and Figure 3-2. Arteriovenous oxygen difference was only calculated in twenty-two mitochondrial patients due to a failure to capture cardiac output (NICOM) data on two participants.

All exercise parameters demonstrated significant differences between the two groups ($p < .05$), with peak exercise capacity ($\dot{V}O_{2PEAK}$) and the anaerobic threshold (AT) in mitochondrial participants being 31% and 26% lower than in sedentary controls and peak workload being 42% lower.

Table 3-4: Exercise variables of mitochondrial patients and controls from a peak exercise test.
*Data is reported as means (standard deviations). ** p<.01, * p<.05*

Variable	**Control group**	**Mitochondrial group**	***p* value**
Peak Heart rate	186 (16)	155 (23)	< .001**
$\dot{V}O_2$ (l/min)	2.2 (0.7)	1.2 (0.5)	< .001**
Peak exercise capacity ($\dot{V}O_{2PEAK}$) (ml.kg.min.)	27.9 (6.6)	19.3 (5.7)	< .001**
% predicted $\dot{V}O_2$ achieved	92% (20)	58% (21)	
Anaerobic Threshold (AT) (ml.kg.min)	16.3 (4.62)	12.1 (4.0))	.006**
AT as % of predicted $\dot{V}O_{2MAX}$	54% (13)	36% (15)	
Peak workload (watts)	176 (41)	83 (41)	< .001**
Peak workload (watts/kg)	2.2 (0.5)	1.3 (0.5)	< .001**
Arteriovenous difference (mlO_2/dl)	11.2 (4.8)	8.8 (2.7)	.01*

Figure 3-2: Scatterplots of individual exercise variables of all participants. (Error bars denote means and standard deviations).



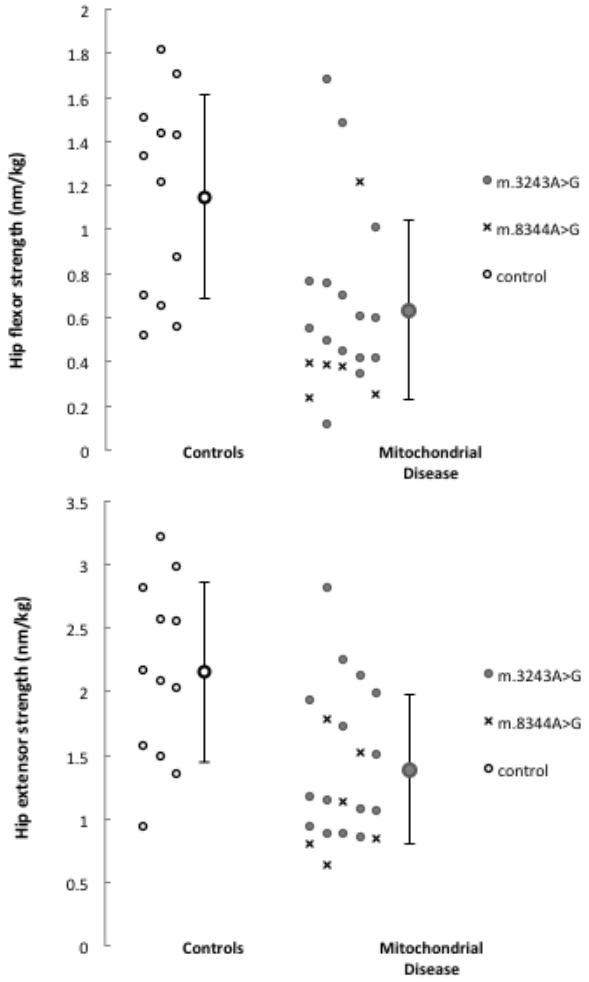
3.5.4 Muscle strength

Proximal muscle strength was different between the groups in both hip flexor and extensor muscle groups with hip flexor strength (nm/kg) in mitochondrial participants reported as 45% lower than the sedentary control group (Table 3-5, Figure 3-3). Proximal muscle power followed the expected hierarchy with hip extensor strength being greater than hip flexor strength (Cahalan *et al.*, 1989).

Table 3-5: Table comparing muscle strength in patients with mitochondrial disease and sedentary controls.
*Data reported as medians and quartile ranges. ** p<.01*

Variable	**Control group (n = 12)**	**MD Group (n = 21)**	**p value**
Hip flexor strength (nm)	106 (51, 119)	28 (22,45)	< .001**
Hip extensor strength (nm)	175 (148, 225)	64 (52, 106)	< .001**
Hip flexor strength (nm/kg)	1.3 (0.7, 1.5)	0.5 (0.4, 0.8)	.002**
Hip extensor strength (nm/kg)	2.1 (1.6, 2.8)	1.1 (0.9, 1.8)	.003**

Figure 3-3: Graphs of individual participants proximal muscle strength. (Error bars denote means and standard deviations).



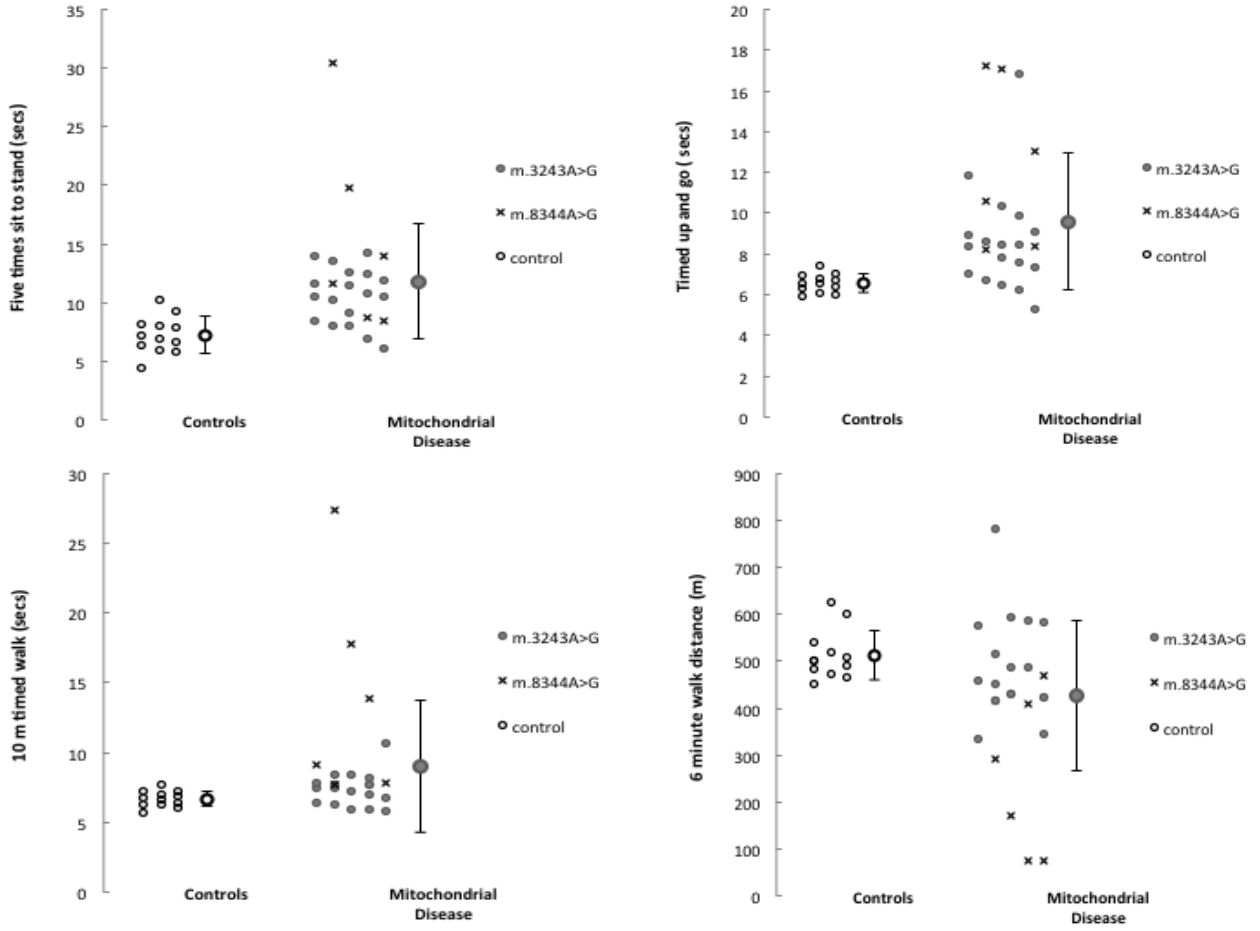
3.5.5 Functional Ability

The functional outcome measures used were able to detect significant differences between the two groups ($p < .05$). The outcome measures demonstrated that mitochondrial patients performed the functional tasks slower than the control group and walked a shorter distance over 6 minutes (Table 3-6, Figure 3-4).

Table 3-6: Functional outcome measure results of control and mitochondrial patients.
*(MD, Mitochondrial Disease).Data is reported as medians (quartiles). ** p<.01, *p<.05*

Variable	**Median (Quartiles)**		***p*-values**
	Control	**MD**	**MD-control**
5 times Sit to Stand (secs)	7 (6, 8)	12 (9, 13)	< .001**
Timed up and Go (secs)	7 (6, 7)	8 (7, 10)	.001**
10m timed walk (secs)	7 (6, 7)	8 (7, 8)	.006**
6 minute walk distance (metres)	500 (476, 535)	430 (361, 508)	.013*

Figure 3-4: Graphs of individual performances of functional outcome measures. (Error bars denote means and standard deviations).



3.5.6 The relationships between biological, clinical and functional measures in patients with mitochondrial disease

Correlations were performed between the three types of measures chosen (disease burden, exercise capacity and functional ability) to identify relationships between measures. First to be explored were the relationships between disease severity and the exercise and functional measures. The results of these initial correlations demonstrated that functional measures produced moderate correlations with disease severity; therefore further correlations were performed between the functional measures to assess if the functional measures used showed relationships with each other. The same procedures were repeated with the exercise measures. As multiple comparisons were performed statistical significance was assumed when the *p value* < .01.

Associations between disease severity, physical activity, functional capacity and exercise performance

A direct relationship was seen between the two measures of disease severity (mitochondrial mutation load and disease severity measured using the NMDAS), demonstrating that an increase in disease burden was shown with an increased mutation load (r=0.6, *p= .001*).

Disease severity using both the clinical disease scale (NMDAS) and mutation load showed moderate correlations with number of steps taken in a day, with increased disease severity showing a reduction in daily steps (NMDAS vs. daily step count; r =-0.6, *p*= .003**, mutation load vs. daily step count r =-0.5, *p*= .017*). Although, no relationship was demonstrated between disease severity and physical activity when energy expenditure is used as a variable

(NMDAS; r = 0.04, *p*= .854, mutation load r = 0.2, *p*= .352). Due to the discrepancy in the relationships between the two measures of physical activity and disease severity, further correlations were performed showing only a moderate relationship between the two measures of physical activity (step count vs. energy expenditure; r = 0.6, *p*= < .001).

When investigating the two measures of disease severity and exercise and functional measures no relationships were evident between mutation load and any of the exercise and functional parameters measured, except the TUG (r=0.5)(Table 3-7)

Table 3-7: Relationships between mutation load (heteroplasmy) and measures of physical activity, exercise capacity and functional outcome measures. Significant results are denoted in bold.

Domain	**Parameter**	**Correlation coefficient (*p* value)**
Physical Activity	Energy expenditure	0.2 (.352)
	Steps taken per day	-0.5 (.017)*
Exercise capacity	vO_{2PEAK} (ml.kg.min)	-0.2 (.430)
	Anaerobic threshold (ml.kg.min)	-0.1 (.713)
	Peak workload (W/kg)	-0.3 (.146)
	Arteriovenous difference (ml O_2/dl)	-0.2 (.317)
Muscle Strength	Hip flexor strength (nm/kg)	-0.3 (.905)
	Hip extensor (nm/kg)	-0.1 (.525)
Functional Measures	10m timed walk (secs)	0.3 (.172)
	Timed up and Go (secs)	**0.5 (.020)***
	5 times sit to stand (secs)	0.3 (.162)
	6 minute walk distance (secs)	-0.2 (.329)

Disease severity when measured using the Newcastle Mitochondrial Disease Adult Scale (NMDAS) showed relationships with all exercise parameters investigated, with peak workload (nm/kg) showing the strongest correlation (r = -0.6, *p* = *.001*). These associations demonstrated that exercise capacity reduced with increased disease severity. Moderate relationships were also evident between clinically measured disease severity (NMDAS) and all functional outcomes except the 5 times sit to stand (5XSTS). Whereas hip muscle strength correlated poorly with disease severity (NMDAS) (r=0.3).

These correlations are represented graphically in Figure 3-5 and are summarised in Table 3-8.

Figure 3-5: Scatterplots of the relationships between disease severity and exercise parameters.

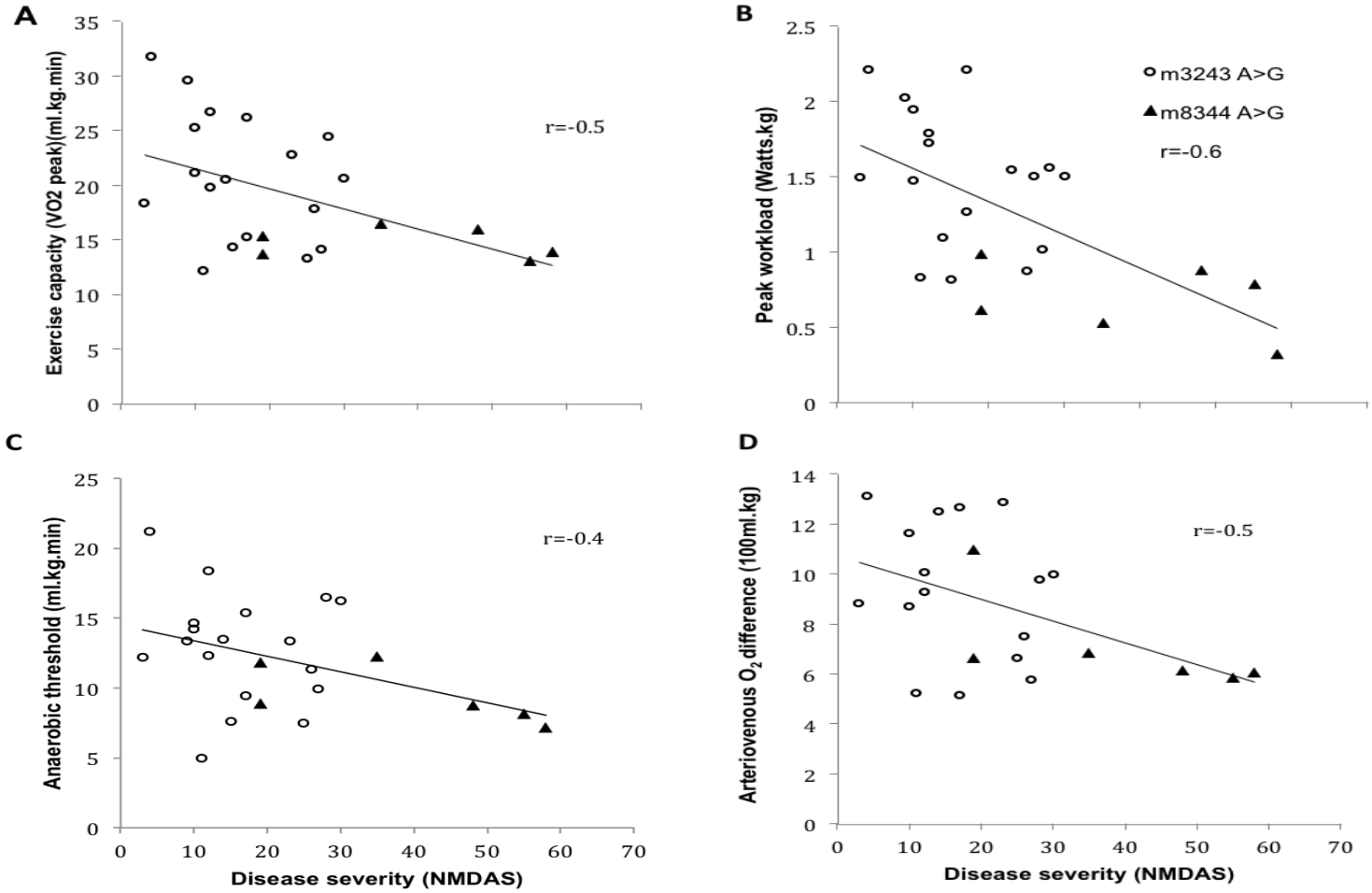


Table 3-8: The relationships between disease severity, physical activity, exercise capacity and functional outcome measures.
**p< .05, ** p< .01. Statistical significant results are shown in bold type.*

Domain	Parameter	NMDAS vs domain Correlation coefficient (*p* value)
Physical Activity	Energy Expenditure	0.04 (.854)
	Steps taken per day	**-0.6 (.003)****
Exercise capacity	$\dot{V}O_{2PEAK}$ (ml.min.kg)	-0.5 (.015)*
	Anaerobic Threshold (ml.min.kg)	-0.4 (.035)*
	Peak workload (Watts)	-0.5 (.013)*
	Peak workload (W/kg)	**-0.6 (.001)****
	Arteriovenous difference (ml O2/dl)	-0.5 (.019)*
Muscle strength	Proximal hip muscle strength (nm/kg)	-0.3 (.136)
Functional measures	5 times sit to stand (secs)	0.3 (.266)
	Timed up and go (secs)	**0.6 (.001)****
	10m timed walk (secs)	**0.6 (.001)****
	6 minute walk distance (m)	-0.5 (.018)*

Associations between exercise parameters and physical activity

Peak exercise capacity showed a direct relationship with both step count and energy expenditure as measures of physical activity (steps r = 0.7, $p < .001$**, energy expenditure r = 0.6, $p = .006$**). Demonstrating mitochondrial patients with higher physical activity levels had a greater exercise capacity.

As all exercise parameters had demonstrated similar correlations to disease severity and physical activity, further investigations were undertaken to assess the relationships between these exercise parameters. The results demonstrated that all exercise parameters correlated strongly with each other (r ≥ 0.7) (Table 3-9).

*Table 3-9: Relationships between measures of exercise performance. Correlation coefficients (p values) ** p< .01.Statistically significant results are shown in bold type.*

Exercise parameter	**$\dot{V}O_{2PEAK}$ (ml.min.kg)**	**Anaerobic Threshold (ml.min.kg)**	**Peak workload (w/kg)**	**Arteriovenous difference (ml O2/dl)**
$\dot{V}O_{2PEAK}$ (ml.min.kg)		**0.9 (< .001)****	**0.9 (< .001)****	**0.8 (< .001)****
Anaerobic Threshold (ml.min.kg)			**0.8 (< .001)****	**0.8 (< .001)****
Peak workload (w/kg)				**0.7 (< .001)****

Associations between Functional outcome measures and physical activity

Functional outcome measures also demonstrated relationships with the daily step count (Timed up and go, r =-0.5, $p = .009$**, 10 m timed walk r =-0.5, $p = .019$*), although this relationship was not replicated when considering energy expended (r< 0.4). As all but one of the functional measures had demonstrated similar relationships between disease severity and physical activity, further correlations between the functional measures were also performed. Unsurprisingly all the walking tests showed strong relationships with each other. Whereas the 5 times sit to stand test only showed an association with the

Timed up and go test. This is also unremarkable as both measures include the sit to stand movement (Table 3-10).

Table 3-10: Relationships between functional walking tests.
*Correlation coefficients (p values) *p< .05 ** p< .01. Statistically significant results are shown in bold type.*

Walking Test	Timed up and go	10m timed walk (single)	6 minute walk distance	5 times sit to stand
Timed up and go		**0.8 (<.001)****	**-0.6 (.001)****	**0.6 (.003)****
10m timed walk			**-0.9 (<.001)****	0.4 (.038)*
6 minute walk distance				-0.3 (.142)

Associations between physiological and functional measures

To investigate the relationships between the exercise and functional measures further correlations were performed and are summarised in Table 3-11. Strong relationships were seen between proximal muscle strength and all exercise parameters ($r \geq 0.7$). The only significant relationship between proximal muscle strength and functional measures was demonstrated with the 6-minute walk distance ($r = 0.6$). These results demonstrate that the mitochondrial participants with stronger proximal muscles or lower levels of myopathy had better exercise capacity and were able to walk further over 6 minutes. The remaining walking measures also showed significant relationships with the exercise measure of peak workload (Timed up and go; $r = -0.5$, 10 metre timed walk; $r = -0.6$, 6 minute walk distance; $r = 0.6$).

Table 3-11: Relationships between functional and exercise test parameters.
*(*p< .05, **p< .01). Statistically significant results (p< .01) are shown in bold type.*

	Correlation coefficient (*p value*)				
Parameter	**$\dot{V}O_{2\ PEAK}$ (ml.min.kg)**	**Arteriovenous difference (mlO_2/dl)**	**Peak workload (w/kg)**	**Anaerobic threshold (ml.min.kg)**	**Hip flexor strength (nm/kg)**
5 times sit to stand (secs)	-0.2 (.305)	0.1 (.811)	-0.3 (.191)	-0.1 (.597)	-0.4 (.086)
Timed up and go test (secs)	-0.4 (.049*)	-0.1 (.524)	**-0.5 (.009**)**	-0.4 (.71)	-0.4 (.099)
10m timed walk (single) (secs)	-0.4 (.037*)	-0.3 (.232)	**-0.6 (.002**)**	-0.4 (.035*)	-0.5 (.014*)
6 minute timed distance (m)	0.4 (.030*)	0.4 (.074)	**0.6 (.002**)**	0.5 (.026*)	**0.6 (.003**)**
Hip flexor strength (nm/kg)	**0.8 (< .001**)**	**0.7 (.001**)**	**0.7 (< .001**)**	**0.8 (< .001**)**	

Exploration of individual items of the Newcastle Mitochondrial Disease Adult Scale (NMDAS) relationships with exercise and functional measures

As the NMDAS investigates many aspects of mitochondrial disease correlations for each sub section of the NMDAS were performed. This enabled investigation of the relationships between different aspects of mitochondrial disease and the measures performed in the study.

The NMDAS scale consists of 29 questions, which represent different aspects of mitochondrial disease. These aspects are listed in the first column of Table 3-12. Table 3-12 reports the highly significant correlations highlighted in bold text. From these correlations it can be seen that self-reported exercise tolerance showed a strong relationship with measured exercise capacity (peak workload; r=-0.8, $p < .001$, peak exercise capacity; r =-0.7, $p < .001$) and proximal muscle strength (r= -0.6, $p = .002$). With those mitochondrial patients reporting subjectively reduced exercise tolerance having reduced measured exercise capacity and proximal muscle strength. Reported exercise intolerance also showed a relationship with two functional measures; the 10m timed walk and the Timed up and go (r= 0.6, r = 0.5, $p< .01$). With those mitochondrial patients reporting impaired exercise tolerance taking longer to perform walking tasks.

All the functional components of the scale (such as dressing and hygiene) showed associations with the walking outcome measures (r values from 0.5 - 0.6). In addition the Timed up and Go demonstrated a relationship with cerebellar signs (r= 0.5, $p < .01$) and cognition (r= 0.5, $p < .01$).

Table 3-12: Correlations of individual sections of the Newcastle Mitochondrial Disease Adult Scale (NMDAS) with physiological and functional measures.
*(** p =0.01,*p=0.05). Statistically significant results are shown in bold text. (CPEO-Chronic Progressive External Opthalmoplegia).*

Spearman correlations (p value)							
NMDAS component	**Peak exercise capacity (ml.kg.min)**	**Peak Workload (w/kg)**	**Timed up and go (secs)**	**10m timed walk (secs)**	**6 minute walk distance (m)**	**Hip flex strength (nm/kg)**	**Five times sit to stand (secs)**
Total NMDAS	-0.5*(.015)	**-0.6**(.001)**	**0.6**(.001)**	**0.6**(.001)**	-0.5*(.018)	-0.3 (.136)	0.3 (.266)
Vision	-0.1 (.622)	-0.1 (.447)	0.2 (.286)	0.4*(.044)	-0.4 (.090)	-0.1 (.561)	0.01 (.968)
Hearing	0.1 (.770)	0.03(.860)	0.1 (.589)	0.1 (.605)	0.04 (.843)	0.1 (.662)	-0.1 (.697)
Speech	-0.3 (.970)	-0.5*(.015)	0.5*(.026)	0.5*(.022)	-0.4 (.080)	-0.3 (.241)	0.2 (.375)
Swallowing	0.3 (.222)	-0.2 (.435)	0.2 (.338)	0.3 (.210)	-0.3 (.175)	-0.3 (.215)	0.2 (.251)
Handwriting	-0.2 (.248)	-0.5*(.026)	0.5*(.012)	**0.6**(.003)**	-0.5*(.011)	-0.3 (.223)	0.2 (.331)
Cutting	-0.4 (.053)	-0.5*(.024)	0.5*(.025)	**0.5**(.008)**	**-0.6**(.002)**	-0.5*(.012)	0.2 (.282)
Dressing	-0.4 (.065)	-0.5*(.026)	**0.5**(.010)**	0.5*(.010)	**-0.6**(.005)**	-0.5*(.027)	0.3 (.092)
Hygiene	-0.4 (.066)	-0.5*(.029)	**0.5**(.010)**	**0.5**(.010)**	**-0.6**(.005)**	-0.5*(.030)	0.3 (.095)
Exercise	**-0.7**(<.001)**	**-0.8**(<.001)**	**0.5**(.009)**	**0.6**(.001)**	-0.5*(.005)	**-0.6**(.002)**	0.2 (.358)
Gait	-0.4*(.038)	-0.5*(.013)	**0.6** (.003)**	**0.5**(.009)**	-0.5*(.020)	-0.4 (.099)	0.2 (.331)
Psychiatric	-0.3 (.103)	-0.5*(.017)	0.3 (.164)	0.4 (.069)	-0.2 (.322)	-0.1 (.597)	-0.02 (.941)
Migraine	-0.1 (.590)	-0.1 (.627)	-0.01 (.979)	-0.2 (.320)	0.3 (.157)	0.3 (.161)	-0.1 (.520)
Seizures	-0.4 (.071)	-0.5*(.017)	0.5*(.012)	-0.4*(.032)	-0.4*(.047)	-0.3 (.274)	0.3 (.169)

Spearman correlations (p value)							
NMDAS component	**Peak exercise capacity (ml.kg.min)**	**Peak Workload (w/kg)**	**Timed up and go (secs)**	**10m timed walk (secs)**	**6 minute walk distance (m)**	**Hip flex strength (nm/kg)**	**Five times sit to stand (secs)**
Stroke like episodes	0.1 (.595)	0.2 (.413)	-0.1 (.692)	-0.01 (.966)	0.1 (.486)	-0.2 (.466)	0.1 (.513)
Encephalopathic	0.1 (.613)	0.2 (.416)	-0.1 (.686)	-0.02 (.920)	0.2 (.445)	0.2 (.450)	0.1 (.543)
Gastro-intestinal	-0.2 (.368)	0.1 (.501)	-0.3 (.192)	-0.4 (.053)	0.4 (.090)	0.02 (.926)	-0.2 (.360)
Diabetes	0.3 (.173)	0.2 (.389)	-0.01 (.942)	0.03 (.906)	-0.01 (.972)	0.1 (.525)	-0.03 (.883)
Respiratory	0.1 (691).	0.1 (.763)	-0.3 (.175)	-0.1 (.643)	-0.03 (.907)	-0.1 (.687)	0.1 (.799)
Cardiovascular	-0.2 (.389)	-0.2 (.264)	0.4*(.029)	0.4*(.028)	-0.5*(.013)	-0.3 (.137)	-0.3 (.103)
Visuals	-0.3 (.129)	-0.4 (.069)	0.3 (.215)	0.4 (.058)	-0.4*(.083)	-0.3 (.173)	-0.1 (.812)
Ptosis	0.2 (.289)	-0.1 (.810)	-0.1 (.695)	-0.2 (.402)	0.2 (.259)	-0.1 (.809)	-0.1 (.540)
CPEO	-0.4*(.047)	-0.3 (.108)	0.4 (.089)	0.4 (.080)	-0.4 (.052)	-0.4 (.053)	0.1 (.525)
Dysphonia/Dysarthria	-0.3 (.152)	-0.5*(.020)	0.3 (.096)	0.4*(.046)	-0.4*(.035)	-0.3 (.212)	0.1 (.592)
Myopathy	-0.5*(.019)	-0.5*(.023)	0.2 (.247)	0.4 (.056)	-0.3 (.223)	-0.5*(.038)	0.1 (.623)
Cerebellar	-0.3 (.123)	-0.4 (.060)	**0.5**(.008)**	0.4*(.047)	-0.4 (.072)	-0.3 (.224)	0.1 (.572)
Neuropathy	-0.4 (.052)	0.5 (.011)	0.5*(.002)	0.5*(.014)	-0.4 (.072)	-0.4 (.073)	**0.5**(.008)**
Pyramidal	-0.1 (.762)	-0.02 (.920)	0.2 (.477)	0.1 (.686)	0.01 (.960)	0.04 (.863)	-0.1 (.762)
Extrapyramidal	-0.1 (.727)	-0.1(.522)	0.3 (.132)	0.3 (.097)	-0.3 (.113)	-0.1 (.522)	-0.02 (.922)
Cognition	-0.4*(.04)	**-0.5**(.008)**	**0.5**(.007)**	0.5*(.016)	-0.3 (.103)	-0.2 (.418)	0.3 (.114)

3.6 **Discussion**

The key findings of the study were that both exercise and functional measures were able to differentiate between participants with mitochondrial disease and sedentary controls; with disease severity demonstrating strong relationships with both exercise and functional measures. In addition this study demonstrated that dynamometry was able to detect impaired proximal muscle strength and this reduction in strength had a strong relationship with the peak exercise performance of participants with mitochondrial disease. Finally the study confirmed previous findings that the level of mitochondrial mutation load related to disease severity, exercise capacity and daily activity levels (Chinnery *et al.*, 1997; Jeppesen *et al.*, 2003; Taivassalo *et al.*, 2003; Whittaker *et al.*, 2009; Apabhai *et al.*, 2011).

3.6.1 Physical Activity

Despite the level of physical activity not being a primary hypothesis of this study discussion of the differences demonstrated between the activity levels of the two groups is essential, as the low levels of physical activity are likely to impact on the levels of exercise capacity and potentially the functional ability of participants with mitochondrial disease.

This study reported that patients with mitochondrial disease had low levels of day-to-day physical activity when measured objectively. This finding is in line with previous studies in people with mitochondrial and other neuromuscular conditions and is related to disease severity (Phillips *et al.*, 2009; Tudor-Locke *et al.*, 2009; Apabhai *et al.*, 2011). Levels of physical activity reported in this study in the mitochondrial group also related to the level of peak exercise capacity. It is not possible to report that the lower activity levels in the

mitochondrial patients resulted in reduced exercise capacity, although previous exercise studies have indicated that the exercise capacity within a mitochondrial population can be improved with increased activity in the form of exercise (Taivassalo *et al.*, 2001; Pilar *et al.*, 2005; Jeppesen *et al.*, 2006b).

A study by Apabhai *et al* investigating physical activity in a larger cohort of patients with mitochondrial disease participants reported low levels of physical activity alongside increased obesity (Apabhai *et al.*, 2011). This finding was not replicated in this study where the group with mitochondrial disease did not show signs of obesity and in fact had significantly lower BMI's than the control group. The difference in weight between the two studies of patients with mitochondrial disease is probably due to the restriction in this study to two specific genotypes, level of disease burden and sample size. The population used in this study was smaller than in the study by Apabhai *et al* and hence may not be representative of the total population of mitochondrial disease patients. Gastro intestinal symptoms are a common feature of patients with the m.3243A>G genotype (Chinnery *et al.*, 1997) and were noted in ten of our participants with this genotype, which also may have contributed to a lower weight in this study's population. In addition the mitochondrial population used in this study were all independently ambulant which may have resulted in lower body mass. From the results of this study it is impossible to ascertain whether the lower weight of participants reported in this study was due to the higher disease burden, or type of mitochondrial genotype.

It is already known that low levels of physical activity are detrimental in people with neuromuscular disease who are at increased risk of coronary heart disease, obesity, osteoporosis and depression (McDonald, 2002 ; Abresch *et al.*, 2009). Increased activity has specific disease benefits for patients with mitochondrial

disease over and above the known general health benefits. These benefits include increased to mitochondrial biogenesis in muscle (Taivassalo *et al.*, 2001), improved heart function (Bates *et al.*, 2013) and improved quality of life (Taivassalo *et al.*, 2006). The reasons for reduced physical activity in mitochondrial disease are likely to be multifactorial and require further investigation to fully understand the relationship between disease burden and daily physical activity. Understanding this relationship may enable more appropriate interventions to be developed to increase physical activity in this group and ameliorate the effects of reduced levels of physical activity.

3.6.2 Disease severity and exercise capacity

Disease severity in this study was reported in two ways; by use of Newcastle Mitochondrial Disease Adult Scale (NMDAS) (Schaefer *et al.*, 2006) and % mitochondrial mutation load in epithelial cells of urine (Whittaker *et al.*, 2009). The relationship between disease severity and mutation load is in agreement with previous work that reported an increased mutation load associated with increased disease severity, reduced oxidative capacity (Jeppesen *et al.*, 2003; Jeppesen *et al.*, 2006a) and increased clinical features in mitochondrial disease (Chinnery *et al.*, 1997). However the relationship is only moderate and therefore does not tell the full story. This is highlighted in two participants from this study who had the lowest disease severity scores (NMDAS) despite having very different mutation loads, one being 34% and the other 80%. The presence of clinical features in this study group at low mutation levels also supports previous findings that reported symptoms at mutation loads as low as 40% in the m.3243A>G mutation (Jeppesen *et al.*, 2006a). These findings support the adoption of a cautious approach when trying to predict clinical severity by

mutation load alone, as mutation levels vary from tissue to tissue and genotype to genotype (DiMauro and Schon, 2003).

Comparing disease severity and exercise capacity in this study to previous exercise studies is difficult. This is because previous studies included either multiple genotypes with a wide variety of mutation loads and clinical features, or very small numbers of patients with one genotype (large-scale single deletion) (Jeppesen *et al.*, 2003; Taivassalo *et al.*, 2003; Trenell *et al.*, 2006). Our participants despite being recruited to take part in an exercise study had relatively high mutation levels ranging from 22 to 94%. These mutation levels are similar to previous studies that report mutation loads (Taivassalo *et al.*, 2003; Jeppesen *et al.*, 2006a) and are more reflective of the clinical caseload seen in our clinics than some previous studies, as none of the participants had extremely low mutation loads and non were asymptomatic (Jeppesen *et al.*, 2006b).

The restriction to two genotypes follows recommendations made in the recent Cochrane review to reduce the number of genotypes within studies in mitochondrial disease (Pfeffer *et al.*, 2012). Despite restricting the study to two genotypes the variability in symptoms and exercise performance reported remained as high as previous exercise studies with a wider range of genotypes. The wide variety of performance was disappointing but highlights the variability of disease burden and clinical features within genotypes and confirms the previously reported differences between genotypes (Taivassalo *et al.*, 2003; Cejudo *et al.*, 2005).

The lower peak exercise capacity (58% vs. 92%), anaerobic threshold (36% vs. 54%) and arteriovenous oxygen difference reported in this study's patient group

support the findings of previous studies and indicate a reduction in mitochondrial function in the muscles of patients with mitochondrial disease (Taivassalo *et al.*, 2003). These lower results are due to muscles reverting to non-aerobic methods of energy production earlier in exercise testing as the mitochondrial respiratory chain fails to keep up with the energy requirements of the cell placed upon it by exercise. The exercise response seen in patients replicated previously reported linear responses of increased oxygen consumption with increasing workload with the limiting factor being oxygen extraction (Taivassalo *et al.*, 2003). It is difficult to ascertain how much of the reduction in exercise performance can be attributed to the primary biological deficit as the mitochondrial group also reported lower levels of physical activity. Therefore some impairment in exercise capacity is likely to be due to deconditioning.

This study demonstrated that objectively measured exercise capacity had a moderate relationship with self-reported exercise capacity, myopathy, chronic progressive external ophthalmoplegia and gait, within the disease severity scale. This is unsurprising as all are muscle related symptoms. These findings support the continued use of exercise testing as a non-invasive measure of mitochondrial function within muscle in the mitochondrial disease population. Widespread adoption of exercise testing is however hampered, as facilities and expertise are limited to specialist centres.

In summary the exercise capacity of subjects with mitochondrial disease in this study was similar to previous studies. Despite the cohort not including candidates with low mutation loads and only including patients with two genotypes with demonstrable clinical signs of disease, no reduction in the variability of exercise response was reported. Lower activity levels in the

mitochondrial group may have also further attenuated the exercise performance within the patient group compared to controls. Therefore the current treatment advice given to mitochondrial patients to participate in regular exercise should continue, as physical activity has been shown to improve exercise capacity and reduce exacerbation of muscle symptoms due to de-conditioning.

3.6.3 Disease severity and functional ability

Alongside exercise capacity this study investigated the use of clinically based functional outcome measures in participants with mitochondrial disease. Functional outcome measures are frequently used to evaluate therapy by physiotherapists (Yoward *et al.*, 2008). Functional outcome measures have been used in numerous studies involving neurological, neuromuscular and elderly populations, to measure a variety of parameters from motor impairment and functional mobility to disease severity and also to predict future disability (Takeuchi *et al.*, 2008; Ries *et al.*, 2009; McDonald *et al.*, 2010). Functional outcome measures are being shown to be reliable in an increasing number of neurological and neuromuscular conditions (Steffen *et al.*, 2002; Flansbjer *et al.*, 2005; Andersson *et al.*, 2006; Katz-Leurer *et al.*, 2008; McDonald *et al.*, 2010) and their ease of use (Whitney *et al.*, 2005) in both the clinical and research setting make investigation of their possible use in mitochondrial disease imperative.

Many outcome measures are available for use in the clinical and research areas in neurological conditions. The measures used in this study were chosen because they were clinically relevant to this population and are commonly used by physiotherapists working in Neurology (Yoward *et al.*, 2008). In this study all the Functional Outcome Measures (FOM) used were able to detect differences

between the participants with mitochondrial disease and controls. Three of the FOMs used in this study involved walking and therefore unsurprisingly demonstrate strong relationships with each other. Strong relationships were also demonstrated between the sit to stand measure and the Timed up and go, which both contain the movement of sit to stand. The 10m timed walk and the Timed up and go test in this study demonstrated very strong correlations with the total disease severity score, exercise capacity (measured and reported) and functional tasks within the Newcastle Mitochondrial Disease Adult Scale. In addition the Timed up and go showed an association with Cerebellar symptoms in the scale. For each outcome measure the following paragraphs will discuss their previous use and the results of this study.

The Timed up and go was originally used to test functional mobility in the elderly frail population (Podsiadlo D *et al.*, 1991) and provided timed cut-offs for independent living. As the measure included a move from sitting to standing and a change of direction it was thought to also test elements of balance ability and has been used to assess patients risk of falling (Shumway-Cook *et al.*, 2000). These attributes support the finding in this study of a relationship between cerebellar signs and the Timed up and go. Unfortunately no other balance tests were performed in the study due to time constraints so this observation cannot be confirmed. The TUG has been shown in other populations to assess balance (Brusse *et al.*, 2005; Katz-Leurer *et al.*, 2008; Schepens *et al.*, 2010), but further studies are required comparing this measure with other methods of assessing balance to confirm this in a mitochondrial population.

The 10m Timed Walk has been used previously to calculate gait speed and has been described as an almost perfect measure (Wade, 1992). Many

neurological and neuromuscular populations have used this measure and have reported relationships to lower limb strength (Scott *et al.*, 1982) community ambulation (Busse *et al.*, 2006; Vandervelde *et al.*, 2009) and subsequent disability (Guralnik *et al.*, 2000; Studenski *et al.*, 2003). Despite the strong correlations with disease severity and exercise capacity this study failed to demonstrate any relationship between gait speed and muscle strength when measured by dynamometry or reported muscle strength in the disease scale. This conflicting finding may be due to the multitude of symptoms seen in this cohort of mitochondrial patients (Table 3-1). It may also be that muscle weakness is not such a prominent symptom as reported in other neuromuscular conditions such as Spinal muscle atrophy and the muscular dystrophies and also not the primary cause for reduced function (Scott *et al.*, 1982; Merlini *et al.*, 2004).

The 6-minute walk distance was able to discriminate between the two groups but showed weaker relationships with disease severity and only correlated significantly with peak exercise workload and proximal muscle strength. The 6-minute walk distance was originally devised as a field test for exercise capacity (Society, 2002) and is described as a non-specific and non-diagnostic test which can be used in a multitude of conditions. The 6-minute walk distance (6MWD) has been used in numerous neuromuscular trials to measure a variety of parameters including disease progression, exercise capacity, functional mobility and fatigue (Takeuchi *et al.*, 2008; Montes *et al.*, 2010; Hashizume *et al.*, 2012). It has been reported to evaluate the global and integrated response of all body systems involved in walking, including neuromuscular, pulmonary, and cardiovascular systems and is therefore considered to be a valid clinical endpoint in neuromuscular studies (Florence *et al.*, 2008; Montes *et al.*, 2010;

McDonald *et al.*, 2013; Willis *et al.*, 2013). The lack of strong relationships between the 6MWD and exercise parameters in this study was unexpected given the original use of the measure. Possible explanations for this result may have been: that only one attempt at the 6 minute walk test was completed, this was despite knowing that an increased distance is usually covered on a second attempt (2002). Also the mitochondrial group may have performed the walk at a lower self-selected speed to prevent any negative effects they may have previously experienced when walking long distances. Only one test was performed in this study to reduce the risk of fatigue affecting subsequent trials in the MD group. The performance of one attempt of the 6-minute walk was justified, as it is in line with previous studies in neuromuscular conditions where fatigue is perceived as a limiting factor (Andersson *et al.*, 2006; Montes *et al.*, 2010). To ensure a maximal performance of a 6-minute walk in this disease group with only one attempt future studies may require additional encouragement to obtain an accurate result (McDonald *et al.*, 2010; Lerario *et al.*, 2012).

Despite the 6-minute walk test taking more time, requiring more space to perform and producing similar results to the other walking tests it is not superfluous. The 6-minute walk test did show a stronger relationship to muscle strength than the other functional tests, which is in line with previously reported studies in other neuromuscular diseases (Lerario *et al.*, 2012).

As proximal myopathy is a common symptom reported in studies of these two genotypes (Chinnery *et al.*, 1997) a functional activity was chosen that measured lower limb strength. This was a measure of the movement from sit to stand, which requires greater lower limb strength than walking and stair climbing (Berger *et al.*, 1988). This study demonstrated that participants with

mitochondrial disease had weaker hip muscles and were significantly slower to get in and out of a chair. Despite these findings the two measures only demonstrated a weak relationship to each other and reported muscle strength within the NMDAS. The failure of the 5 times sit to stand test to demonstrate a relationship with any aerobic exercise measures is unremarkable as this test is reported to measure lower limb strength. However it was surprising that only a weak relationship was demonstrated to proximal muscle strength, which conflicts with previous work investigating the sit to stand movement (Nitz *et al.*, 1997; Inkster *et al.*, 2003). A possible explanation for this lack of a relationship may be due to the sit to stand motion being more than just a measure of lower limb strength, but also a measure of the efficiency of other systems such as balance and sensation in providing constant feedback throughout the sit to stand movement (Lord *et al.*, 2002). All of these systems can be affected in this disease group and may have had a bearing on the results.

In summary functional outcome measures were able to discriminate between the mitochondrial disease and control groups. They were quick and easy to perform and showed good relationships to disease severity in mitochondrial patients. The variety of relationships reported between functional and exercise measures used in this study highlight that these outcomes appear to measure different aspects of mitochondrial disease. With functional measures allowing measurement of impairment in multiple systems simultaneously whereas physiological measures likely to be limited to measuring one specific system.

3.6.4 Proximal muscle strength

Although proximal myopathy is a common symptom of mitochondrial disease this is the first study to show that the muscle strength was nearly 50% lower within the mitochondrial group compared to a disease free control group.

The use of dynamometry to measure muscle strength was developed in orthopaedic conditions and is seen as the gold standard for measuring muscle strength. Normative values of dynamometry values for hip muscle strength are limited and previous work has reported variable reproducibility (Burnett *et al.*, 1990; Boling *et al.*, 2009; Julia *et al.*, 2010). Although no previous studies to our knowledge report measuring hip muscle strength in mitochondrial disease, dynamometry has been used in other neuromuscular (Burnett *et al.*, 1990; Tiffreau *et al.*, 2007) and elderly populations (Bohannon, 1998). The use of dynamometry in other neuromuscular diseases has demonstrated that it is capable of; i) measuring the extremely low torques produced by participants with severe muscle disease, ii) disease progression and, iii) relate to functional abilities (Tiffreau *et al.*, 2007; Lerario *et al.*, 2012; El Mhandi and Bethoux, 2013). To overcome the lack of comparable data this study included a control group of comparable age. Both disease and control groups performed the same protocol in a lying as opposed to standing and were assessed by the same tester at a similar time of day (Julia *et al.*, 2010; Maffiuletti, 2010).

The strong relationship between exercise capacity and strength reported in this study has been seen previously in mitochondrial patients where improvements in muscle strength were reflected in improvements in peak exercise capacity (Murphy *et al.*, 2008). Results from this study demonstrate that limitations in peak exercise capacity in mitochondrial patients may not be solely due to

aerobic capacity and impaired mitochondrial function but also reduced muscle strength. It should be noted that the strong relationship between proximal muscle strength and peak exercise capacity may have been heightened by the use of a recumbent bike, which required increased muscle strength when reaching higher wattages (Walsh Riddle and Blumenthal, 1989). These results highlight that the relationship between aerobic and strength performance of muscles in MD patients requires further investigation.

Unfortunately the study was unable to demonstrate a relationship between measured muscle strength using dynamometry and clinical testing of muscle strength recorded within the NMDAS. Muscle strength in the disease severity scale was measured using manual muscle testing (Frese *et al.*, 1987). Differences in accuracy between the two methods of muscle testing may explain why no relationship was demonstrated. Previous studies have reported that patients can lose up to 50% of muscle power and still be reported as having normal muscle strength on clinical testing (Watkins *et al.*, 1984; Sapega, 1990). These findings are in agreement with the results, of this study where a great variability in proximal muscle strength was seen using dynamometry, yet all MD participants reported muscle strength of grade 4 or above on manual muscle testing, where a score of 5 is normal.

As reported in the previous section a moderate relationship was seen between muscle strength and only one functional measure(6MWD), despite two functional tests (TUG and 5XSTS) including a movement of sit to stand which requires an increased lower limb strength (Berger *et al.*, 1988). Reasons for the poor correlations between tests with a sit to stand component and hip strength are likely due to the Timed up and go test allowing the use of upper limbs which will compensate for lower limb weakness and, considerable loss of power being

required prior to a loss of function being observed (Lexell, 2000). Also as discussed in the previous section, although the sit to stand test has been reported to be a functional marker of lower limb strength in the elderly, or people with proximal myopathy (Bohannon, 1995; Jones *et al.*, 1999; Merlini *et al.*, 2004; Busse *et al.*, 2006) the sit to stand movement is more complicated than originally thought and requires the integration of balance systems, muscle power and sensory feedback to perform efficiently (Lord *et al.*, 2002). These systems are likely to be affected in patients with mitochondrial disease and impact on the sit to stand movement. Therefore the poor relationship with lower limb strength and functional tests is unsurprising. The poor correlation demonstrates that reduced muscle power is not the primary contributor to difficulties in performing the sit to stand movement and it is likely that patients adopt compensatory mechanisms to allow them to continue to perform the functional task of sit to stand.

In summary, although the measurement of muscle strength by dynamometry is considered a gold standard, further work is required to establish robust normative values and verify its reproducibility in the testing of hip muscles. The use of dynamometry in research investigating the muscle strength of neuromuscular conditions should be continued due its high levels of precision (Boling *et al.*, 2009), reliability (Julia *et al.*, 2010) and its ability to measure muscle strength over a wide range of ability (Tiffreau *et al.*, 2007). It should be borne in mind that measurement of muscle strength provides limited information on the functional consequences of muscle weakness. Finally the use of dynamometry in a clinical setting is limited due to expense and limitations in space. Consequently other methods of measuring muscle strength, such as

hand held dynamometry may need to be considered for more inclusive trials (Reed *et al.*, 1993).

3.7 **Limitations**

Although this study is one of the largest studies into the exercise capacity of a clinically affected group of mitochondrial patients with to two specific genotypes, the sample size remains small. The sample size was chosen to detect a change in exercise capacity in a population with mitochondrial disease following an exercise study, not to detect between group differences, which may have required a larger sample size. The restriction to only two genotypes unfortunately did not reduce the heterogeneity in the mitochondrial patient's performance of both exercise and functional tests. Despite the levels of heterogeneity reported, this study was still able to demonstrate differences between the two groups. The variety of responses reported in the study is unavoidable in such a heterogeneous disease and is acceptable as it reflects the true nature of the disease presentation in these two genotypes. Mitochondrial disease remains a rare condition and therefore an increase in numbers would only have been possible with the inclusion of multiple sites, which was beyond the scope and funding of this study.

A potential bias in the mitochondrial population studied may have occurred as the participants were recruited as part of a study investigating cardiac adaptations to an aerobic training programme and therefore participants had to be capable of completing a 4 month training programme independently. These inclusion requirements may have resulted in a high functioning sub-set of patients being included, although this is not reflected in the high mutation levels and low levels of daily activity reported in this study's population.

The low levels of physical activity in the mitochondrial population resulted in the two groups having very different levels of daily physical activity. The difference

between the group's activity levels was difficult to overcome due to the severely low levels of activity in some of the mitochondrial population, which is typical of this disease. Controlling for the low levels of activity would have been inappropriate due to the small numbers included in the study.

The number of correlations performed on the sub sections of the disease severity scale resulted in multiple comparisons being performed, which may have resulted in some spurious results due to type 1 error. The number of comparisons could have been reduced by choosing only the subsections thought to relate to functional and exercise data, but for completeness it was felt that inclusion of all comparisons was more transparent. To overcome spurious results the significance value was increased to a *p* value of <.01.

The extensive protocol and high participant burden meant that it was not possible to assess the reliability of functional measures in this population, although they have all been shown to be reliable in other populations (Tyson and Connell, 2009). To ensure maximum reproducibility of tests in this study the same practitioners performed all the tests, they were performed at a similar time of day and all exercise tests were analysed by one exercise physiologist.

This study tested lower limb functional tests and no testing of upper limb function was possible due to time constraints. It was, however, interesting to note that functional tasks using upper limbs in the disease severity scale (handwriting, dressing) related to the walking tests. This is likely to be due to the global nature of functional tests and mitochondrial disease.

3.8 **Conclusions**

In summary this data shows that exercise and functional measures are able to detect impaired performance of exercise and functional tasks in mitochondrial patients with these two specific genotypes. The functional measures showed relationships with exercise capacity and disease severity. Further investigation into these measures is required to assess their reliability, sensitivity and reproducibility over time. The use of functional measures within a clinical situation is possible due to the lack of specialist equipment required and the speed of execution. Future use of functional measures in clinical settings will provide additional information concerning disease severity and the impact of mitochondrial disease on our patient's daily lives.

Taken in combination, these data suggest that clinical care teams should explore the utility of functional outcome measures in routine clinical assessment as a means to better understand the functional impact of mitochondrial disease.

Chapter 4 Gait characteristics in mitochondrial disease. A cross sectional study of two mitochondrial genotypes

4.1 **Introduction**

Mitochondrial disease as described previously is a disease that has multisystem involvement which results in symptoms such as muscle weakness, pain, cardiac impairment, fatigue, ataxia, and visual disturbance. The varied presentations of symptoms have made study of disease progression and the effect of interventions difficult. The multitude of symptoms seen in mitochondrial diseases has led to characterisation of different phenotypes into syndromes such as MELAS (mitochondrial encephalopathy lactic acidosis and stroke-like episodes syndrome) and MERRF (myoclonic epilepsy with ragged red fibres) or by genetic mutation (m.3243A>G and m.8344A>G). These characteristics however are of little benefit to the patient or clinician in predicting the course of the disease due to the heterogeneity between genotype and phenotype.

Clinical measures used currently are unable to accurately discriminate between levels of heteroplasmy and genotype and therefore are of limited use in understanding disease progression, underlying pathology and the benefits of interventions. A recent Cochrane review concurred with this opinion reporting that the wide variety of outcomes used in clinical trials limited the comparability of trials and that the outcomes used provided no evidence to support the use of any intervention in mitochondrial disease (Pfeffer *et al.*, 2012).

Symptoms such as myopathy and exercise intolerance have previously been measured in studies in mitochondrial disease (Jeppesen *et al.*, 2003; Taivassalo *et al.*, 2003), whereas fatigue, although a prominent feature of mitochondrial disease affecting between 20-60% of patients has had limited research (Mancuso *et al.*, 2012; Gorman *et al.*, 2015). The definition of fatigue encompasses concepts such as tiredness and clinically relevant symptoms

such as exercise intolerance and muscle fatigue. Fatigue is a complex, multi-faceted symptom that encompasses physical, central, and general fatigue. Due to its complex and subjective nature fatigue remains difficult to evaluate.

Gait is becoming a useful tool in identifying markers of pathology, disease progression and in measuring the efficacy of interventions (Lord *et al.*, 2013a). Discrete features of gait, such as stride-to-stride variability, are highly sensitive to aging, pathology and early (pre-clinical) symptoms (Baltadjieva *et al.*, 2006; Verghese *et al.*, 2007; Rochester *et al.*, 2012; Rochester *et al.*, 2014b). To date, however, no detailed investigation of gait in patients with mitochondrial disease has been performed. Therefore this study provides a preliminary investigation into the use of gait measures in two mitochondrial genotypes. The study investigates whether gait characteristics relate to genotypes, heteroplasmy and disease burden, and then assesses whether gait characteristics are altered with the addition of a secondary task or fatigue. The results may provide a robust and sensitive measure of pathology, disease burden and treatment efficacy in this disease that will be useful to researchers and clinicians alike.

4.2 **Study aims and hypotheses**

Aims

1. To describe the pattern of gait impairments within two genetic types of mitochondrial disease (m.3243A>G and m.8344A>G).
2. To explore the relationships between gait characteristics, disease severity and other measures of physical performance such as exercise capacity and strength.

Primary Hypothesis

Gait characteristics will differ between mitochondrial and control groups.

Secondary hypotheses

1. The pattern of gait impairments will differ between the two genotypes.
2. Gait characteristics will be associated with underlying markers of disease severity (e.g. heteroplasmy) and its pathological consequences (e.g. proximal myopathy and ataxia).
3. Gait characteristics will be able to indicate fatigue within mitochondrial participants.

4.3 **Subject and Methods**

4.3.1 Subjects and study design

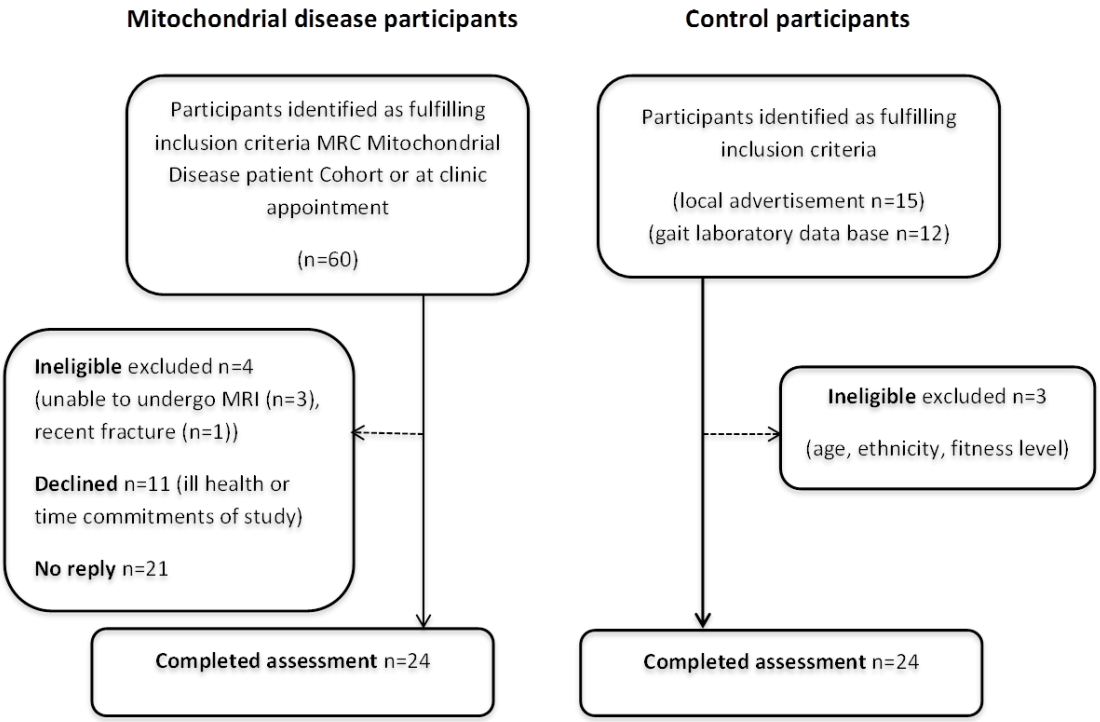
Patients with mitochondrial disease due to two genotypes (m.3243A>G and m.8344A>G) were recruited from the Newcastle Mitochondrial Disease database (Figure 4-1).

All eligible patients were invited to participate based on the following clinical inclusion criteria: (i) clinical stability for >6 months; (ii) ability to use an upright stationary bicycle ergometer; (iii) no current participation in regular physical activity (≤1 weekly session); (iii) reported exercise intolerance and fatigue, and (iv) ability to perform the walking assessment safely.

Exclusion criteria were the presence of known cardiac involvement, co-morbidities precluding exercise training, and contra-indications to Magnetic Resonance Imaging.

Twenty-four mitochondrial participants with the two genotypes (m.3243A>G, n=18; m.8344A>G, n=6) were recruited to participate from consecutive attendees at a mitochondrial clinic as part of an exercise intervention study (Bates *et al.*, 2013). A sample of age and sex matched control participants with normal ECG and no history of cardiovascular or metabolic disease were also recruited from local advertisement and the gait laboratory database (Rochester *et al.*, 2014b).

Figure 4-1: Consort Diagram for the cross sectional study of gait characteristics in mitochondrial disease.



The following sections include brief explanations of the methods used in the study. Full details of the investigations performed can be found within Chapter 2.

All assessments were undertaken between the two centres listed in the methods section over a period of two days. This was necessary due to the distance required to travel to the centres by some of the mitochondrial participants.

4.3.2 Disease Burden

All subjects underwent a physical examination by an experienced clinician, disease severity was assessed and disease burden was established using the Newcastle Mitochondrial Disease Adult Scale (Schaefer *et al.*, 2006) and mutation load.

4.3.3 Physical Activity

Physical activity and energy expenditure were assessed objectively using a validated multi-sensor array (SenseWear Pro_3, Bodymedia Inc, Pennsylvania, USA 2.5)(St-Onge *et al.*, 2007).

4.3.4 Anthropometry

Body weight (kg) and standing height (m) were measured. Whole body composition was determined using air displacement plethysmography using a BodPod (Life Measurement Inc., Concord, CA, USA).

4.3.5 Progressive Exercise testing

A stepped incremental workload test was performed using analysis of expired air gases and non-invasive bio reactance cardiac output. The test was performed on an electronically braked stationary bike at a steady cadence

between 60 and 80 rpm to elicit a symptom limited maximum oxygen uptake and heart rate response.

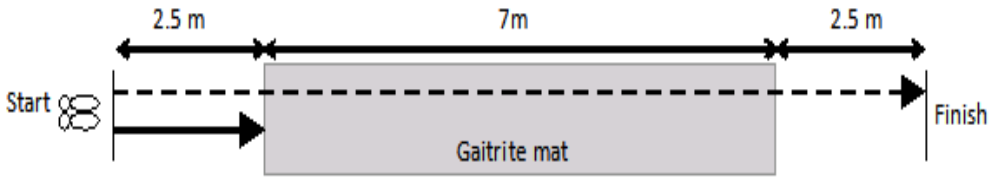
4.3.6 Muscle Strength

Proximal muscle power of the hip flexor and extensor muscles was measured using an isokinetic dynamometer (CSMI HUMAC®/NORM testing and rehabilitation system). Average peak torque was calculated for each muscle group from averaging the middle four repetitions.

4.3.7 Quantitative gait assessment

Gait was assessed in a gait laboratory setting along with other functional outcome measures (reported in Chapter 3). Gait was assessed using a 7m long × 0.6m wide instrumented mat (Platinum model Gaitrite, software version 4.5, CIR systems, USA). The mat was placed in the centre of the 12 m walkway to ensure that steady gait speed was measured (Figure 2-4).

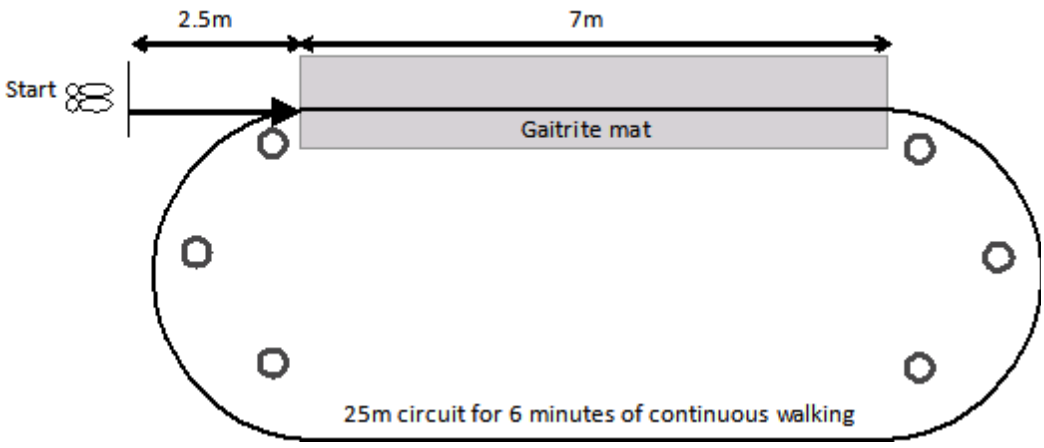
Figure 4-2: Gait laboratory set up for testing single and dual walks.



Participants were asked to perform 3 different walking tasks (single task, dual task and a continuous walk over 6 minutes). During the single task walks participants were instructed to perform three 12m walks at their “comfortable pace”. The concurrent task for the dual task walks consisted of a digit span task.

The 6 minute walk involved participants walking continuously around a 25m oval circuit. Instructions provided were as stated for the 6 minute timed walk, where participants were encouraged to walk as far as possible in 6 minutes (Society, 2002). Gait was repeatedly recorded as they walked over the mat placed on one side of circuit (Figure 4-3).

Figure 4-3: Gait laboratory set up for continuous 6-minute walk.



Gait was quantified using a predefined model hypothesized to reflect independent features of neural control and characterise the features of gait associated with mitochondrial disease and its genotypes (Lord *et al.*, 2013b). This model included: pace (step velocity and step length); rhythm (cadence); variability (step time and step length variability); asymmetry (step time asymmetry); and postural control (step width, step width variability, step length asymmetry). Gait variability, otherwise known as gait dynamics, was measured to characterise the step–to step fluctuations over the walking task. Gait variability was calculated for step length, step time and step width.

4.4 **Statistical analysis**

Due to the small numbers and variability in some parameters a conservative approach to statistical analysis was undertaken. Non-parametric Kruskal-Wallace tests were used to test for group differences in gait characteristics. Post-hoc Mann-Whitney *U* tests are recorded as significant post Holm-Bonferroni corrections (Rice, 1989).

Within group comparisons were performed using Wilcoxon Signed rank tests to assess the effect of performing a dual task on gait characteristics.

Associations between gait and disease severity, mutation load and pathophysiological variables were assessed using Spearmen Rho correlations. To avoid misleading findings we limited the correlation analysis between gait and NMDAS (total NMDAS score and subsections concerning, exercise tolerance, gait stability, cerebellar ataxia and myopathy). To accommodate for multiple correlations, only correlations with a $p < 0.01$ were considered to be statistically significant.

For completeness comparisons of all sections of the NMDAS are included in Appendix G.

4.5 **Results**

All consented participants attended for all gait assessments. Two mitochondrial participants were unable to complete the walks with the addition of a dual task due to an increased risk of falling. The same participants stopped walking prior to the end of the continuous 6-minute walk; therefore their data was excluded from continuous walk data. Three mitochondrial participants were unable to complete dynamometry testing due to ill health (diarrhoea, migraine and a negative response to exercise test earlier in the day). Data was available only for 12 control participants performing the 6 minute continuous walk as the 12 control participants obtained from the gait laboratory database had only completed single and dual task walks.

Of the 24 mitochondrial participants, 18 presented with the m.3243A>G mutation and 6 with the m.8344A>G mutation (as reported in chapter 3). Participant demographic and clinical symptoms are presented in Table 4-1. Both the NMDAS score and urinary mutation load were significantly lower in the m.3243A>G genotype than the m.8344A>G genotype (NMDAS, p = .023*, mutation load p = .001**).

Table 4-1: Individual and group demographic characteristics of patients with mitochondrial disease and control subjects. Data presented as median and (quartiles).

Group	Age	Sex	Height (cm)	Body mass (kg)	BMI (kg.m^{-2})	Mutation load (%)	NMDAS	Phenotype
m.3243A>G	50	f	169	63.4	22.2	34	3	Migraine, fatigue, hypothyroidism, myalgia, constipation, hypertension, dyslipidaemia, coeliac disease
	58	f	173	52.1	17.4	59	12	Hearing loss, ataxia, constipation, underweight, myopathy, myalgia, exercise intolerance
	58	m	186	89.8	25.9	66	30	Hearing loss, diabetes, ataxia, retinopathy, constipation, hypertension, dysarthria, myopathy, neuropathy, exercise intolerance
	39	m	172	74.6	25.2	80	17	Hearing loss, diabetes, migraine, fatigue, hypothyroidism, ataxia, constipation, exercise intolerance
	37	f	164	50.7	18.9	48	10	Hearing loss, diabetes, exercise intolerance, ataxia, dysarthria, asthma
	42	m	176	83.7	27.0	82	12	Hearing loss, diabetes, migraine, ataxia, depression, exercise intolerance
	42	f	154	49.0	20.7	43	15	Hearing loss, diabetes, ataxia, constipation, depression, exercise intolerance, short stature
	47	m	182	64.0	19.3	63	28	Hearing loss, diabetes, fatigue, ataxia, myalgia, depression, myopathy, neuropathy, ptosis, PEO
	38	f	164	54.2	20.1	53	11	Hearing loss, migraine, constipation, myopathy, fatigue, exercise intolerance, asthma
	22	m	183	59.2	17.7	89	17	Hearing loss, migraine, epilepsy, ataxia, constipation, underweight, exercise intolerance, fatigue
	53	f	164	59.1	22.0	22	10	Hearing loss, ataxia, retinopathy, constipation, myopathy, exercise intolerance
	25	m	174	49.8	16.5	90	26	Hearing loss, migraine, ataxia, retinopathy, constipation, depression, dysarthria, myopathy, exercise intolerance, short stature, asthma
	18	f	154	39.5	16.7	59	9	Hearing loss, retinopathy, underweight, myopathy, exercise intolerance, short stature
	24	f	155	64.0	26.7	72	27	Hearing loss, migraine, ataxia, retinopathy, constipation, depression, dysarthria, myopathy, exercise intolerance, short stature
	55	m	179	104.5	32.6	76	14	Hearing loss, diabetes, depression

	36	m	179	72.4	22.6	80	4	Migraine, exercise intolerance
	50	m	162	64.6	24.6	87	23	Hearing Loss, exercise intolerance, ataxia, myopathy, fatigue, depression, retinopathy, epilepsy, encephalopathy, cognitive decline, stroke-like episodes
	55	f	153	45.8	19.5	68	25	Hearing loss, diabetes, myopathy, exercise intolerance, ataxia, constipation, depression, retinopathy, PEO, ptosis, short stature, mild dysphagia, hypertension
m.8344A>G	46	m	163	70.9	26.7	77	19	Mild concentric LVH, lipomata, exercise intolerance, dysphagia, myoclonic jerks, ataxia, seizures, neuropathy, fasciculation's, constipation, hearing loss, myopathy, parenchymal lung disease
	28	m	173	51.7	17.3	95	55	Hearing loss, fatigue, epilepsy, ataxia, retinopathy, constipation, dysarthria, myopathy, myoclonus, neuropathy, exercise intolerance, underweight
	59	f	162	69.8	26.6	75	19	Myoclonus, deaf, diabetes, lipomata , myopathy, mild concentric LVH
	25	m	176	68.0	22.0	94	48	Epilepsy, ataxia, retinopathy, depression, dysarthria, exercise intolerance, myopathy, myoclonus
	28	m	180	56.2	17.3	93	35	Hearing loss, migraine, epilepsy, ataxia, constipation, underweight, depression, dysarthria, myopathy, neuropathy, exercise intolerance
	38	m	160	76.5	29.9	94	58	Lipomata, hearing loss, migraine, epilepsy, ataxia, retinopathy, depression, dysarthria, myopathy, myoclonus, neuropathy, exercise intolerance, short stature
m.3243A>G	42 (36 ,52)	f9 m9	170 (162 ,178)	61.3 (51.0 ,70.4)	21.3 (18.9 ,25.1)	67.0 (51.8 ,80.5)	15 (10, 25)	
m.8344A>G	33 (28 ,44)	f1 m5	168 (162 ,175)	68.9 (59.1 ,70.6)	24.3 (18.5 ,26.7)	93.5 (76.5 ,94.3)	42 (19 ,56)	
Control	41 (32 ,51)	f10 m14	170 (163 ,178)	75.4 (69.7 ,84.4)	25.2 (24.1 ,28.9)			

4.5.1 Gait impairments associated with mitochondrial disease

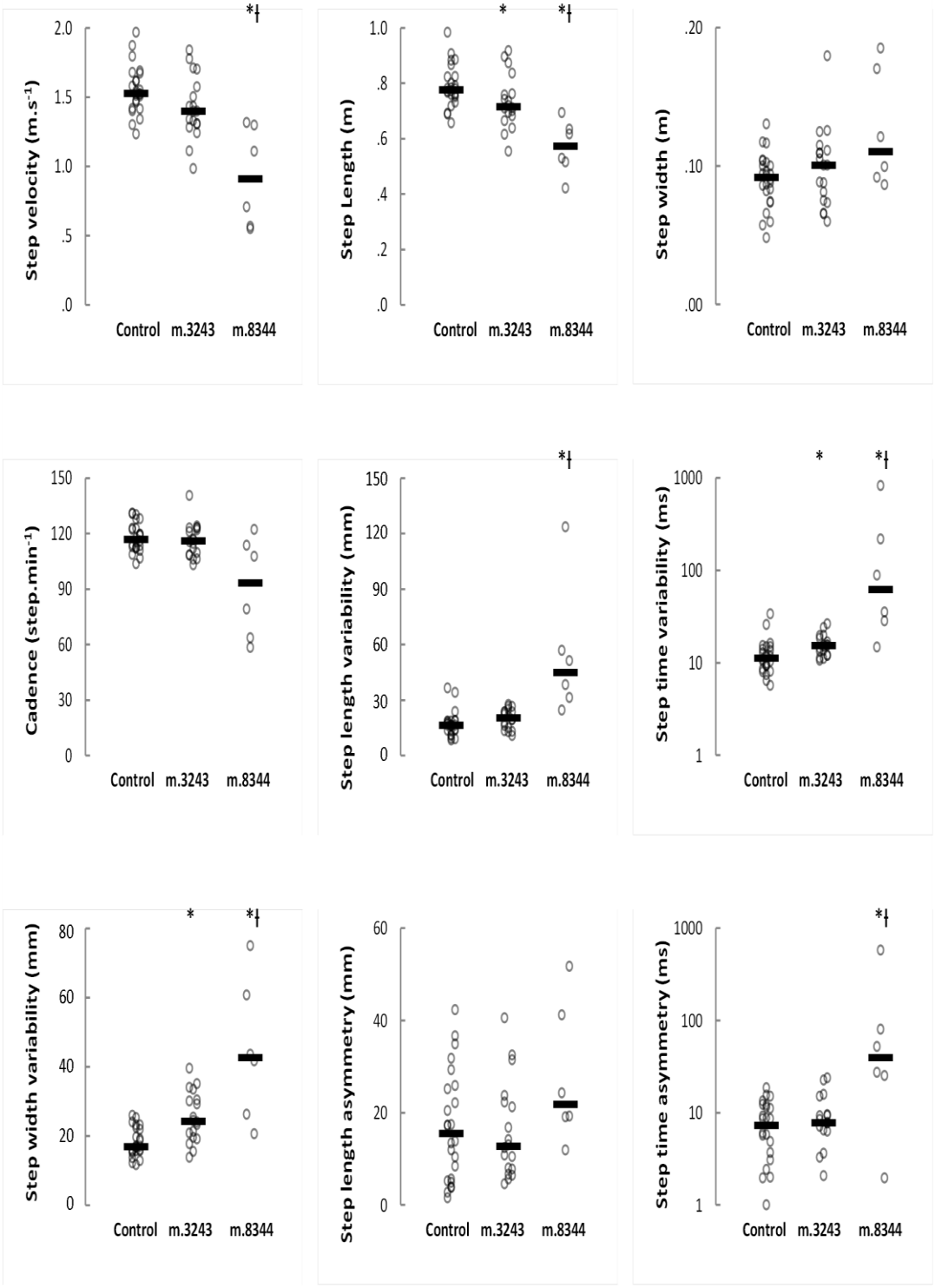
Gait characteristics in single task walking.

Participants with mitochondrial disease when compared to the control group demonstrated significantly reduced gait speed, step length and increased variability in step time, step length and step width when performing a single task walk (Table 4-2, Figure 4-4). However, when described by genotype, more selective gait impairments are seen, with the m.8344A>G participants demonstrating more globally impaired gait characteristics in all variables except cadence, step width and step length asymmetry when compared to both the control and the m.3243A>G group (Table 4-2, Figure 4-4). The m.3243A>G group demonstrated differences in only three gait variables when compared to controls, with a shorter step length and increased variability in step time and step width.

Table 4-2: Gait performance whilst performing a single task walk.
(MD n=24, controls n=24). Data presented as group medians (quartiles), raw p values presented. Significant group differences after Holm-Bonferroni correction are highlighted in bold type.

Gait Domain	Gait Variables	Controls	m.3243A>G	m.8344A>G	Kruskal-Wallis (*p*)	Mann Whitney U tests (*p*)			
						Controls & mtDNA disease	Controls & m.3243A>G	Controls & m.8344A>G	m.3243A>G & m.8344A>G
Pace	*Step velocity (m.s^{-1})*	1.52 (1.43, 1.66)	1.40 (1.30, 1.60)	0.91 (0.56, 1.30)	**.002**	**.002**	.042	**<.001**	**.003**
	Step length (m)	0.78 (0.75, 0.82)	0.71 (0.68, 0.78)	0.57 (0.49, 0.65)	**.001**	**.001**	**.024**	**<.001**	**.001**
Rhythm	*Cadence (steps.min^{-1})*	117 (112, 123)	116 (108, 123)	93 (62, 116)	**.046**	.155	.525	.021	.040
Asymmetry	*Step time asymmetry (ms)*	7.3 (2.6, 11.9)	7.8 (3.5, 10.8)	39.4 (19.2, 202.6)	**.035**	.149	.629	**.006**	**.009**
Variability	*Step length variability (mm)*	16.3 (13.5, 18.5)	20.3 (14.5, 23.9)	44.8 (29.6, 73.4)	**<.001**	**.004**	.067	**<.001**	**<.001**
	Step time variability (ms)	11.1 (8.6, 14.6)	15.2 (11.9, 18.5)	61.5 (24.8, 368.1)	**.005**	**< .001**	**.004**	**<.001**	**.002**
Postural control	*Step width (m)*	0.092 (0.076, 0.102)	0.100 (0.075, 0.112)	0.110 (0.090, 0.174)	.220	.112	.297	.065	.251
	Step width variability (mm)	16.9 (15.6, 22.3)	24.2 (19.4, 31.2)	42.6 (24.9, 64.3)	**.016**	**<.001**	**.001**	**<.001**	**.022**
	Step length asymmetry (mm)	15.5 (5.3, 25.6)	12.7 (7.4, 22.6)	21.7 (17.3, 43.8)	**.019**	.536	.980	.116	.056

*Figure 4-4: Individual and group gait performance of single task walking. The solid bar represents the median value with individual scores displayed around the median. * Indicates a significant difference compared to control participants. ł Indicates a significant difference between m.3243A>G and m.8344A>G genotypes. A p value<.05 is considered as significant after Holm-Bonferroni corrections were performed.*



Gait characteristics performing a dual task walk

Dual task walking was performed in 22 mitochondrial and 24 control participants (two mitochondrial participants with the m.8344A>G did not perform the dual task walk due to an undue risk of falling). No further gait characteristics were noted to be different between the groups than was reported performing single task walking. The m.8344A>G group continued to demonstrate a globally impaired gait pattern, with the m.3243A>G group demonstrating differences in step time and width variability when compared to controls (Table 4-3, Figure 4-5).

To investigate if performing another task whilst walking resulted in interference of gait, the study compared gait characteristics during single and dual task walking. Two gait characteristics, step velocity (p = 0.031) and step length (p = 0.026) demonstrated differences between the two walking tasks, with a reduction in step velocity and step length being reported on the performance of a secondary task (Table 4-4). To investigate if this interference was unique to the mitochondrial group the dual task interference value was calculated for these two variables. Dual task interference was calculated using the equation shown below (Rochester *et al.*, 2014a):

$$Dual\ task\ interference = \frac{dual\ task - single\ task}{single\ task}$$

No differences were demonstrated between the mitochondrial and control groups dual task interference scores (Step velocity: MD; median value, -0.06, IQR (-0.13, 0.05), Control; -0.06 (-0.12, 0.04), (p= .809). Step length: MD; -0.04 (-0.1, 0.02), Control; -0.03 (-0.12, 0.04), (p= .930).

Table 4-3: Gait performance whilst performing a dual task walk.
(MD n=22, Controls n=24). Data presented as group medians (quartiles) raw p values presented. Significant group differences after Holm-Bonferroni correction are highlighted in bold type.

Gait Domain	Gait Variables	Controls	m.3243A>G	m.8344A>G	Kruskal-Wallis (*p*)	Mann Whitney U tests (*p*)			
						Controls & mtDNA disease	Controls & m.3243A>G	Controls & m.8344A>G	m.3243A>G & m.8344A>G
Pace	*Step velocity (m.s^{-1})*	1.46 (1.36, 1.53)	1.35 (1.18, 1.48)	1.12 (0.59, 1.33)	**.016**	.021	.104	**.004**	.098
	Step length (m)	0.75 (0.69, 0.80)	0.71 (0.63, 0.75)	0.62 (0.52, 0.65)	**.005**	**.008**	.057	**<.001**	.066
Rhythm	*Cadence (steps.min^{-1})*	115 (109, 121)	116 (107, 120)	107 (66, 124)	.324	.218	.374	.186	.434
Asymmetry	*Step time asymmetry (ms)*	5.2 (2.6, 9.9)	8.1 (3.9, 16.1)	15.1(12.6, 29.16)	**.032**	.062	.232	.013	.066
Variability	*Step length variability (mm)*	17.9 (14.6, 21.5)	21.7 (16.9, 26.5)	27.2 (23.7, 43.3)	**.022**	.029	.127	**.007**	.098
	Step time variability (ms)	12.65 (10.15, 14.86)	15.3 (13.0, 21.7)	25.1 (18.7, 169.9)	**.002**	**.002**	**.013**	**.001**	**.042**
Postural control	*Step width (m)*	0.091 (0.079, 0.105)	0.096 (0.075, 0.120)	0.092 (0.088, 0.106)	.780	.482	.509	.728	.967
	Step width variability (mm)	17.2 (13.7, 21.2)	25.7 (19.2, 29.9)	27.9 (22.2, 33.6)	**.001**	**<.001**	**.001**	**.005**	.652
	Step length asymmetry (mm)	16.3 (2.9, 26.9)	15.8 (4.5, 23.0)	23.1 (8.6, 50.1)	.627	.598	.819	.355	.484

Figure 4-5: Individual and group gait performance of dual task walking.
*The solid bar represents the median value with individual scores displayed around the median. * Indicates a significant difference compared to control participants. ǂ Indicates a significant difference between m.3243A>G and m.8344A>G genotypes. A p value<.05 is considered as significant after Holm-Bonferroni corrections were performed.*

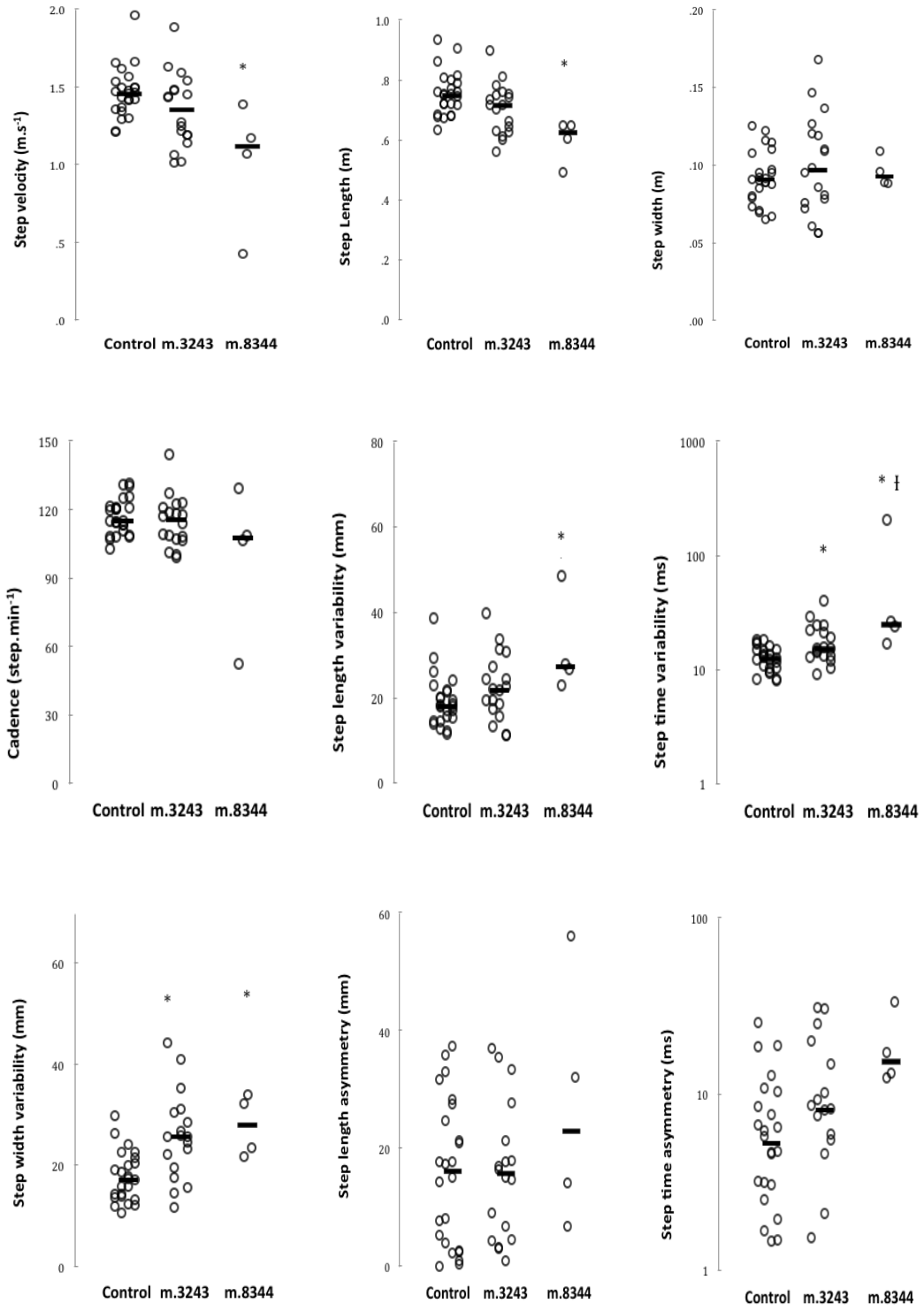


Table 4-4: Comparisons of single and dual task walking on gait characteristics of mitochondrial participants.
A p value<.05 is considered significant and is shown in bold type.

Gait variables	Median (Quartiles)		Wilcoxon rank test
	Single task walking	**Dual task walking**	***P* value**
Step Velocity (m.s^{-1})	1.33 (1.27, 1.52)	1.26 (1.12, 1.48)	**.031**
Step length (m)	0.70 (0.64, 0.76).	0.68 (0.62, 0.75)	**.026**
Cadence (steps min^{-1})	115 (108, 123)	112 (107, 121)	.131
Step time asymmetry (ms)	8.1 (3.5, 17.3)	9.0 (5.3, 17.9)	.733
Step length variability (mm)	22.8(15.9, 26.5)	22.7(18.0, 28.5)	.485
Step time variability (ms)	15.4 (13.0, 20.9)	16.5 (13.0, 24.0)	.758
Step width (m)	0.099 (0.079, 0.112)	0.095 (0.077, 0.119)	.987
Step width variability (mm)	24.9 (20.4, 33.6)	25.7 (21.1, 31.4)	.506
Step length asymmetry (mm)	15.4 (7.9, 23.8)	15.8 (6.2, 28.9)	.758

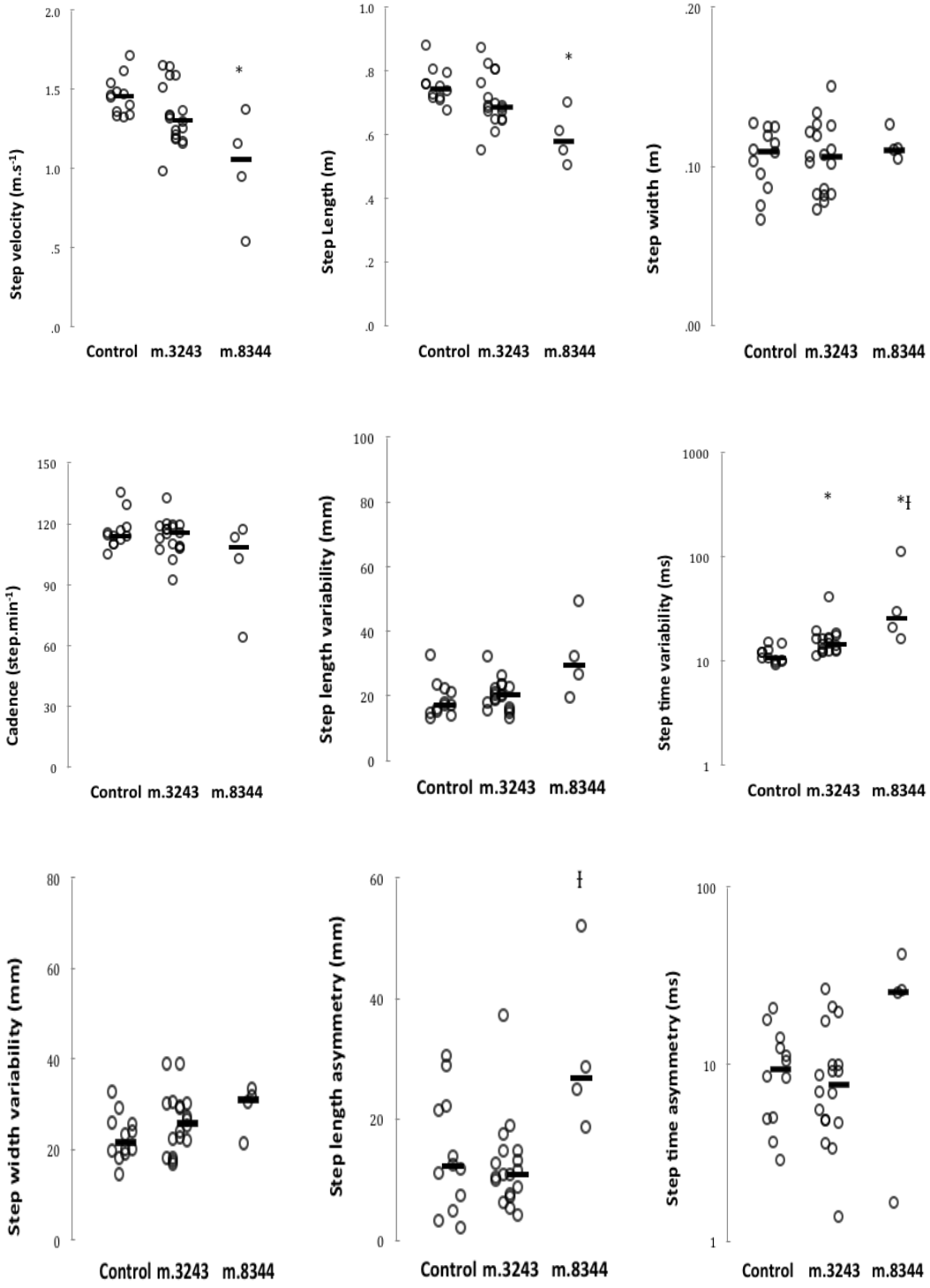
Gait characteristics during a continuous 6 minute walk.

Twelve control and 22 mitochondrial participants performed the continual walk over 6 minutes. Step time variability remained as a gait characteristic demonstrating differences between the control and m.3243A>G group. Other variables such as step length and velocity remained different but failed to reach statistical significance due to multiple comparisons. The m.8344A>G group continued to demonstrate differences in step time variability, step velocity and step length (Table 4-5, Figure 4-6).

Table 4-5: Differences in gait performance when performing a 6-minute walk. (MD n=22, controls n=12). Data presented as group medians (quartiles) raw p values presented. Significant group differences after Holm-Bonferroni correction are highlighted in bold type.

Gait Domain	Gait Variables	Controls (n=12)	m.3243A>G (n=18)	m.8344A>G (n=4)	Kruskal-Wallis (*p*)	Mann Whitney U tests (*p*)			
						Controls & mtDNA disease	Controls & m.3243A>G	Controls & m.8344A>G	m.3243A>G & m.8344A>G
Pace	*Step velocity ($m.s^{-1}$)*	1.46 (1.34, 1.53)	1.31 (1.19, 1.48)	1.05 (0.64, 1.32)	**.011**	**.011**	.035	**.013**	.066
	Step length (m)	0.75 (0.72, 0.79)	0.69 (0.65, 0.75)	0.58 (0.52, 0.68)	**.009**	**.010**	.043	**.002**	.081
Rhythm	*Cadence ($steps.min^{-1}$)*	114 (111, 118)	116 (108, 119)	108 (74, 116)	.340	.534	.787	.212	.195
Asymmetry	*Step time asymmetry (ms)*	9.4 (4.9, 13.6)	7.8 (4.8, 9.9)	25.6(7.5, 38.0)	.269	1.000	.602	.170	.166
Variability	*Step length variability (mm)*	17.1 (14.7, 22.0)	20.2 (16.2, 22.7)	29.6 (21.5, 45.2)	**.042**	.094	.232	.030	.042
	Step time variability (ms)	10.61 (9.95, 12.56)	14.52 (12.39, 16.48)	25.44 (17.47, 90.67)	**<.001**	**<.001**	**<.001**	**.001**	**.014**
Postural control	*Step width (m)*	0.109 (0.088, 0.122)	0.107 (0.082, 0.125)	0.111 (0.106, 0.123)	.753	.790	.917	.599	.484
	Step width variability (mm)	21.6 (19.2, 25.9)	25.9 (21.1, 29.8)	31.1 (23.6, 32.9)	.115	.102	.215	.058	.227
	Step length asymmetry (mm)	12.1 (5.5, 22.0)	10.7 (7.4, 14.4)	26.7 (20.1, 46.2)	**.033**	.845	.642	.058	**.003**

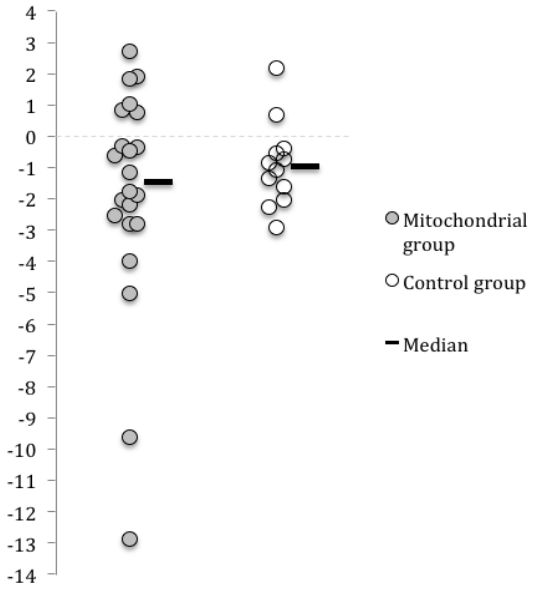
Figure 4-6: Individual and group gait performance of a long continuous walking task.
*The solid bar represents the median value with individual scores displayed around the median. *Indicates a significant difference compared to control participants. ł Indicates a significant difference between m3243A>G and m.8344A>G genotypes.*



4.5.2 Changes in gait characteristics over a 6-minute continuous walk.

To investigate whether gait characteristics in the mitochondrial group altered over a prolonged walk (6 minutes) due to muscle fatigue, comparisons were made between gait characteristics during the first and last two minutes of a six-minute walk. The gait characteristic of cadence was the only characteristic to report a statistical difference between these two time points (p = .022) with a reduction in cadence seen over time (Table 4-6, Figure 4-7).

Figure 4-7: Cadence change scores over a 6-minute walk.



A change in cadence was also reported in the control group (p = .041). Comparison of the change in cadence between both groups was unable to demonstrate a difference between groups (p = .736). However four participants with mitochondrial disease demonstrate a decrease in cadence greater than seen in the control group.

Table 4-6: Changes in gait characteristics in participants with mitochondrial disease over a six-minute walk. Data presented as group medians (quartiles), raw p values presented.

Gait domain	Gait variables	Minute 1&2	Minute 5&6	*p* value
Pace	Step Velocity (ms^{-1})	1.31 (1.19, 1.39)	1.27 (1.15, 1.42)	0.223
	Step length (m)	0.68 (0.64, 0.72)	0.69 (0.62, 0.74)	0.685
Rhythm	Cadence (steps min^{-1})	115 (107, 119)	114 (106, 118)	0.022
Asymmetry	Step time asymmetry (ms)	10.56 (5.28, 17.16)	8.49 (1.35, 20.25)	0.884
Variability	Step length variability (mm)	21.1 (17.5, 28.1)	19.8 (15.2, 26.6)	0.223
	Step time variability (ms)	17.88 (14.33, 20.66)	16.85 (12.55, 20.23)	0.355
Postural Control	Step width (m)	0.107 (0.089, 0.120)	0.110 (0.086, 0.124)	0.189
	Step width variability (mm)	27.1 (21.3, 34.5)	24.4 (21.2, 30.0)	0.211
	Step length asymmetry (mm)	12.8 (5.8, 23.3)	11.7 (7.8, 22.13)	0.709

4.5.3 Relationships between gait characteristics and clinical disease burden

To examine the relationship between gait characteristics of participants with mitochondrial disease and clinically measured disease burden the data from both genotypes were pooled (n = 24). The NMDAS scale has 29 questions investigating all aspects of mitochondrial disease. In this study relationships were investigated between gait characteristics, total disease burden and sections of the NMDAS that were hypothesised to affect gait characteristics. This limited the number of comparisons performed. Analyses on all sections of the NMDAS scale were performed for completeness and are shown as an appendix (Appendix G).

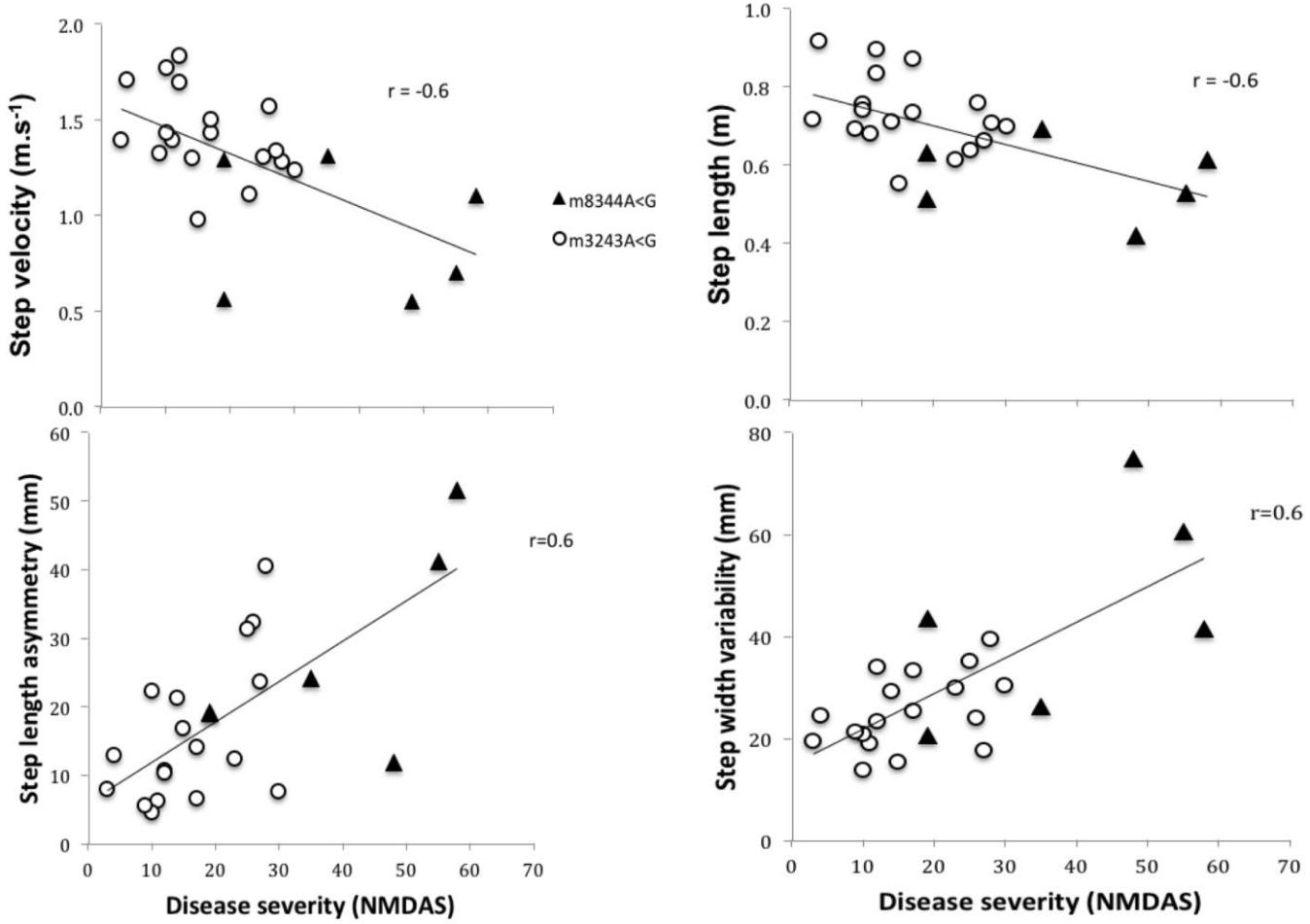
Disease burden (total NMDAS score) showed a moderate indirect relationship with walking speed (r = -0.6) and step length (r = -0.6), both within the pace domain. This, therefore, demonstrated that mitochondrial subjects with a higher disease burden walked at a slower speed with a shorter step length. Direct relationships were also shown between disease burden and the postural control domain, with associations demonstrated with step width variability (r =0.6) and step length asymmetry (r = 0.6). These relationships demonstrated that gait became more variable and asymmetric with increased disease burden (Table 4-7, Figure 4-8).

Investigations of the sub sections within the NMDAS highlighted additional gait features in respect to variability and asymmetry (Table 4-7). In addition cerebellar ataxia and gait stability demonstrated moderate correlations with all but two gait variables.

Table 4-7: Relationships between gait characteristics, disease severity (total NMDAS score) and individual NMDAS subsections. Significant correlations are presented in bold type (p ≤ 0.01, two-tailed)

Gait Domain	Gait Variables	NMDAS	NMDAS Subscale			
			Exercise tolerance	Gait stability	Myopathy	Cerebellar ataxia
Pace	*Step Velocity (ms^{-1})*	**-0.6 (.001)**	**-0.7 (<.001)**	**-0.6 (.006)**	-0.4 (.054)	-0.4 (.039)
	Step length (m)	**-0.6 (.003)**	**-0.7 (<.001)**	**-0.6 (.004)**	-0.5 (.014)	-0.4 (.068)
Rhythm	*Cadence (steps min^{-1})*	-0.4 (.042)	**-0.3 (.096)**	-0.4 (.048)	-0.1 (.937)	-0.3 (.160)
Asymmetry	*Step time asymmetry (ms)*	0.5 (.024)	0.5 (.016)	0.4 (.029)	**0.5 (.007)**	0.4 (.034)
Variability	*Step length variability (mm)*	0.3 (.110)	0.5 (.010)	0.5 (.024)	0.2(.374)	**0.5 (.009)**
	Step time variability (ms)	0.1 (.506)	0.5 (.012)	0.3 (.114)	0.1 (.626)	0.5 (.010)
Postural control	*Step width (m)*	0.4 (.081)	0.4 (.087)	0.4 (.033)	0.3(.214)	0.4 (.039)
	Step width variability (mm)	**0.6 (.002)**	0.4 (.078)	**0.6 (.002)**	0.4 (.071)	**0.6 (.001)**
	Step length asymmetry (mm)	**0.6 (.001)**	0.4 (.032)	0.3 (.174)	**0.6 (.001)**	0.4 (.029)

Figure 4-8: Scatter plots of the relationships between total disease severity score (NMDAS) and gait characteristics.



4.5.4 Relationships between gait characteristics and pathophysiological measures of disease burden.

Explorations of a number of pathophysiological markers of mitochondrial disease with respect to gait characteristics were performed. Increased mutation load demonstrated a moderate relationship with increased step time asymmetry ($r = 0.6$) and step width variability ($r = 0.6$) (Table 4-8). The relationships between gait characteristics, energy expenditure, peak exercise capacity and muscle strength were less clear. Moderate relationships were seen between step length and velocity with impaired proximal muscle strength, peak exercise capacity and energy expenditure, denoting a relationship with the domain of pace (Table 4-8). However, these were not deemed to be significant due to multiple comparisons. No other gait variables demonstrated relationships with the pathophysiological characteristics of disease.

Table 4-8: Relationships between mutation load, energy expenditure, peak exercise capacity muscle strength variables and gait outcomes in people with mitochondrial disease.
Significant correlations are presented in bold type (p ≤ 0.01, two-tailed)

Gait Domain	Gait Variables	Mutation load (%)	Community energy expenditure (calories)	Exercise capacity (peak oxygen consumption) (ml.kg.min)	Hip flexor strength (nm/kg)	Hip extensor strength (nm/kg)
Pace	*Step velocity*	-0.3 (.164)	0.3 (.157)	0.5 (.019)	0.4 (.045)	0.5 (.039)
	Step length	-0.2 (.285)	0.4 (.039)	**0.6 (.002)**	0.5 (.017)	0.5 (.019)
Rhythm	*Cadence*	-0.4 (.049)	<-0.1 (.918)	0.2 (.405)	0.1 (.773)	0.2 (.404)
Asymmetry	*Step time asymmetry*	**0.6 (.004)**	-0.1 (.673)	-0.3 (.109)	-0.3 (.128)	-0.5 (.037)
Variability	*Step length variability*	0.4 (.064)	-0.2 (.396)	-0.2 (.328)	-0.2 (.430)	-0.2 (.454)
	Step time variability	0.2 (.474)	-0.3 (.239)	-0.2 (.473)	-0.3 (.159)	-0.2 (.378)
Postural control	*Step width*	0.1 (.674)	0.2 (.359)	<-0.1 (.990)	-0.1 (.767)	<-0.1 (.929)
	Step width variability	**0.6 (.002)**	0.1 (.754)	-0.2 (.440)	-0.3 (.140)	-0.3 (.205)
	Step length asymmetry	0.4 (.058)	0.1 (.723)	-0.3 (.129)	-0.2 (.431)	-0.2 (.369)

4.6 Discussion

Mitochondrial diseases result in a wide variety of symptoms that can lead to gait impairment; previous studies in this patient group have looked at the clinical manifestations of disease by assessing exercise capacity and muscle strength. This study is the first to attempt to quantify gait in a group with genetically confirmed mitochondrial disease. A previously reported model was used to capture the complexity of gait. The use of this model enabled the identification of patterns of gait deficits that reflect the underlying pathophysiology and differentiate between a group of participants with mitochondrial disease and disease free controls, and between different mitochondrial genotypes.

The key findings of the study are that gait characteristics differed in a sensitive and selective manner between patients with mitochondrial disease and disease free participants. Gait characteristics were also able to discriminate between the two mitochondrial genotypes; those with the m.8344A>G demonstrating global gait impairment whereas, within a mildly affected m.3243A>G subgroup, this was reduced to a discreet number of gait variables. These variables did not change with the addition of another task whilst walking or when the length of the walk was extended. The relationships demonstrated between gait, disease severity, and pathophysiological markers, support the future use of gait analysis as a robust clinical measure of disease severity in mitochondrial patients.

4.6.1 Gait deficits associated with mitochondrial disease and genotype.

Differences in gait characteristics were demonstrated in both the mitochondrial disease group and the two genotypes when compared to controls. The m.8344A>G genotype group was globally impaired with differences evident in

all gait characteristics except cadence, step width and step length asymmetry. The global impairment in the m.8344A>G group is unremarkable given the high number of clinical symptoms and high level of disease burden reported in this group. The m.3243A>G group however demonstrated fewer gait characteristics that differed from the control group, reflecting their higher level of ability. Despite their high functioning, differences were highlighted in maintaining adequate pace (step length), low levels of variability (step time variability) and postural control (step width variability) (Galna *et al.*, 2013a). These results suggest that different pathological processes may result in gait impairments even in a mildly affected group of the m.3243A>G genotype and these patients can be differentiated by their gait characteristics.

When participants performed a secondary task whilst walking, no further gait characteristics were observed to be different between the groups. The addition of a dual task whilst walking unsurprisingly affected pace in the mitochondrial group, but a similar response was also reported in the control group and has been reported in other populations (Kelly *et al.*, 2012a).

The addition of another task whilst walking aims to provide further stress to the systems controlling locomotion. This added stress aims to reduce any cognitive compensation employed to maintain walking performance and hence dual task walking is able to reveal latent motor deficits (Yogev-Seligmann *et al.*, 2008; Kelly *et al.*, 2012b). The use of dual task testing during gait assessment and how it can be interpreted remains under debate, with the type of dual task and the emphasis given to the dual task during walking affecting results (Kelly *et al.*, 2010; Al-Yahya *et al.*, 2011). Walking at a pace other than preferred speed has also been shown to be a more sensitive measure of pathology and reveal

deficits in motor ability (Mirelman *et al.*, 2011; Verghese *et al.*, 2012). Whilst the dual task testing appears to provide consistent results in conditions such as Parkinson Disease, with resultant increases in variability and reduced speed (Kelly *et al.*, 2012a), dual task interference is also a feature of healthy ageing and its value in predicting clinical problems such as falls has been questioned (Smulders *et al.*, 2012).

An explanation for the lack of any further gait impairment becoming apparent with dual tasking in this study may be due to the type and difficulty of the dual task and the attention given to that task. The dual task performed may not provide enough stress to reduce any cognitive compensation in participants. This is unlikely in this study as the difficulty of the cognitive task was calculated for each participant, in a non-challenging sitting position, prior to walking. Also participants in this study were not instructed to give more attention to the cognitive task whilst walking. Despite this an error rate of only 23% was reported in recalling the digit span, which demonstrates that attention was given to the cognitive task whilst walking. Another possible reason for the lack of effect of a dual task was the removal of two of the most affected participants (both with the m.8344A>G mutation) from performing the dual task walk due to a risk of falling. Inclusion of these participants in the single task data may have increased the gait disturbances reported during single task walking, although this would not explain why no further changes in gait were observed when only the m.3243A>G group were considered. It should also be noted that the m.3243A>G group were a high functioning group and therefore cognitive compensation may not have been necessary to maintain gait performance.

In summary the performance of a dual task did not appear to provide any additional information concerning gait impairments in this cohort of patients. Further work may be warranted to investigate if this remains the case in all genotypes and also whether the type of dual task performed has an effect.

The measurement of gait characteristics during a continuous walk were performed to reveal whether gait altered as walking became more automatic during a longer walk versus a short intermittent walk (Bilney *et al.*, 2003; Lindemann *et al.*, 2008; Paterson *et al.*, 2009) and to investigate if the characteristics of gait changed with fatigue. Although fewer gait impairments were noted during a continuous walk, this was not due to changes in gait characteristics within the group of mitochondrial patients, but due to an increased variability in a smaller control group performing the continuous walk test (n = 12 vs. n = 24). The difference in the performance of the smaller control group makes comparisons between the three walking tasks more difficult, although gait characteristics across all walking tasks appear to be similar.

The similarities in the gait deficits reported across the three walking tasks provide reassurance as to the robust nature of gait assessment under different situations. These similarities may allow us to reduce participant burden in future investigations by reducing the number of walks performed. In a patient group that has a high incidence of reported fatigue this may prove beneficial.

4.6.2 Does gait reflect the burden of disease?

The exploration of relationships between gait and clinical disease severity (NMDAS) for the total mitochondrial group (m.3243A>G and m.8344A>G) demonstrated patients with a higher disease burden had greater gait

impairment. Moderate associations (r = 0.4 - 0.6) were demonstrated with all gait characteristics except step time variability, although some failed to reach statistical significance due to the conservative approach to statistical analysis. The gait characteristics demonstrating significant associations with disease burden were not the same characteristics that were able to differentiate between the mitochondrial and control groups. This is likely to be due to the fact that they are measuring different impairments. However, gait speed, step length and step width variability were able to differentiate between the mitochondrial disease group and controls, and were associated with disease burden. Hence these three variables provide a selective group of characteristics that may be valuable when assessing relationships between gait and disease burden in patients with mitochondrial disease.

The examination of associations between gait and individual questions within the NMDAS demonstrated relationships between exercise capacity, gait and cerebellar sub-scales highlighting the underlying pathophysiological contribution. Cerebellar ataxia demonstrated moderate relationships with all variables within the asymmetry, variability and postural control domains. The reporting of gait asymmetry as the only significant characteristic to demonstrate a relationship with myopathy is difficult to explain, especially as the associations reported between hip muscle strength and pace seem to contradict this finding. These conflicting results may be due to the low level of sensitivity within the myopathy question of the NMDAS, where muscle strength is measured using manual muscle tests (Sapega, 1990) as opposed to the gait measures. Another possible explanation may be the inclusion of upper and lower limb muscle

strength in the rating scale, although this is unlikely as myopathy in mitochondrial disease is usually widespread and not limited to isolated limbs.

4.6.3 Does gait reflect the pathological consequences of mitochondrial disease?

To explore the relationship between gait characteristics and pathology the study examined the relationships between gait and mutation load, baseline community energy expenditure, exercise capacity and hip strength. The m.3243A>G group were a high performing group demonstrating normal gait speed and therefore early gait impairments may relate to disease pathology, unlike the m.8344A>G group who were globally impaired which reflects a combination of disease pathology and subsequent compensations. Therefore this study concentrated on the m.3243A>G group for the exploration of pathophysiological consequences of disease.

Relationships were reported between heteroplasmy levels and the gait characteristics of increased step width variability and step time asymmetry. Step width variability was also significantly greater in the m.3243A>G when compared to controls. It is possible that this feature of gait may reflect the underlying pathological changes and warrants further investigation.

Ataxia is a common clinical symptom in patients with mitochondrial disease (Turnbull *et al.*, 2010) and cerebellar impairment is a major cause of gait ataxia. Previous gait studies have demonstrated that balance impairment and increased gait variability are important findings in people with ataxia (Morton and Bastian, 2004; Ilg *et al.*, 2007; Serrao *et al.*, 2012; Rochester *et al.*, 2014b). Gait variability describes the size of stride-to-stride fluctuations in walking and is

typically less than 5% in a healthy population with a smooth and consistent stepping motion (Schniepp *et al.*, 2012). The cerebellum plays a key role in the control of timing and coordination of gait, the finding of increased gait variability is a possible result of cerebellar atrophy (Spencer *et al.*, 2003; Diedrichsen *et al.*, 2007). The findings of this study imply that cerebellar impairment is underpinning the gait disturbance observed in mitochondrial disease, with early changes in the generation of consistent steps leading to increased variability. The increased variability in step width demonstrates lateral instability and is implicated as a marker of impaired postural control within the gait model and studies within older adults (Brach *et al.*, 2005). Given the clinical presentation of these genotypes (Chinnery *et al.*, 1997; Mancuso *et al.*, 2013; Nesbitt *et al.*, 2013) it is probable that muscle pathology and ataxia, either in isolation or in combination, are likely to contribute to the subtle gait changes observed. This is supported by the significant correlations seen with the cerebellar domain within the NMDAS and the selective association with mutation load.

Muscle strength is often reflected in gait by step length (Schulz *et al.*, 2007) which enables an adequate pace to be maintained during walking. Reduced step length in the mitochondrial group is likely to be a consequence of muscle weakness. This is supported by the moderate relationships of peak exercise capacity and hip muscle strength with step length, although it must be noted that hip muscle strength, despite demonstrating a moderate relationship with step length, did not demonstrate a significant relationship due to multiple comparisons. The lack of significant correlations with hip strength could be a result of the robust nature of gait to muscle weakness (van der Krogt *et al.*, 2012) and the position used for muscle strength testing (e.g. lying as opposed

to standing), which may have affected results. However, these results suggest that adequate muscle strength contributes to the maintenance of step length and walking speed in participants with mitochondrial disease.

4.6.4 Does fatigue affect gait characteristics?

This study hypothesized that gait characteristics would be able to detect muscle fatigue over a prolonged walk. Despite fatigue or exercise intolerance being present in all of the participants with mitochondrial disease, this study was unable to detect changes in gait characteristics over a 6-minute walk. Within an Italian cohort of mitochondrial patients fatigue was reported as the 4th most common symptom, with 35% of patients with the m.3243A<G mutation reporting fatigue (Mancuso *et al.,* 2012). A Newcastle cohort reported fatigue that was even higher at 62 % (unpublished data). The differences in the prevalence of fatigue between these two cohorts is likely due to the different methods of assessing “fatigue” which remains difficult to define. The inability of gait variables to detect fatigue was disappointing but may be due to a number of factors: Firstly participants were asked to walk as far as they could in 6 minutes and provided with a prompt every minute. Receiving a verbal external prompt may have allowed the participants to pace themselves over the 6-minute walk. This pacing may have led to a submaximal performance of the test and therefore participants did not exhibit fatigue. The submaximal performance of the 6-minute walk by neuromuscular participants has been reported previously and protocols therefore have been adapted to include increased verbal encouragement, to ensure a maximal performance (McDonald *et al.*, 2010). Secondly, the length of walk may also have been a limiting factor in such a high functioning patient group but a more lengthy investigation was not possible in

this study due to the already high participant burden. Therefore, fatigue remains difficult to assess, further work is required before measurement of gait can be used as a potential marker of fatigue.

4.7 **Limitations**

Although this study is the first to provide a description of gait impairment in mitochondrial disease, it is still unknown how gait deteriorates over the lifetime of the patient or how responsive gait impairments are to potential therapeutic intervention. Also as part of this study it was only possible to investigate two genotypes and in very small numbers therefore it is not possible to extend the findings to other forms of mitochondrial disease. However, given that ataxia is documented in a third of our centre's cohort of patients with less than half of these cases attributable to the genotypes studied here, it is possible that the findings are transferable to other genotypes. Further investigation is, however, required.

This study was limited to two genotypes to reduce heterogeneity within the mitochondrial group; despite this the two genotypes appeared to present very differently when gait was assessed. This limitation was addressed by looking at the genotypes together and also separately. This method of analysis had the consequences of reducing the number in the m.8344A>G group dramatically and resulting in some comparisons being non-significant due to multiple comparisons.

4.8 **Conclusion**

As commented in the Cochrane review, identification of sensitive outcomes is essential for trials investigating mitochondrial disease. This data shows that gait deficits are associated with mitochondrial disease and genotype in a selective pattern. These gait deficits also seem to reflect the clinical burden of disease and its pathological consequences.

Gait is easily quantifiable and sensitive to change in other clinical populations. Therefore, quantification of discrete gait characteristics in patients with mitochondrial disease may improve the measurement of disease burden and provide further information concerning underlying pathology and progression. The results of this study suggest that further longitudinal investigations of gait are warranted as well as the investigation of the effect of gait deficits in mitochondrial disease on community mobility.

Chapter 5 An overview of the measures used in the observational studies included in this thesis

5.1 Introduction

The measures used in the studies in this thesis span the International Classification of Functioning, Disability and Health (World Health Organization, 2001), with measurements extending from impairments such as exercise intolerance, to activities such as walking, and participation, via quality of life questionnaires. The relationships of these measures to disease severity have already been discussed in the previous results chapters. Their ability to detect change post an exercise intervention will be evaluated in the next two chapters.

This chapter will review the accuracy of the clinical measures used in this thesis in recognising patients with mitochondrial disease and differentiating between mitochondrial genotypes. The results will provide valuable information concerning the sensitivity and specificity of measures and will help researchers and clinicians to use appropriate measures in future trials.

5.2 Subjects and methods

The subjects and methods used are reported in chapter 3 and 4 of this thesis. The measures evaluated are: Exercise capacity, proximal muscle strength, functional outcome measures (5XSTS, TUG, 10MTW, 6MWD) and the gait characteristics at baseline.

5.3 Statistical analysis

The sensitivity (true positive rate, TPR) and specificity (true negative rate, TNR) of the outcomes measured in the studies were analysed using Receiver operating characteristic (ROC) curve analysis.

Sensitivity was calculated using the equation below (TP = true positives, FN = false negatives);

$$TPR = \frac{TP}{TP + FN}$$

Specificity was calculated using the equation below (TN= true negative, FP = false positive);

$$TNR = \frac{TN}{TP + FP}$$

From this the area under the curve (AUC) was calculated using SPSS version 20.

Accuracy (ACC) was derived using the following equation (P = positives, disease group, N=negatives, disease free group);

$$Acc = \frac{TP + TN}{P + N}$$

5.4 **Results**

As reported previously all participants attended for all assessments although three participants were unable to complete muscle strength testing due to ill health.

The following tables and graphs will report the sensitivity, specificity and accuracy of measures in predicting whether participants have mitochondrial disease and also if the measures have the ability to distinguish between the two mitochondrial genotypes. Table 5-1 demonstrates that a number of measures can be described as good or excellent (AUC > 0.8), with the measure of sit to stand showing high levels of sensitivity and specificity. When investigating the

ability of these measures to discriminate between genotypes four measures were deemed excellent (AUC > 0.9).

Table 5-1: The relative accuracy, sensitivity and specificity of measures used within this thesis to discriminate between the control and disease groups.
ŧCut off chosen at the point of maximum accuracy. Where more than one point had the same accuracy, the most sensitive cut-off value was chosen. AUC - Area under the curve. (MD; n=24, Controls; n=12)

Variable	Cut off point ŧ	Accuracy	Sensitivity	Specificity	Area Under the Curve	p value
Five times Sit to Stand (secs)	> 8	89%	92%	83%	0.934	< .001
Arteriovenous O_2 difference (ml O_2/dl)	<19.4	83%	96%	58%	0.848	.001
Timed Up and Go (secs)	> 7	81%	79%	83%	0.840	.001
Peak Exercise Capacity (ml/kg/min)	< 23.5	81%	83%	75%	0.826	.002
Hip flexor strength (nm/kg)	<1.2	81%	91%	58%	0.819	.003
Step length (m)	<0.76	81%	83%	75%	0.813	.003
Step width variability (mm)	>17	78%	92%	50%	0.809	.030
Hip extensor strength (nm/kg)	<2.0	81%	86%	67%	0.804	.004
Step velocity ($m.sec^{-1}$)	<1.51	78%	80%	75%	0.802	.004
10m walk duration (secs)	>7	78%	70%	92%	0.778	.007
Anaerobic threshold (ml/kg/min)	<17.6	75%	91%	42%	0.754	.016
6 minute walk distance (m)	<440	86%	100%	54%	0.752	.015
Step time variability (ms)	>11	78%	100%	33%	0.719	.035
Cadence ($steps.min^{-1}$)	<127	69%	96%	17%	0.660	.123
Step length variability (mm)	>19	72%	71%	75%	0.632	.202
Step time asymmetry (ms)	>3	72%	100%	17%	0.618	.254
Step width (m)	>0.05	69%	93%	83%	0.563	.546
Step length asymmetry (mm)	>36	72%	100%	17%	0.559	.568

Figure 5-1: Area under the curve plots for functional outcome measures and exercise capacity and hip muscle strength to differentiate between mitochondrial disease patients and controls.
The open circle marker denotes the cut-off point of highest accuracy.

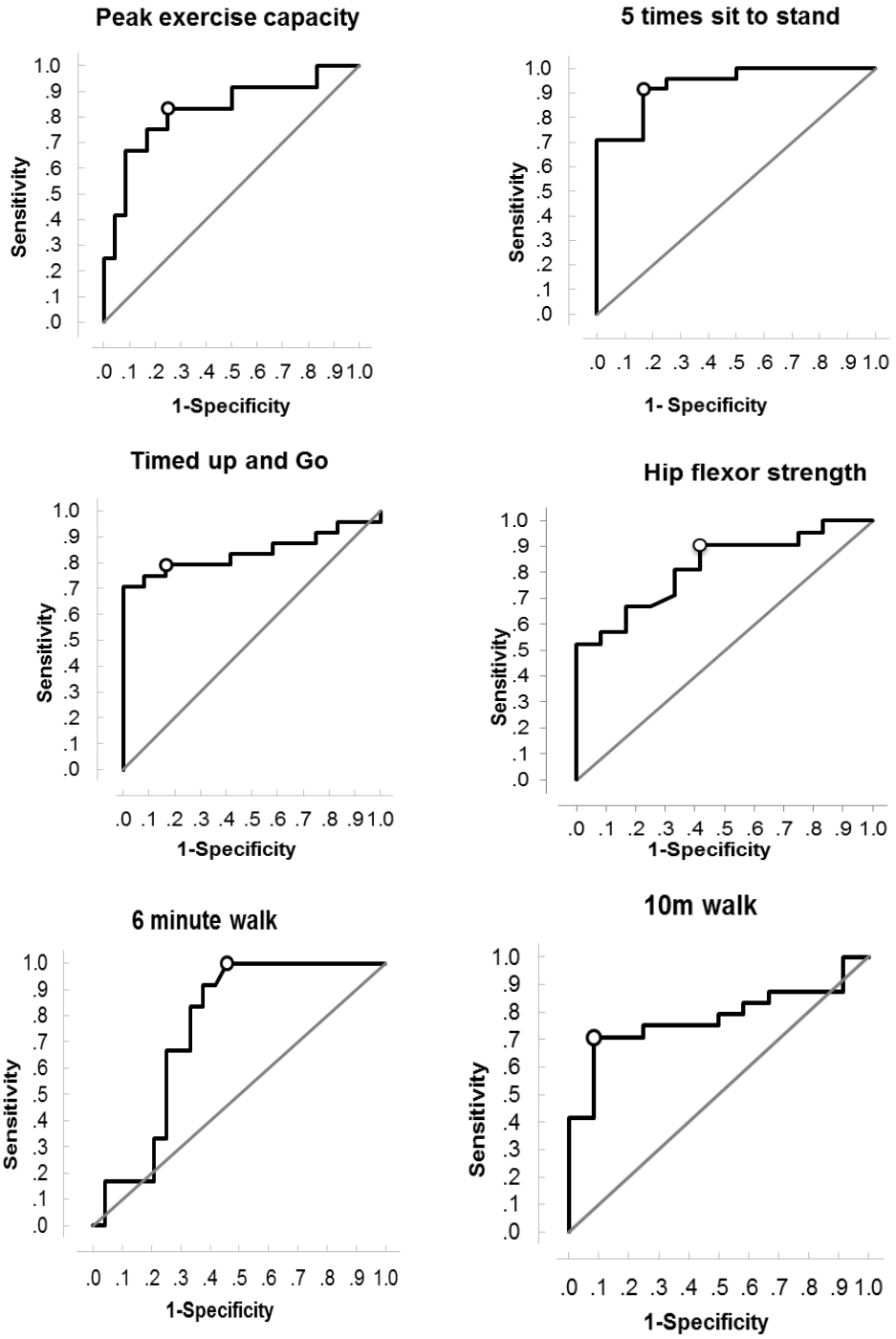


Figure 5-2: Area under the curve plots for a discrete number of gait characteristics to differentiate between mitochondrial disease patients and controls.
The open circle marker denotes the cut-off point of highest accuracy. (MD: n=24, Controls: n=12)

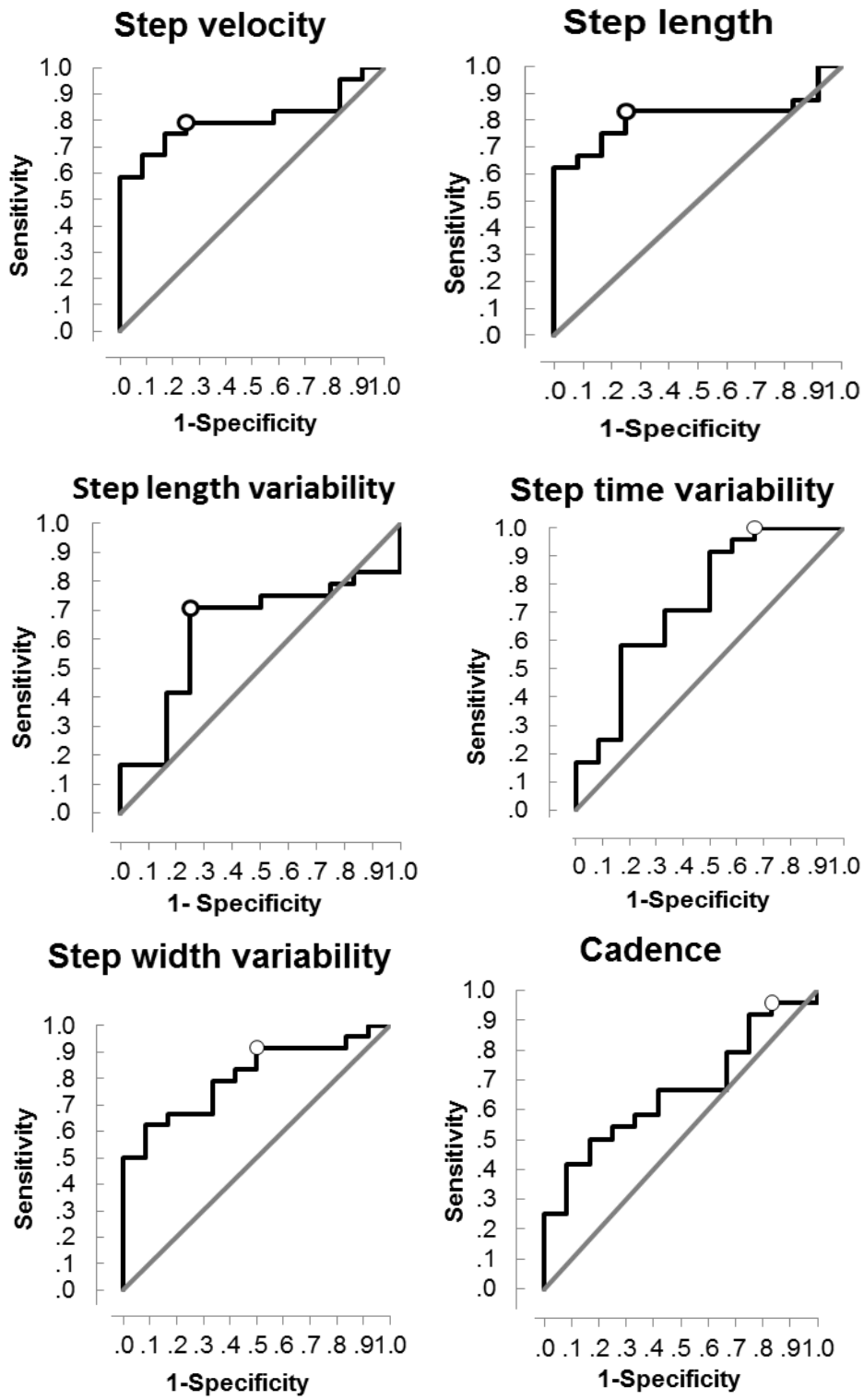


Table 5-2: The relative accuracy, sensitivity and specificity of measures used in this thesis to discriminate between the two genotypes (m.3243A>G and 8344A>G).
[ł] Cut off chosen at the point of maximum accuracy. Where more than one point had the same accuracy, the most sensitive cut-off value was chosen. AUC - Area under the curve. (m.3243A>G: n=18, m.8344, n=6).

Variable	Cut off point [ł]	Accuracy	Sensitivity	Specificity	Area Under the Curve	p value
Step length variability (mm)	29	96%	100%	83%	0.963	.001
Step length (m)	0.54	88%	100%	50%	0.917	.003
Step time variability (ms)	27	96%	100%	83%	0.907	.003
10m walk duration (secs)	12	88%	100%	50%	0.903	.004
Step velocity ($m.sec^{-1}$)	0.84	88%	100%	50%	0.898	.004
6 minute walk distance (m)	313	92%	100%	67%	0.889	.005
Timed Up and Go (secs)	10	92%	100%	67%	0.861	.009
Step time asymmetry (ms)	24	96%	100%	83%	0.852	.011
Step width variability (mm)	41	92%	100%	67%	0.815	.023
Peak Exercise Capacity (ml.min.kg)	14	79%	89%	50%	0.815	.023
Cadence ($steps.min^{-1}$)	92	88%	100%	50%	0.787	.039
Anaerobic threshold (ml/kg/min)	9.18	79%	83%	67%	0.787	.039
Step length asymmetry (mm)	40.82	83%	100%	33%	0.769	.053
Hip flexor strength (nm/kg)	0.4	88%	87%	83%	0.767	.062
Hip extensor strength (nm/kg)	0.9	88%	100%	50%	0.722	.119
Step width (m)	0.18	79%	100%	17%	0.667	.230
Five times Sit to Stand (secs)	18.28	83%	100%	33%	0.634	.334
Arteriovenous O_2 difference (ml O_2/dl)	9.45	71%	94%	0%	0.521	.883

Figure 5-3: Area under the curve plots for functional outcome measures and exercise capacity and hip muscle strength to differentiate between two mitochondrial genotypes.
The open circle marker denotes the cut-off point of highest accuracy.

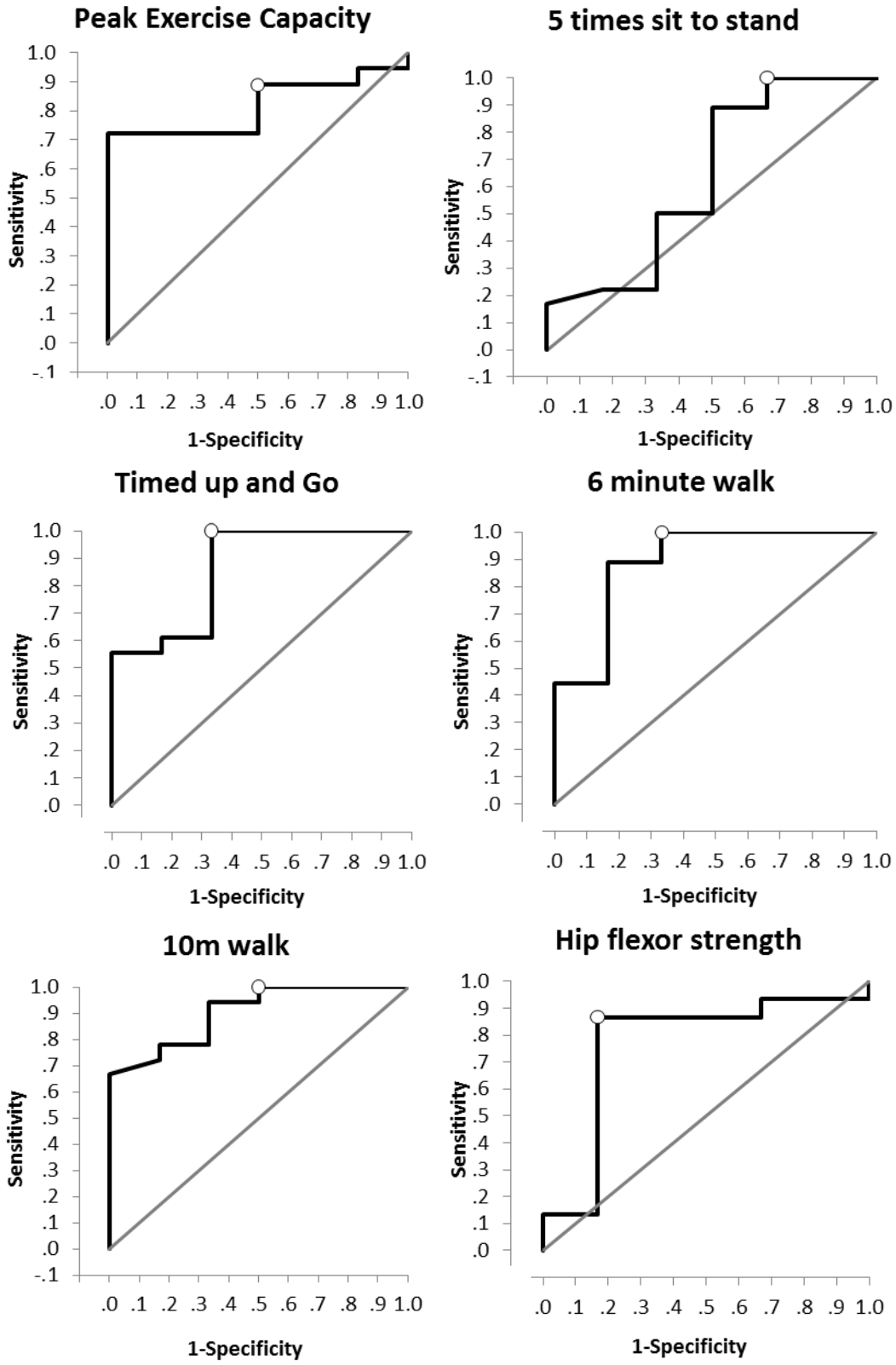
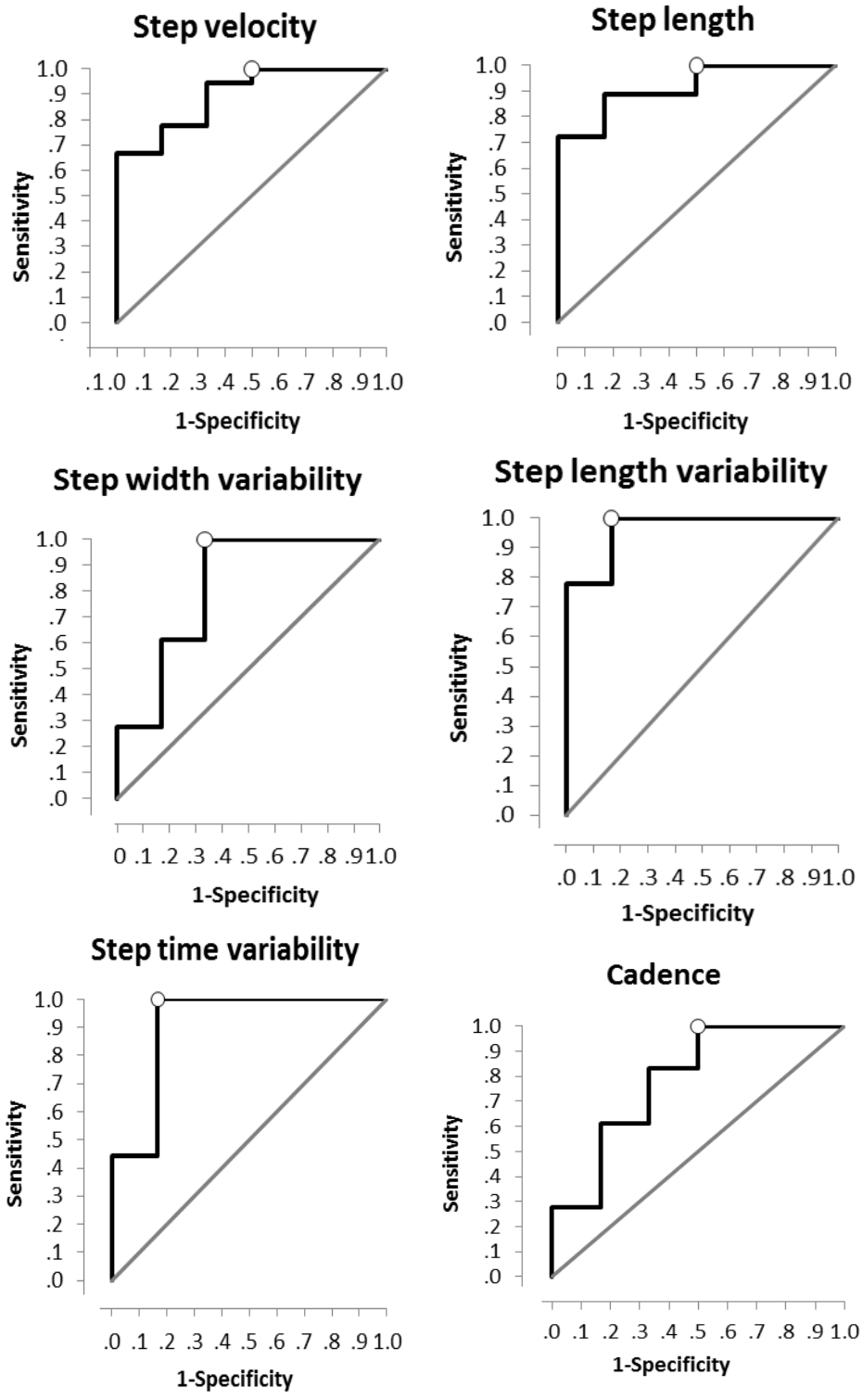


Figure 5-4: Area under the curve plots for a discreet number of gait characteristics to differentiate between two mitochondrial genotypes.
The open circle marker denotes the cut-off point of highest accuracy.



5.5 **Discussion**

Research thus far in Mitochondrial disease has led to a greater understanding of mitochondrial function and the genetic causes of mitochondrial disease. The study of disease progression and the evaluation of interventions has proven less successful due to the complex and varied nature of mitochondrial disease. This thesis has used a number of well-known clinical outcome measures to evaluate different aspects of disease as recommended by the World Health organisation's International Classification of impairments, disabilities and handicaps. Comparison of all the measures used can assist in the recommendation of a refined battery of measures to be used in future clinical and research work. Careful selection of measures is imperative if disease progression and the effects of interventions are to be measured accurately. The correct use of measures will; i) enable early detection of impairments while they are still amenable to treatment; ii) discriminate between controls and patients with mitochondrial disease and iii) potentially between different mitochondrial genotypes.

When discriminating between groups with and without mitochondrial disease the studies demonstrated that two exercise parameters (a-vO_2 diff, $\dot{V}O_{2PEAK}$), hip muscle strength, two functional measures (5XSTS, TUG) and 3 gait characteristics (step velocity, step length and step width variability) were deemed to have good discriminative abilities. Differing levels of sensitivity and specificity were noted, however, which is likely to be due to the arbitrary nature of cut off values chosen.

Previous evaluations of exercise testing as a means of diagnosis in mitochondrial disease have reported levels of sensitivity of 63 - 75% and specificity of 70 - 90% (Tarnopolsky, 2004). These figures are similar to our findings despite our results only including maximal exercise testing. The high level of accuracy of oxygen extraction and peak exercise capacity identifying patients with mitochondrial disease found in this thesis is reassuring as these measures have been commonly used in previous exercise studies (Taivassalo *et al.*, 2003; Taivassalo *et al.*, 2006) and were surmised to reflect mitochondrial function. Although these measures were able to discriminate well between a healthy and diseased population they were less able to discriminate between different mitochondrial genotypes. This is unsurprising as both mitochondrial genotypes have impaired mitochondrial function and are therefore likely to have impaired exercise capacity.

The poor ability of exercise testing to discriminate between different types of mitochondrial disease has also been alluded to in previous studies when comparing different myopathic diseases with mitochondrial disease (Dandurand *et al.*, 1995; Dysgaard Jeppesen *et al.*, 2003). These studies reported that exercise testing was not a sensitive diagnostic test for mitochondrial myopathies. Despite these findings it was agreed that exercise capacity is significantly reduced in patients with mitochondrial disease and that exercise capacity is a useful research outcome in therapeutic trials, especially ones where exercise is the intervention. The authors also reported that the specificity and sensitivity of the test was not as important as its reliability and reproducibility (Tarnopolsky, 2004).

The different types of exercise testing combined with the many exercise variables reported in exercise studies do make comparisons between studies difficult. A consensus regarding the type of exercise test and the appropriate measures to report would improve comparisons between studies and allow improved analysis over a larger number of patients in these rare conditions. The demonstration of two exercise variables (a-vO_2 diff, $\dot{V}O_{2PEAK}$) with good levels of accuracy will hopefully lead to fewer exercise parameters needing to be reported in future studies.

Despite the nonspecific nature of functional outcome measures two of the measures used in this thesis had high levels of accuracy (5XSTS, TUG). These findings are harder to explain, as the previous results chapter (Chapter 3) reported that although all the functional measures were both able to demonstrate a difference between control and disease groups, the 5XSTS did not relate to the level of disease burden, exercise capacity or proximal muscle strength. It should be remembered that this measure, along with other functional measures, tests the composite result of multiple elements of a movement such as: strength, postural control, sensation. Due to the composite nature of functional outcomes, they are unable to indicate which impairment results in a limitation of function. It would seem that something about the sit to stand movement included in both of these measures is indicative of mitochondrial disease, even within a high functioning group of patients where myopathy was minimal and a relationship to disease burden could not always be exhibited.

The simplicity, along with the lack of requirement for specialised equipment, of these functional measures makes their use in clinical practice possible.

However, the lack of sensitivity to change may be a barrier to use in a research environment.

The 6MWD despite being a field measure of exercise capacity was unable to discriminate between the two groups (AUC < 0.8). This result supports the findings in the previous results chapter and the hypothesis that impaired walking in patients with mitochondrial disease is likely to be a result of multiple system involvement, as well as secondary deconditioning, and not just impaired mitochondrial function within muscle leading to reduced exercise capacity.

Three gait characteristics (step length, step length variability and time variability) demonstrated excellent power (AUC>0.9) when discriminating between the two mitochondrial genotypes, highlighting the sensitive and selective nature of gait characteristics. This further emphasises the differences that have been previously reported in descriptions of the two genotypes. The gait variables reported represent the gait domains of pace and variability and may be indicators of primary pathological changes as opposed to secondary compensations.

Unfortunately time constraints and participant burden meant that the reliability and reproducibility of measures used in these studies were not assessed. The reliability and reproducibility of a number of functional measures have been demonstrated in other disease populations, however, and hopefully this was maintained in these studies by limiting the number of assessors and following strict protocols.

Investigating the accuracy of the measures used in this study has highlighted that, as expected, different measures were better at measuring different disease

attributes. In clinical trials it is imperative that the correct measures are used to measure what a researcher expects to be different, either between groups, over a time span, or following an intervention. As a result of these studies, future studies can explore the use of a more refined number of measures in these genotypes to enable discrimination between: Patients with mitochondrial disease and controls; different genotypes, disease burden and mutation load Table 5-3. The small sample size and the use of only two mitochondrial genotypes means that results should not be extrapolated to a larger more varied cohort. Further investigation into the use of clinical outcome and gait measures is required to confirm that functional outcome measures and analysis of gait are valid and accurate measures in other mitochondrial disorders.

Table 5-3: Review of key clinical outcomes used in baseline studies. (X- AUC >0.9).

Domain	Variable	Able to discriminate between control and MD group (AUC > 0.8)	Able to discriminate between genotypes (AUC > 0.8)	Correlates to diseases severity (NMDAS)	Correlates to % mutation load
Exercise capacity	a-vO_2 difference	X		X	
	Peak exercise capacity	X	X	X	
	Anaerobic threshold		X	X	
Muscle Strength	Hip flexor strength	X			
	Hip extensor strength	X			
Gait	Step length	X	X	X	
	Step velocity	X	X	X	
	Step time variability		X		
	Step length variability		X		
	Step width variability	X	X	X	X
	Step time asymmetry		X	X	X
Clinical Functional measures	5XSTS	X			
	TUG	X	X	X	X
	10MTW		X	X	
	6MWD		X	X	

5.6 **Conclusion**

The studies in this thesis have demonstrated that a limited number of gait and functional measures are capable of discriminating between disease and control groups with varying levels of accuracy. The wider use of these measures will enable a better clinical picture of mitochondrial disease to be gained without the use of laboratory or expensive and extensive testing. The use of these measures in larger cohorts of patients will enable links between primary pathological changes and their likely effects on function to be clarified.

Chapter 6 The effect of a structured exercise intervention on the exercise and functional capacity of people with mitochondrial disease

6.1 Introduction

Currently no clear evidence is available to support the use of any intervention in the treatment of mitochondrial disease (Pfeffer *et al.*, 2012). Many of the interventions routinely used in the clinical management of patients with mitochondrial diseases remain essentially supportive, with treatments being aimed at the medical management of symptoms of the disease such as diabetes, epilepsy and cardiac impairment.

Medical treatments for the muscle symptoms related to mitochondrial disease, such as reduced exercise capacity, fatigue and muscle weakness, have been tried, but as yet there have been no randomised controlled trials that have reported any benefits (Pfeffer *et al.*, 2012). The use of aerobic exercise has been shown to have long-term health benefits in the general population (Fletcher, 1999; Haskell *et al.*, 2007; Lee *et al.*). Patients with mitochondrial disease are at greater risk of cardiovascular disease due to disease pathology and low levels of habitual activity (Apabhai *et al.*, 2011), therefore aerobic exercise has been proposed as a potential treatment in this disease group (Pfeffer *et al.*, 2012).

Previous studies investigating the effects of exercise in mitochondrial disease have demonstrated improvements in muscle function by performing exercise tests and muscle biopsies (Cejudo *et al.*, 2005; Taivassalo *et al.*, 2006; Trenell *et al.*, 2006). These studies have either included a wide variety of genotypes, or patients with a single deletion that have a myopathic phenotype (Taivassalo *et al.*, 2001; Cejudo *et al.*, 2005; Taivassalo *et al.*, 2006). Until now the use of functional outcome measures to evaluate the effects of an exercise intervention

in mitochondrial disease has been limited and results inconclusive (Jeppesen *et al.*, 2006b; Trenell *et al.*, 2006).

This case controlled interventional study will provide further evidence that exercise is safe and beneficial in another discrete group of mitochondrial patients. The use of simple functional outcome measures alongside traditional measures of exercise capacity will be able to demonstrate that improvements in muscle function translate into improvements in functional activities and quality of life in patients with mitochondrial disease.

6.2 **Study aims and hypothesis**

Aims

1. To determine the effects of aerobic exercise on the exercise capacity of two specific mitochondrial genotypes (m.3243A>G, m.8344A>G).
2. To investigate if changes in exercise capacity result in changes in the performance of day-to-day activities.

Primary hypothesis

Exercise capacity will improve within a discrete group of mitochondrial patients after an aerobic exercise intervention.

Secondary hypotheses

Improvements in exercise capacity will translate to improvements in functional tasks.

Disease burden, quality of life and cognitive ability of participants with mitochondrial disease will improve following an aerobic exercise intervention.

Muscle strength will remain unchanged following an aerobic exercise intervention.

6.3 **Subjects and Methods**

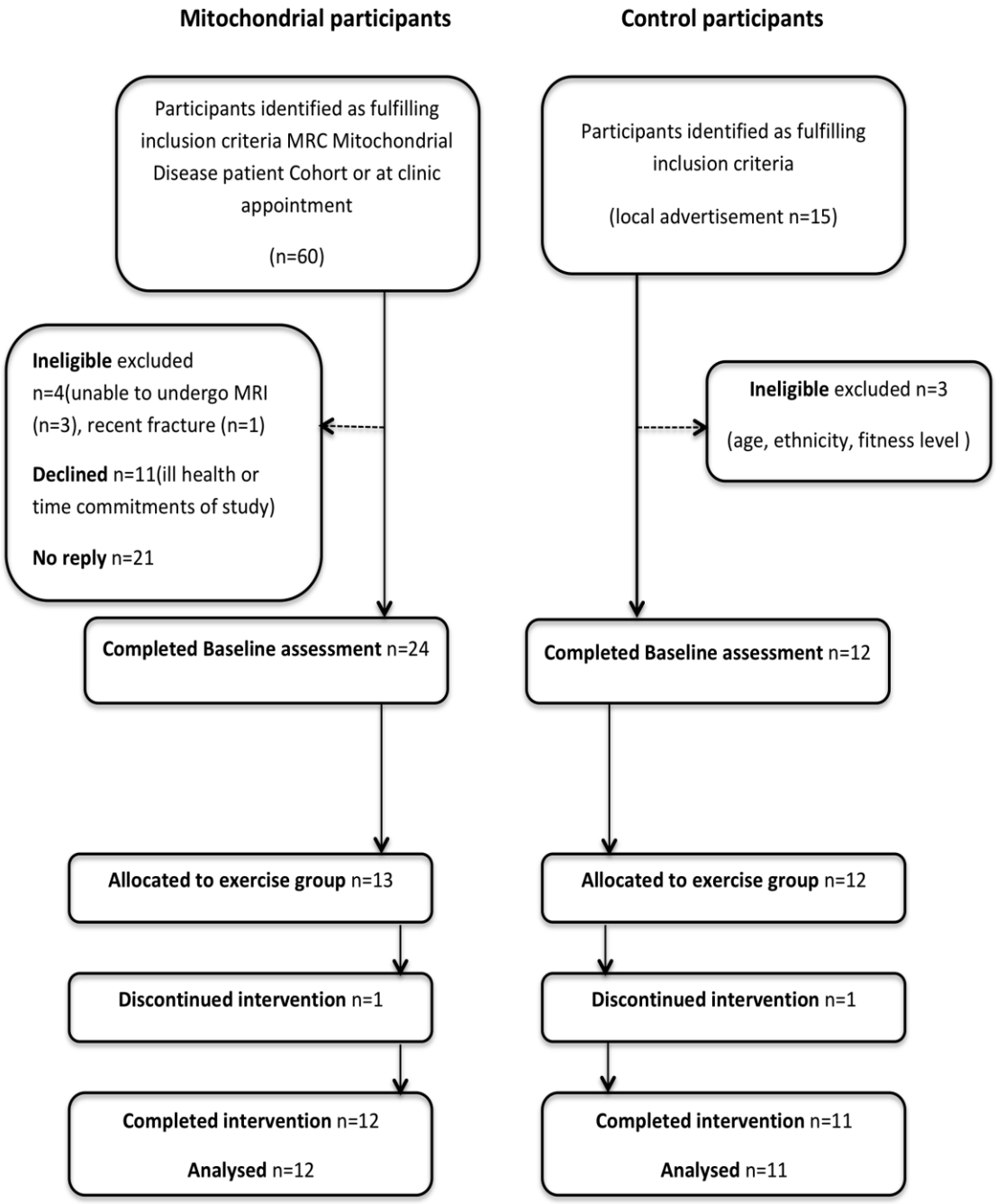
Sixty patients with mitochondrial disease were invited to participate in the exercise study from consecutive attendees at a specialist outpatient clinic between August 2010 and July 2011. Patients were clinically identified as having the m.3243A>G or m.8344A>G mitochondrial DNA mutation (Figure 6-1).

All eligible patients were invited to participate based on the following clinical inclusion criteria: (i) clinical stability for >6 months; (ii) able to use an upright stationary bicycle ergometer; (iii) no current participation in regular physical activity (≤1 weekly session) and (iv) reported exercise intolerance and fatigue. Exclusion criteria were the presence of known cardiac involvement, co morbidities precluding exercise training, and contra-indications to Magnetic Resonance Imaging.

A power calculation was determined from the change in peak oxygen consumption following a similar exercise intervention (Taivassalo *et al.*, 2006). Mean 1 =1.36 (SD 0.4), Mean 2 = 1.51 (SD 0.4), Alpha =5%, Beta =80%. This calculated a sample size of nine. A minimum of twelve people per treatment arm were recruited to allow 3 participants from each group to leave the study and retain sufficient power for intervention evaluation.

From the 36 participants in a previous observational study (chapter 3) a convenience sample of thirteen patients with mitochondrial disease and all the 12 sedentary control participants were allocated to the exercise intervention study. One participant from each group failed to return for final testing due to time commitments.

Figure 6-1: Consort diagram for the study of the effect of aerobic exercise on physiological and functional measures in mitochondrial disease.



All visits were undertaken between the two centres listed in chapter 2 over a period of two days. Over the two days both disease and control groups underwent; assessments of baseline physical activity, anthropometry, resting metabolism, peak exercise capacity, proximal muscle strength and functional assessment using recognised clinical functional outcome measures. In addition the disease burden of the mitochondrial participants was assessed, whilst the sedentary control volunteers underwent a review of their medical history, physical examination and completed the Physical Activity Readiness Questionnaire.

After completion of a 16-week aerobic exercise intervention all initial assessments (except physical activity measurement) were repeated. An additional visit was performed midway through the intervention, to monitor that no adverse responses to exercise had occurred and to reassess the exercise capacity of participants.

The following section includes brief explanations of the methods of assessment used in this study (Full details can be found in Chapter 2).

6.3.1 Disease burden

All subjects underwent a physical examination by an experienced clinician. Disease severity was assessed and disease burden was established using the Newcastle Mitochondrial Disease Adult Scale (NMDAS), a validated scoring system (Schaefer *et al.*, 2006). Mutation load, an established marker of clinical outcome was derived from urinary epithelial cells (McDonnell *et al.*, 2004; Shanske *et al.*, 2004; Whittaker *et al.*, 2009).

6.3.2 Anthropometry

Body weight (kg) and standing height (m) were measured before and after the exercise intervention. Whole body composition was determined using air displacement plethysmography using a BodPod (Life Measurement Inc., Concord, CA, USA).

6.3.3 Physical Activity

Physical activity and energy expenditure were assessed objectively at baseline using a validated (St-Onge *et al.*, 2007) multi-sensor array (SenseWear Pro_3, Bodymedia Inc, Pennsylvania, USA 2.5).

6.3.4 Resting Substrate metabolism

Following an overnight fast (≥ 8 hours with no food or beverages) resting substrate oxidation was determined by expired gas analysis (CORTEX Biophysik, Leipzig, Germany) using a Hans Rudolf breathing mask while participants lay supine for 30 minutes in a quiet room..

6.3.5 Progressive Exercise testing

A peak exercise test was performed as described in section 2.3.8. A number of parameters including VO_{2PEAK}, anaerobic threshold (AT), peak workload (W) and systemic arteriovenous oxygen difference (a-vO_2 diff) were measured.

6.3.6 Dynamometry

Proximal muscle power was measured using isokinetic dynamometry of the hip flexor and extensor muscles (CSMI HUMAC®/ NORM testing and rehabilitation system).

6.3.7 Functional assessment

Functional outcomes measures were performed in the Clinical Ageing Research Unit, Newcastle University. The following functional outcome measures were performed: Six-minute walk distance (American Thoracic Society Statement, 2002); 10 metre timed walk (Bohannon, 1997); timed up and go test (Podsiadlo D *et al.*, 1991) and the 5 times sit to stand (Lord *et al.*, 2002; Whitney *et al.*, 2005). The order of tests was randomized except for the six-minute walk distance, which was always performed last to reduce the effects of fatigue on other tests.

6.3.8 Cognitive Ability

Cognitive function was tested using the revised Addenbrookes Cognitive Examination (ACE-R). A cut off 88 was used as an indication of mild cognitive impairment (Eneida *et al.*, 2006).

6.3.9 Quality of Life

Quality of life was assessed using the short form (SF12) health survey (Ware *et al.*, 1996) which already forms part of the Newcastle Mitochondrial Disease Adult Scale (Schaefer *et al.*, 2006).

6.4 **Aerobic training intervention**

Upon completion of baseline tests, participants undertook 16 weeks of aerobic exercise training. Exercise sessions were conducted at a local gym or at home. The first exercise session was supervised by a qualified physiotherapist. The exercise programme was based on the individual's performance of the symptom limited peak exercise test. Participants were initially asked to perform 20 minutes of cycling within a heart range that was equivalent to 60-70% of their VO_{2PEAK}. If participants were unable to perform 20 minutes of continuous exercise they were encouraged to cycle for as long as they could with rests, until they had performed 20 minutes of cycling in total. Participants exercised (cycling) 3 times a week for the 16 weeks (48 sessions in total). All participants received weekly contact via phone or email. The intensity of the exercise sessions were tailored to the individual and increased progressively over the 16 weeks. Progression was dictated by the participant, initially participants were encouraged to cycle for 20 minutes at designated heart rate range, and this was then increased by 5 minutes until they were able to perform 45 minutes of exercise. Participants were encouraged to increase resistance on bike to maintain heart rate within training range. Once 45 minutes continuous exercise was achieved at the designated heart rate range the range could be increased as able. Exercise was recorded in an exercise diary and monitored retrospectively via a POLAR watch monitor.

Midway through the training period participants attended the clinical research facility to repeat the progressive exercise test performed at baseline. Training heart rates were adjusted to maintain training at a heart rate≈70% VO_{2PEAK} for the remainder of the exercise intervention.

6.5 **Statistical Analysis**

Normality of distribution was assessed using a Kolmogorov-Smirnov test. To compare within group differences before and after the exercise intervention, paired student t tests, or Related-Samples Wilcoxon Signed Rank Tests, were performed dependant on normality.

To compare the responses to exercise of the mitochondrial and control groups individual change scores were calculated by subtracting the pre intervention score from the post intervention score of each participant. Individual change scores of the control and mitochondrial disease groups were then compared using independent sample t-tests or Mann-Whitney U tests for variables that had demonstrated a significant change following the intervention.

Data is presented as means ± SD for continuous data and as numbers or percentages for categorical data unless otherwise stated. Statistical significance was achieved with a *p value* of < .05, or < .01 where multiple comparisons were performed.

6.6 **Results**

All participants attended baseline testing (Mitochondrial disease group n=13, control group n=12). At baseline one mitochondrial disease patient's body composition data from the Bodpod was unable to be retrieved and one mitochondrial disease patient was unable to complete dynamometry due to an adverse response to the peak exercise test.

One participant from each group was unable to complete the final test visit and therefore their data was excluded from the final analysis (Mitochondrial disease group n=12, control group n=11). All participants returning for the final assessment had completed the exercise intervention, with all participants completing ≥ 80% of the exercise sessions and no adverse events being reported. On review of exercise diaries two participants with mitochondrial disease were unable to cycle for 20 minutes continuously, but performed 20 minutes with 1 or 2 rests. These participants also reported muscle discomfort post exercise on occasions. Recording of exercise in diaries was good but numerous reports were noted concerning POLAR watch recording, therefore participants were required to use heart rate monitor on cycles to monitor heart rate. Therefore numerous sessions were not captured by POLAR watch by both groups.

Two mitochondrial patients were unable to complete the 6-minute walk on the final visit due to a risk of falling; one of these participants also was also unable to perform the other walking tests.

The following sections will report the baseline anthropological characteristics of the participants, followed by the responses to exercise of both groups. Finally a comparison of the response to exercise of the two groups is summarised.

6.6.1 Characteristics of participants

The groups were well matched for age (mitochondrial group 40 ± 11 vs. control 38 ± 12, *p= .671*). The groups did not remain matched for sex post intervention due to the drop out of one person in each group (mitochondrial group; four women and eight men, control group; five women and six men). Baseline characteristics of the two groups are summarised in Table 6-1. Differences between the two groups are reported in both body weight and activity levels, with both being lower in the mitochondrial group. Despite the activity levels measured in the mitochondrial group being lower than the control group, when asked to self-report their activity levels the mitochondrial group reported much higher levels of activity. These results are in line with the larger cross-sectional study (chapter 3).

Table 6-1: Baseline characteristics of participants.

Variable	Mitochondrial Group n=12	Control Group n=11	*p* value
Age (Years)	40 (11)	38 (12)	.671
Weight (Kg)	62 (12)	79 (15)	.005**
BMI (Kg/m^2)	21 (3)	27 (5)	.002**
Daily energy expenditure (cals)	2092 (613)	2505 (362)	.016*
Daily step count	6078 (4935)	9241 (2575)	.004**
IPAQ (met/min/week)	7385 (9629)	3304 (3412)	.604

(IPAQ-International Physical Activity Questionnaire)

The genotypes, symptoms and disease burden levels of the mitochondrial participants are summarised in Table 6-2. At baseline two participants had

cognitive impairment as categorised by an ACE-R score of less than 88, with one participant unable to complete the form due to poor vision.

Quality of Life scores were lower in the mitochondrial group for the physical component as compared to the control group; physical composite score (PCS) (mitochondrial group 40 ± 13 vs. control 55 ± 5, *p*= .004), mental composite score (MCS) (mitochondrial group 46 ± 12 vs. control 48 ± 12, *p*= .588).

Following the exercise intervention the weight of the mitochondrial group increased by 2% (*p*=. 031*) as compared to a 1% loss in the control group (*p*=. 358). No change was seen in the disease severity post exercise intervention (NMDAS; pre-exercise, 22 ±15, post-exercise, 23±17, *p*=. 684). Also no change was reported in cognition and quality of life (ACE-R; 89 ± 11, 89 ±12, *p*= .983, SF12; Physical composite score 41 ± 13, 40 ± 12, *p*= .365; Mental composite score 45 ± 12, 45 ± 13, *p*= .913).

Table 6-2: Baseline disease characteristics of mitochondrial participants.

Genotype	Age	Sex	Height (cm)	Mass (kg)	BMI	Heteroplasmy (%)	NMDAS	Principle clinical features
m.3243A>G	58	F	173	52.1	17.4	59	12	Hearing loss, ataxia, constipation, underweight, myopathy, myalgia, exercise intolerance
	39	M	172	74.6	25.2	80	17	Hearing loss, diabetes, migraine, fatigue, hypothyroidism, ataxia, constipation, exercise intolerance
	37	F	164	50.7	18.9	48	10	Hearing loss, diabetes, exercise intolerance, ataxia, dysarthria, asthma
	42	M	176	83.7	27.0	82	12	Hearing loss, diabetes, migraine, ataxia, depression, exercise intolerance
	47	M	182	64.0	19.3	63	28	Hearing loss, diabetes, fatigue, ataxia, myalgia, depression, myopathy, neuropathy, ptosis, PEO
	38	F	164	54.2	20.1	53	11	Hearing loss, migraine, constipation, myopathy, fatigue, exercise intolerance, asthma
	22	M	183	59.2	17.7	89	17	Hearing loss, migraine, epilepsy, ataxia, constipation, underweight, exercise intolerance, fatigue
	36	M	179	72.4	22.6	80	4	Migraine, exercise intolerance
	50	M	162	64.6	24.6	87	23	Hearing Loss, exercise intolerance, ataxia, myopathy, fatigue, depression, retinopathy, epilepsy, encephalopathy, cognitive decline, stroke-like episodes
	55	F	153	45.8	19.5	68	25	Hearing loss, diabetes, myopathy, exercise intolerance, ataxia, constipation, depression, retinopathy, PEO, ptosis, short stature, mild dysphagia, hypertension
m.8344A>G	28	M	173	51.7	17.3	95	55	Hearing loss, fatigue, epilepsy, ataxia, retinopathy, constipation, dysarthria, myopathy, myoclonus, neuropathy, exercise intolerance, underweight
	25	M	176	68.0	22.0	94	48	Epilepsy, ataxia, retinopathy, depression, dysarthria, exercise intolerance, myopathy, myoclonus

6.6.2 The effects of an exercise intervention on resting metabolism

Resting metabolism was unchanged by the exercise intervention in both groups with the exception of fat oxidation in the mitochondrial group, which was reduced (1.27 to 1.10 mg/kg/min, *p=0.04*). This change was not reflected in a significant change in the respiratory quotient at rest (0.86 to 0.88, *p*= 0.11) (Table 6-3).

Table 6-3: Resting substrate metabolism pre and post exercise intervention.
** p value<.05 and are denoted in bold type.*

Variable	**Mitochondrial Disease (n=12)**		***p* value**	**Control Group (n=11)**		***p* value**
	Pre intervention	**Post intervention**		**Pre intervention**	**Post intervention**	
Ventilation	7.7 (1.8)	7.8 (2.1)	.770	7.55 (1.8)	8.0 (1.9)	.294
Carbohydrate Oxidation (mg.kg.min)	2.77 (0.95)	2.91 (0.86)	.552	2.94 (0.87)	3.17 (0.69)	.439
Lipid Oxidation (mg.kg.min)	1.27 (0.41)	1.10 (0.42)	**.040***	0.79 (0.29)	0.66 (0.3)	.258
Respiratory Quotient	0.86 (0.05)	0.88 (0.05)	.110	0.91 (0.05)	0.93 (0.04)	.244

6.6.3 The effects of an exercise intervention on the exercise and functional performance of both mitochondrial and control groups

All participants were able to complete a progressive exercise test before and after the exercise intervention, all but one participant achieved 80% of their predicted heart rate maximum. The respiratory exchange ratios produced by both groups were similar and greater than 1, demonstrating peak performance was reached.

The effects of an aerobic exercise intervention on exercise performance

The exercise intervention resulted in an improvement in all aspects of exercise capacity in both groups, although some variables failed to reach statistical significance. In the control group this may have been due to one control participant being unable to exercise one week prior to testing (Table 6-4, Figure 6-2). The improvements noted in this study were greater in the mitochondrial group (peak exercise capacity improved by 16 ± 10% vs. 14 ± 30%, anaerobic threshold by 33 ± 30% vs. 24 ± 32%). Despite the greater improvement in the peak exercise capacity of the mitochondrial group it remained lower post intervention than the control group at baseline, increasing from 55 ± 21% to 66 ± 26% of the predicted peak exercise capacity. Whereas the control group at baseline achieved 93 ± 21% of their predicted peak exercise capacity. The anaerobic threshold as a percentage of the predicted peak exercise capacity in the mitochondrial group also remained low post the exercise intervention despite improving from 34 ± 15% to 44± 17%. This compared to a starting anaerobic threshold of 53 ± 14% of peak exercise capacity in the control group. The arteriovenous oxygen difference (a-vO_2 diff) also increased in both groups (mitochondrial group 16% ± 18 vs. Controls 16% ± 38), suggestive of improved

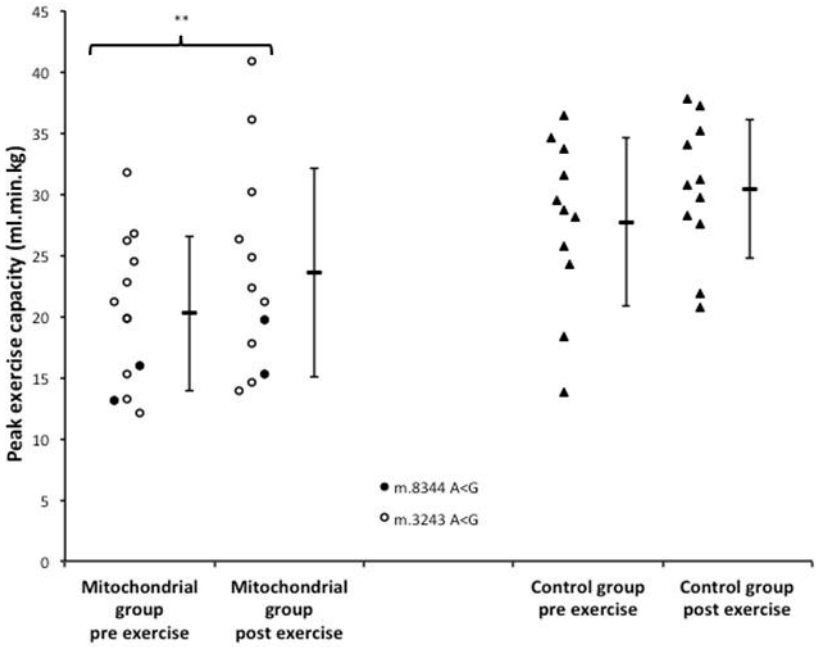
oxygen extraction by muscles in both groups. However, as with other exercise measures, the arteriovenous oxygen difference remained lower in the mitochondrial group post intervention than in the control group at baseline. Peak wattage showed a similar percentage increase in both groups post training (mitochondrial group 14 ± 18% vs. control 13 ± 12%). Despite the increase in peak wattage achieved in testing following training, the post training peak wattage of the mitochondrial group remained approximately 50% of the sedentary controls.

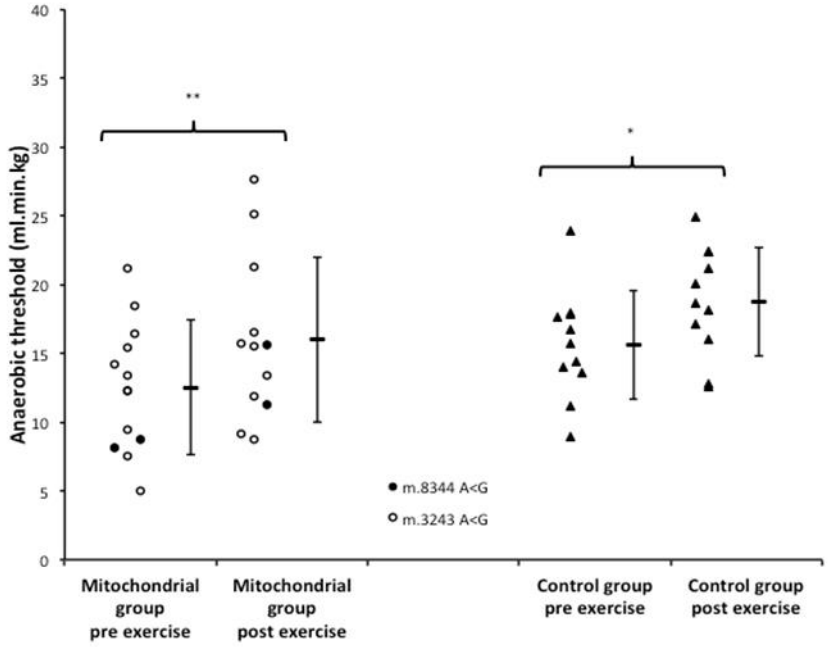
The proximal muscle strengths of both groups are reported in Table 6-5. Due to the large variability in the data the median and inter quartile ranges are reported. The results demonstrate that hip extensors were twice as strong as that of the hip flexors in both groups. The strength of both hip muscle groups in the mitochondrial group was 50% lower than the control group. Following the exercise intervention we saw an increase in the muscle strength in both groups, with a median % increase of >15% in hip extensor strength, despite being a clinically relevant change the change failed to reach statistical significance (p=0.062) (Dvir, 2004).

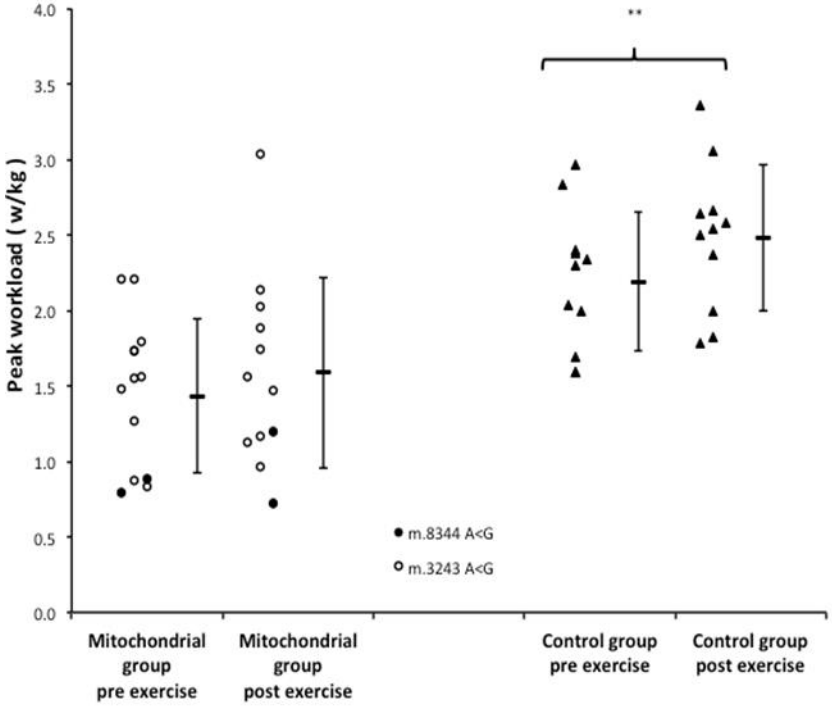
*Table 6-4: Results of progressive exercise tests for mitochondrial and control group's pre and post aerobic intervention. p value <0.5 *, <.01***

	Mitochondrial patients (n=12) Mean ± SD			***p* value**	**Sedentary controls (n=11) Mean ± SD**			***p* value**
	Pre exercise	**Post exercise**	**% change**		**Pre exercise**	**Post exercise**	**% change**	
Peak Heart Rate	162 ± 29	161 ± 17	0	.665	184 ± 16	179 ± 14	3	**.008****
Peak oxygen consumption (l.min)	1.3 ± 0.6	1.6 ± 0.8	19	**.003****	2.2 ± 0.7	2.4 ± 0.6	13	.056
Peak exercise capacity (ml/kg/min)	20.2 ± 6.4	23.6 ± 8.6	16	**.003****	27.7 ± 6.9	30.4 ± 5.6	14	.053
Peak workload (W)	92 ± 45	104 ± 54	14	**.039***	172 ± 41	193 ± 44	13	**.002****
Peak Workload (W/kg)	1.4 ± 0.5	1.6 ± 0.6	12	.060	2.2 ± 0.5	2.5 ± 0.5	14	**.002****
Anaerobic Threshold (ml/kg/min)	12.6 ± 4.9	16.0 ± 6.0	29	**.003****	15.5 ± 4.0	18.8 ± 3.9	32	**.015***
Arteriovenous O2 difference (100ml/kg)	8.8 ± 3.0	10.0 ± 3.1	16	**.007****	11.0 ± 2.8	11.9 ± 1.8	16	.200
Maximum respiratory exchange ratio	1.3 ± 0.1	1.3 ± 0.2	0	.926	1.3 ± 0.1	1.3 ± 0.04	0	.234

*Figure 6-2: Scatter-plots of exercise variables pre and post exercise intervention (Error bars denote means and standard deviations). * denotes significant p values< .05, **p< .01).*






Peak workload (w/kg)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
m.8344 A<G
m.3243 A<G
Mitochondrial group pre exercise
Mitochondrial group post exercise
Control group pre exercise
Control group post exercise

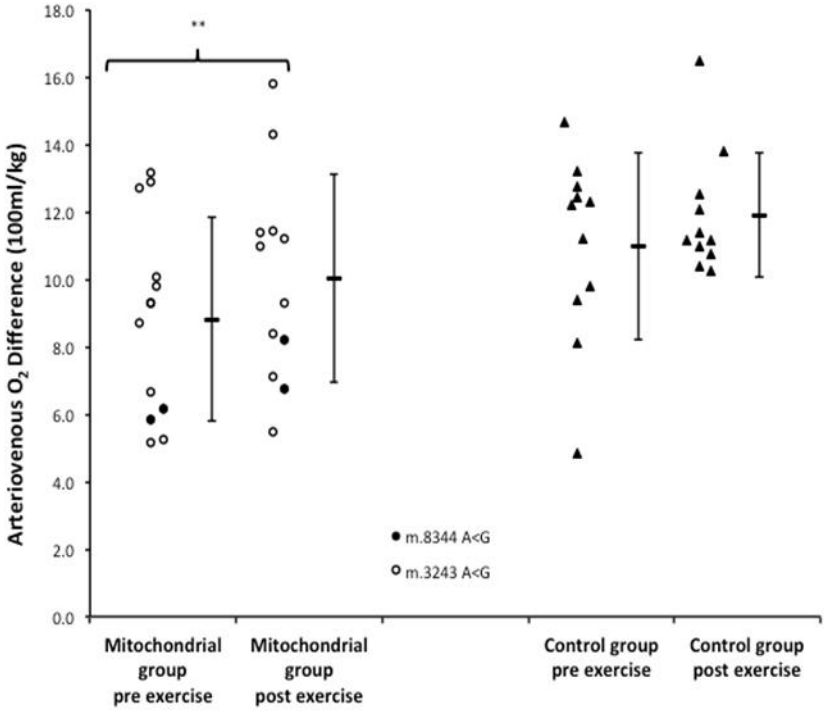

Arteriovenous O_2 Difference (100ml/kg)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
**
m.8344 A<G
m.3243 A<G
Mitochondrial group pre exercise
Mitochondrial group post exercise
Control group pre exercise
Control group post exercise

Table 6-5: Proximal muscle strength pre and post an aerobic exercise intervention.
Data is presented as medians and interquartile ranges.

	Mitochondrial patients (n=11)			*p* value	Sedentary controls (n=11)			*p* value
	Pre exercise	**Post exercise**	**%change**		**Pre exercise**	**Post exercise**	**%change**	
Hip flexor strength (nm)	27 (19-62)	34 (24-63)	19	0.894	104 (50-114)	96 (58-102)	-6	0.230
Hip extensor strength (nm)	60 (48-145)	94 (67-127)	16	0.722	170 (141-226)	189 (155-232)	12	0.062
Hip flexor strength (nm/kg)	0.5 (0.4-0.8)	0.5 (0.4-0.8)	13	0.859	1.2(0.7-1.5)	1.1 (0.8-1.3)	-4	0.397
Hip extensor strength (nm/kg)	1.2(0.9-2.0)	1.6 (1.2-1.9)	5	0.657	2.2 (1.5-2.8)	2.7 (2.2-2.8)	14	0.062

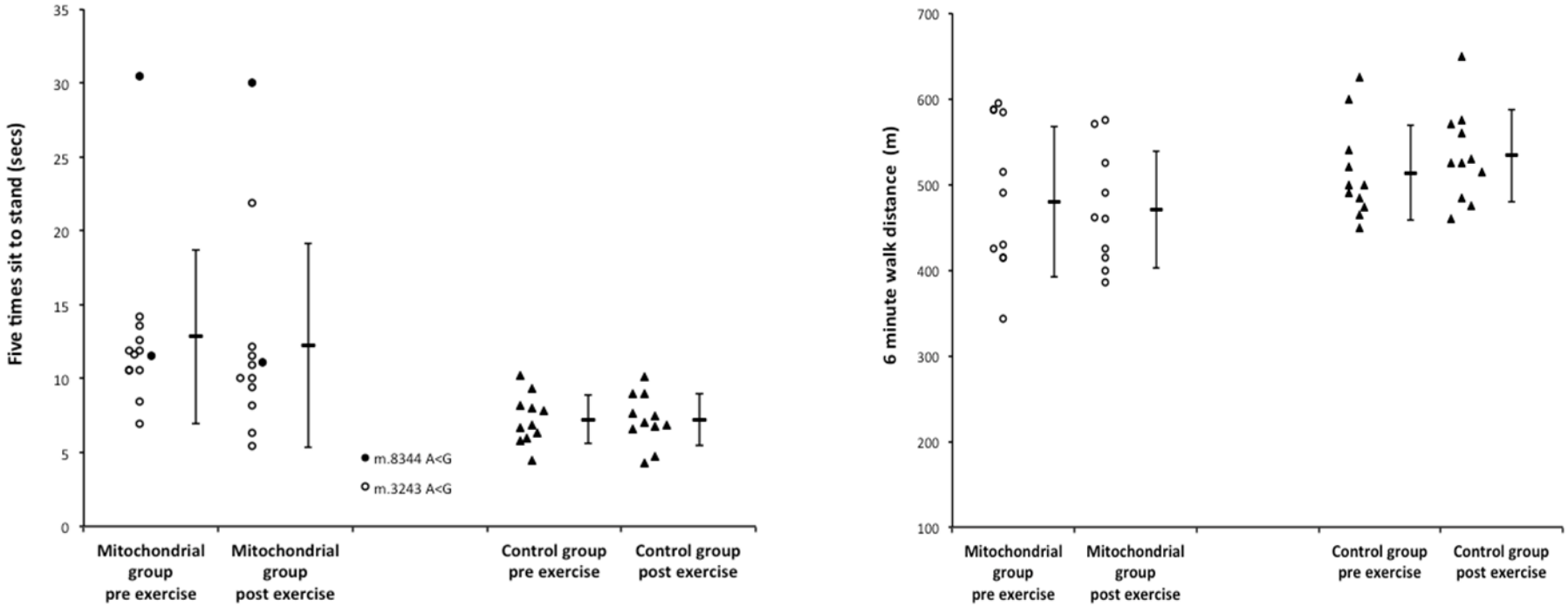
The following section will describe the performance of functional tests at baseline and after the aerobic exercise intervention. Results are summarised in both Table 6-6 and Figure 6-3. The results in Table 6-6 report no clinically measurable or statistically significant changes in the performance of all but one of the functional tests. The 6-minute timed walk distance despite showing an improvement in the control group did not reach a distance deemed to be clinically relevant (13%) (Flansbjer *et al.*, 2005).

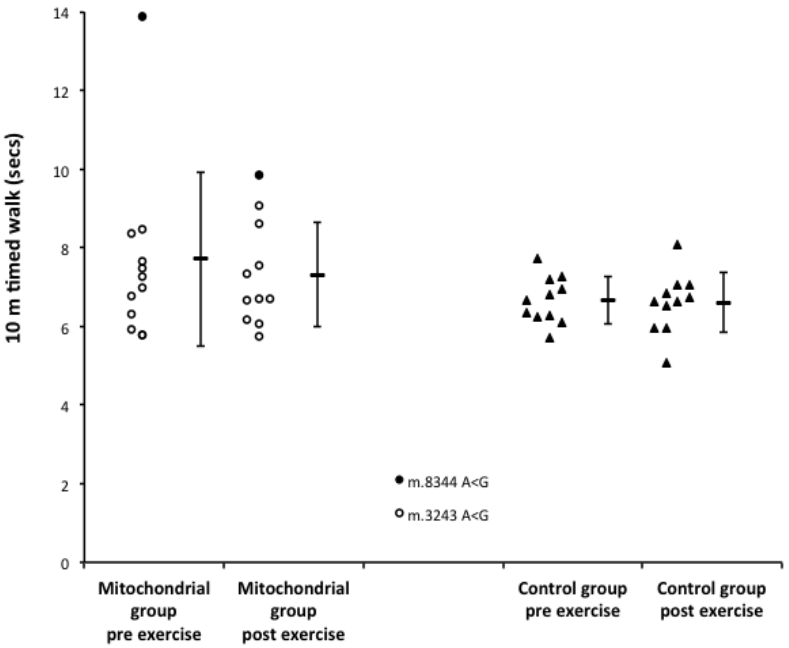
The scatter plots in Figure 6-3 demonstrate the greater heterogeneity within the mitochondrial group compared to the control group at baseline. This remained unchanged by the intervention. Unfortunately one participant with the m.8344A>G mutation was unable to repeat the walking tasks due to a fall resulting from a myoclonic jerk. This participant showed very high levels of walking impairment at baseline (10m timed walk > 25secs), which remained severely impaired after the exercise intervention, although not formally measured.

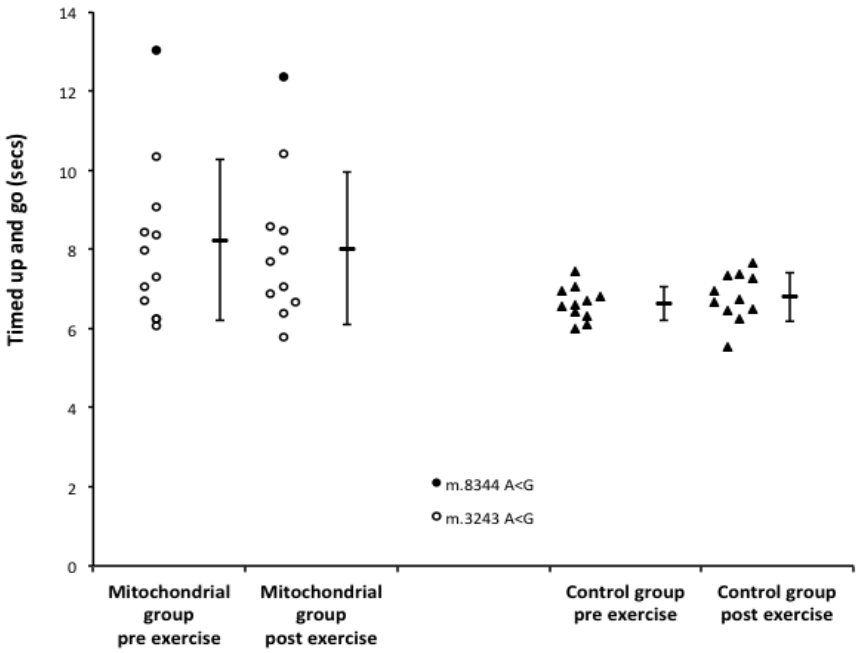
Table 6-6: Functional outcome measures pre and post aerobic exercise intervention .
Data is presented as medians and quartiles . denotes a p value <.01.*

	Mitochondrial patients (n=12)		% change	*p* value	Sedentary controls (n=11)		% change	*p* value
	Pre exercise	**Post exercise**			**Pre exercise**	**Post exercise**		
Five times sit to stand (secs)	12 (11, 13)	10 (9,12)	-11	0.158	7 (6, 8)	7 (7, 8)	9	0.929
Timed up and go (secs) (MD n=11)	8 (7, 9)	8 (7, 9)	-5	0.423	7 (6, 7)	7 (6, 7)	4	0.213
10 metre timed walk (secs) (MD n=11)	7 (6, 8)	7 (6, 8)	-2	0.477	7 (6, 7)	7(6, 7)	<1	1.000
6 minute walk (m) (MD n=10)	459 (415, 568)	461 (418, 516)	-1	0.612	500 (479, 530)	525 (500, 565)	5	**0.032***

Figure 6-3: Scatter-plots for functional outcome measures pre and post exercise intervention. (Error bars denote means and standard deviations)




10 m timed walk (secs)
14
12
10
8
6
4
2
0
m.8344 A<G
m.3243 A<G
Mitochondrial group pre exercise
Mitochondrial group post exercise
Control group pre exercise
Control group post exercise


Timed up and go (secs)
14
12
10
8
6
4
2
0
m.8344 A<G
m.3243 A<G
Mitochondrial group pre exercise
Mitochondrial group post exercise
Control group pre exercise
Control group post exercise

6.6.4 The differences in exercise response between two groups

Further analysis was undertaken to compare the responses of the mitochondrial disease and control groups to the exercise intervention. Where variables had demonstrated a significant change after the exercise intervention the change scores for each group were then compared. The comparison of change scores demonstrated that the response to exercise was similar in both the mitochondrial disease and control groups (Table 6-7). This data should be viewed with caution as the study was designed to demonstrate a change in exercise capacity within the mitochondrial disease group, not to detect the change between the groups.

Table 6-7: Exercise and functional change with an exercise intervention in both control and patient groups. Data presented as mean ± SD.

Variables	Mitochondrial group mean change ± SD	Control group mean change ± SD	Mean difference	*p* value (95% CI of the difference)
Fat oxidation (mg/kg/min)	-0.17±0.25	-0.12±0.34	0.04	0.734 (-0.30, 0.22)
Peak oxygen consumption (l.min)	0.3±0.2	0.2±0.3	0.1	0.477 (-0.1, 0.3)
Peak exercise capacity (ml.min.kg)	3.4±3.0	2.7±4.1	0.7	0.658 (-2.4, 3.8)
Peak workload (Wattage)	12±18	21±17	8	0.263 (-7, 24)
Anaerobic threshold (ml.min.kg)	3.4±3.1	3.2±3.6	0.2	0.888 (-2.7, 3.1)
Arteriovenous O_2 difference (ml/kg/min)	1.2±1.3	0.9±2.2	0.3	0.682 (-1.2, 1.8)
6minute walk distance (m)	-9 ± 60	20±27	30	0.154 (-12, 71)
Hip extensor strength (nm/kg)	0.1±0.6	0.3±0.5	0.2	0.372 (-0.28, 0.73)

6.7 **Discussion**

Mitochondrial disease results in a multitude of symptoms, such as muscle weakness and fatigue, which can lead to increased levels of inactivity. Exercise intervention studies have been shown to improve exercise capacity in patients with mitochondrial disease without adverse events (Taivassalo *et al.*, 1998 ; Taivassalo *et al.*, 2001; Cejudo *et al.*, 2005; Taivassalo *et al.*, 2006; Trenell *et al.*, 2006). These previous exercise studies have confirmed the safety of exercise within mitochondrial populations with multiple genotypes or a discrete population of patients with a single large-scale mitochondrial DNA deletion.

This case controlled intervention study evaluated the effects of aerobic exercise in another discrete group of clinically affected patients with mitochondrial disease due to the m.3243A>G and m.8344A>G point mutations. To our knowledge it is the first to evaluate changes in exercise capacity within this discrete group. Alongside measuring changes in exercise capacity the study investigated whether improvements in exercise capacity were reflected in changes in functional ability.

The key finding of this study was that exercise capacity improved within this discrete population of patients with mitochondrial disease following an aerobic exercise intervention with no adverse effects. This improvement in exercise capacity was not reflected in improvements in the functional measures used in this study.

6.7.1 Changes in the exercise performance of patients with mitochondrial disease

The primary hypothesis in this study was that exercise capacity within this discrete sample of patients with mitochondrial disease would improve following

an aerobic exercise intervention. All aspects of exercise performance in both control and mitochondrial disease participants showed improvement, although statistical significance was not reached in all variables The improvements in peak exercise capacity in the mitochondrial group alongside improvements in anaerobic threshold and oxygen extraction are all in agreement with previous studies (Cejudo *et al.*, 2005; Taivassalo *et al.*, 2006; Jeppesen *et al.*, 2009). Improvements in exercise capacity are presumed to be a result of better oxygen utilization due to mitochondrial biogenesis and increased respiratory enzyme activity as shown by Taivassalo et al and not increased fuel delivery as a result of changes in cardiac function (Taivassalo and Haller, 2004; Bates *et al.*, 2013).

As in previous exercise studies investigating patients with mitochondrial disease, the variation in individual changes in exercise capacity remained wide (change in $VO_{2\ PEAK}$ 7 - 38%). This was despite limiting the study to two genotypes. This varied response is similar to previous studies with a wider genotype inclusion (Cejudo *et al.*, 2005; Jeppesen *et al.*, 2006b). The heterogeneity of response can be observed when examining individual participants; two male participants had mutation loads of 80% but achieved peak exercise capacities of over 35 ml.min.kg, which is comparable to the control subjects and exercise levels of healthy untrained males (Cooper and Storer, 2001). However, two female participants of a similar age to the two high performers with lower mutation loads (48 and 53%) demonstrated much lower exercise capacities (12 and 21 ml.min.kg) that remained low post the exercise intervention (14 and 21 ml.min.kg). These variations in response emphasize the difficulty in predicting the relationships between mutation loads, exercise capacity and the benefits of exercise in this group (Cooper and Storer, 2001; Jeppesen *et al.*, 2003; Taivassalo and Haller, 2004; Jeppesen *et al.*, 2009).

Despite the percentage changes in exercise capacity in the two groups appearing similar, the post training exercise capacity of mitochondrial participants remained lower than the controls, with mitochondrial participants often failing to reach the pre exercise levels of the control group. Parameters such as anaerobic threshold despite not being indicative of pathology post exercise intervention remained at the levels of sedentary individuals (Cooper and Storer, 2001). The reasons for the varied and limited responses of people with mitochondrial disease reported in this and previous studies (Jeppesen *et al.*, 2006b; Taivassalo *et al.*, 2006) are still not fully understood, although it has been assumed that the level of primary biological deficit must play a role. This assumption is not readily supported by the results of this study where 4 participants with mutation loads of over 80% were able to reach over 90% of their predicted peak exercise capacity. One explanation for this discrepancy between mutation load and exercise capacity could be the use of urinary mutation load in this study, which although seen as an accurate predictor of muscle mutation load (Whittaker *et al.*, 2009) in these cases, may not be indicative of the mutation load of muscle tissue. Further investigation of individuals where mutation loads do not appear to relate to disease burden are being explored within the group at Newcastle, to establish if these discrepancies in mutation loads can provide clues to the variability in disease burden reported.

Another reason for the variable and limited response to exercise could be the type and intensity of the exercise intervention. The intensity of exercise sessions in this study was tailored to each participant (60-70% VO_{2PEAK}) to achieve a change in exercise capacity; although in some cases this may not have been sufficient to achieve the maximum improvement possible. A previous

study investigating resistance training also showed an improvement in exercise capacity (Murphy *et al.*, 2008). Interval training has also been reported to be able to optimise the peripheral and central adaptations to exercise and may provide a greater training response (Daussin *et al.*, 2008), but its safety is yet to be proven in patients with mitochondrial disease. Finally it should be considered that the variability of response could just reflect the variability of response that is seen in healthy controls or due to test-retest errors (Bouchard and Rankinen, 2001; Shephard *et al.*, 2004). The variability of response reported in the disease free population may also explain the lack of response of some exercise parameters to training, although adherence to the training program may have been another contributor, with one participant reporting a failure to train one week prior to final testing. Also it should be noted that exercise was individually prescribed for each participant, this may have resulted in participants performing similar relative levels of training but probably very different absolute levels.

The performance of participants with the m.8344A>G mutation is of particular interest to clinical practice; despite both individuals having low exercise capacity levels at baseline they demonstrated an above average response to the exercise intervention with improvements in oxygen consumption of 36 and 24% of baseline. This improvement is similar to changes reported in people without disease (Saltin and Gollnick, 1983) and other disease populations where very de-conditioned individuals demonstrated the most benefits to exercise (Petajan *et al.*, 1996). The improvement seen in the most affected participants is encouraging as it demonstrates that cycling may provide a method of maintaining cardiovascular fitness of patients with mitochondrial disease with a high disease burden (NMDAS scores of 48 and 55). This has been previously

reported in Stroke and Parkinson Disease patients with limited mobility where other methods of training were limited or unsafe (Lee *et al.*, 2008; Lauhoff *et al.*, 2013).

The wide variety of exercise capacities and responses to the exercise seen in this study reiterates the difficulties in researching and reporting on the effects of exercise in this heterogeneous disease group. Further work is still required to establish the dose and type of exercise required to achieve most benefit in this group. Currently trials involving higher intensity stimuli and mixtures of resistance and aerobic exercise alongside muscle biopsy analysis are being undertaken to further understand the mechanisms that lead to the varied responses to exercise, and confirm the safety of more intensive but potentially more beneficial training protocols, in this disease group (Jones. K, unpublished).

Alongside exercise capacity, proximal muscle strength was another impairment investigated in this trial. This is the first study to our knowledge to measure changes in muscle strength using dynamometry in this disease group. Proximal muscle strength improved but failed to reach statistical significance due to the high variability at baseline and variability in the response to the exercise intervention. Previous studies in patients with mitochondrial disease have demonstrated improvements in strength (Cejudo *et al.*, 2005; Murphy *et al.*, 2008). Comparisons with these studies are limited, as the methods of training and methods of measurement were different. Previous studies including resistance, or a mixture of aerobic and resistance training, used different methods of strength measurement and different muscle groups were tested (quadriceps, upper limb muscles). Studies in post Stroke and elderly populations have also demonstrated improvements in muscle strength and power post an aerobic intervention (Keysor and Jette, 2001; Quaney *et al.*,

2009). An improvement in muscle strength was not predicted at the outset of this study as muscle usually adapts specifically to training. Despite this presumption an increase in muscle strength was reported post aerobic training in the hip extensors of participants. This improvement in muscle strength may have occurred partly due to the use of a recumbent bike for training, which required greater lower limb activity (Walsh Riddle and Blumenthal, 1989). The recumbent bike was chosen due to safety requirements and enabled the inclusion of participants with higher disease burden, with symptoms such as reduced balance and ataxia, who would not have been able to use an upright bike safely (Lee *et al.*, 2008). Another potential reason for the improvement in muscle strength was the high levels of weakness found in the mitochondrial population (50% of control group). Therefore, due to the high level of weakness, any exercise intervention would have had an element of resistance training (Dibble *et al.*, 2009). This assumption is supported when examining individual muscle strength responses to exercise; three females with low baseline hip extensor muscle strength managed to increase their hip muscle strength by 100%.

The use of a disease free control group in this study allowed the study to compare the performance of patients with mitochondrial disease and sedentary controls. As numbers were small, and responses variable, the study was unable to state whether the variable level of response was typical. As proximal myopathy is a common manifestation within the two genotypes selected for this study (Chinnery *et al.*, 1997; Kärppä *et al.*, 2005; Mancuso *et al.*, 2013) the variability seen in muscle strength and the wide variety of responses to a low intensity exercise intervention warrants further investigation in a larger cohort of patients. This study chose to investigate hip musculature due to the high

incidence of proximal myopathy in mitochondrial disease. The wealth of normative dynamometry data and ease of testing of knee musculature (Julia *et al.*, 2010) makes it an alternative joint for measurement. Further testing is required to ascertain the merits of measurement at the knee and hip.

This study extends previous knowledge concerning the exercise response of patients with mitochondrial disease by using another largely homogenous group (10 participants with the m.3243A>G point mutation) who have not been previously studied in isolation. This group differs from previous populations studied, as all participants reported symptoms of disease and no unaffected patients with low mutation loads were included (Jeppesen *et al.*, 2006b). Despite all participants with mitochondrial disease being clinically affected they were able to complete the exercise intervention safely without any adverse events.

The improvements seen in exercise capacity replicate the findings reported by other research groups, albeit in another defined mitochondrial group (Taivassalo *et al.*, 2001; Cejudo *et al.*, 2005; Jeppesen *et al.*, 2006b). It is likely that these improvements are due to improvements in oxygen extraction due to mitochondrial biogenesis. Improvements were similar in both groups, confirming that the changes in exercise capacity are likely to be due to reversal of deconditioning, rather than alteration of the primary biological defect. This assumption cannot be confirmed by this study as no muscle biopsy analysis was performed. Improvements reported in muscle strength were unexpected and require further investigation to understand the mechanisms that resulted in this beneficial clinical response.

6.7.2 Did improvements in exercise capacity improve the function of patients with mitochondrial disease?

The discussion so far has been concerned with the improvements of impairments such as exercise capacity and muscle strength. It is recognised that clinical studies should not only assess impairments but other areas included in the international classification of variables and outcomes (Cup 2007). Therefore a secondary aim of the study was to assess whether the expected improvements in exercise capacity were transferable into improvements in functional tasks.

Measurements of function in this study were limited to investigating the effects of exercise on lower limb function due to the type of intervention and time constraints. The functional outcome measures chosen for the study measure common symptoms reported in mitochondrial disease, such as proximal myopathy, exercise capacity and impairments in mobility. The ability to walk and get out of a chair are important activities and are vital to a person's independence (Mitchell, 2006). Unfortunately the measures used in this study were unable to detect changes in these activities despite the improvements noted in exercise capacity and muscle strength. The lack of improvement reported in function in this study is in line with the findings of previous studies in another mitochondrial group, although the use of different functional measures makes direct comparison difficult (Cejudo *et al.*, 2005; Jeppesen *et al.*, 2006b). An exercise trial in mitochondrial disease that did report a change in exercise capacity alongside a change in the functional measure 6MWD performed by Trenell (Trenell *et al.*, 2006). In this trial half the participants had the m.3243A>G mutation and were high functioning. Despite their high level of functioning a statistical improvement was detected by the 6MWD (unlike in this

current study), although this improvement cannot be reported as a clinical change (2002; Flansbjer *et al.*, 2005). These contradictory findings mimic findings in other neurological populations such as stroke, Multiple Sclerosis and Parkinson's disease, with some trials demonstrating an increase in exercise capacity with improvements in functional measures such as the TUG and 6MWD (Keysor and Jette, 2001; Quaney *et al.*, 2009; Sabapathy *et al.*, 2011; Lauhoff P *et al.*, 2013), whilst other studies found no improvement in function (Lee *et al.*, 2008; Collett *et al.*, 2011; Mayo *et al.*, 2013). A number of Cochrane reviews have found no functional benefit from exercise in neuromuscular conditions (Voet *et al.*, 2010; Voet NBM, 2013) whereas other systematic reviews were able to report exercise to be beneficial in improving function in isolated neuromuscular conditions (Ørngreen *et al.*, 2005; Haller *et al.*, 2006; Cup *et al.*, 2007; Abresch *et al.*, 2009). The conflicting results of all these trials are probably due to the functional measures used, as function is unlikely to be changed by improvement in a single impairment such as exercise capacity or muscle strength.

The use of the functional measures performed in this study have been discussed in a previous chapter, where they were reported to relate to community walking ability (Podsiadlo D *et al.*, 1991), activities of daily living (Wade, 1992 ; Potter *et al.*, 1995), risk of falling (Shumway-Cook *et al.*, 2000) and lower limb strength. This study has highlighted the commonly reported problems of floor and ceiling effects when using functional outcome measures (Bohannon and Williams Andrews, 2011). In this study two participants were not able to perform all the walking tasks on repeat testing due to safety issues, and the gait speed of the remaining participants was within the normal range, making demonstration of further improvement difficult. The lack of improvement

in function in this study may be due to a number of reasons including; the floor and ceiling effects reported in the previous paragraph, the use of a small group of patients with a wide variety of disease burden, along with multiple system involvement, and the type of intervention.

The following paragraphs will discuss each functional outcome measure individually and explore possible reasons for the results of this study.

The 6-minute walk test (6MWD) performance of the participants with the m.3243A>G mutation demonstrated that the mitochondrial population covered a distance similar to an elderly population (Bohannon, 2007). Despite the distance being considerably less than the control group, the distance failed to improve with the exercise intervention. The 6-minute walk distance is a field test for sub maximal exercise capacity and therefore this lack of improvement was disappointing. The reason for a lack of improvement is again likely to be due to the small numbers included in the study and wide variations in performance. Walking requires multi-system involvement to perform efficiently (Hausdorff, 2007). Impairments in other systems (such as cerebellar, motor and sensory systems) reported in mitochondrial disease are also likely to limit walking ability, hence the distance covered during the 6 minute walk test. Further understanding of the walking impairments reported in patients with mitochondrial disease and how these impairments affect walking would assist researchers and clinicians in their choice of more appropriate measures to evaluate interventions. The intervention chosen in this study was cycling, which provided no training effect on balance and walking impairments, unlike aerobic training such as walking or treadmill training. Therefore if impairments in balance are significant, the lack of improvement reported in the 6MWD is unsurprising despite the improvement in exercise capacity. However, we should

not rule out the possibility that the lack of improvement may also be due to the adoption of a slower pace to conserve energy and prevent fatigue in the mitochondrial participants, as these are common features of this disease group (Mancuso *et al.*, 2012). If the adoption of a slower pace is an established pattern of behaviour in patients with mitochondrial disease it may necessary to incorporate a behavioural programme alongside an aerobic intervention and provide increased encouragement during testing to achieve a greater change in function. This is currently being studied in other neuromuscular conditions (Voet *et al.*, 2010).

Unlike the 6MWD the 10-metre timed walk (10MTW) found that only 4 participants performed the test at a speed equivalent to an elderly population (Bohannon and Williams Andrews, 2011). Therefore detecting change in this measure may have been impeded by a ceiling effect.

The five times sit to stand measure has previously been reported to not only assess muscle strength, but can also reflect the functioning of multiple systems that include coordination, balance and sensation (Lord *et al.*, 2002) The mitochondrial group performance of the task is similar to an elderly population (Bohannon *et al.*, 2007). Yet again the m.8344A>G subgroup performed the functional task with more difficulty than the participants with the m.3243A>G mutation, but were able to perform this test without risk of falling. The lack of statistical change again could be due to the small numbers involved and the type of intervention with the specificity of response to training likely leading to improvement in exercise capacity but not strength or function (ACSM, 2006). Although improvements in muscle power were seen in the study, it is unlikely that the improvements reported would be large enough to produce a change in the performance of a sit to stand movement. This is supported by Gross et al,

who showed that even though elderly women had half the strength of younger women they were still able to generate enough force to perform a sit to stand movement, and that a critical threshold needs to be crossed before a change in function can be observed (Gross *et al.*, 1998; Lexell, 2000). The results from this study suggest that a relevant cut off point needs to be established to predict at what point the sit to stand movement is compromised and investigate the relative contributions of impairments to the loss of this vital function.

Alongside the limitations of the measures used, the small numbers of participants, and the high level of functioning of the mitochondrial patients, the type and location of the intervention may also have contributed to the lack of change in function reported in the participants. Although improvements in function have been reported in previous studies after an aerobic intervention (Keysor and Jette, 2001; Quaney *et al.*, 2009), many of these studies involved task specific training such as supported treadmill training (Newman *et al.*, 2007). These would have stressed participants balance systems which cannot be said to occur with a cycling intervention (Buchner *et al.*, 1997). Also improvements following an exercise programme in other studies have been reported to be more significant if the exercise is directly supervised or group based, rather than one that is home based or unsupervised (Snook and Motl, 2009; Mayo *et al.*, 2013). Despite the intervention in this study being performed in a supervised environment (local gym), we were unable to directly supervise every training session due to the geographical spread of our participants (ranging from Brighton to Glasgow).

In summary, the functional measures used in this study were unable to detect improvements in function following an exercise intervention. This is likely to be due to i) the small number of participants in the study, which had been designed

to detect a change in exercise capacity, ii) the high level of functioning in all but two of the participants, and iii) the type of intervention. Despite the lack of change in functional tasks, future use of functional measures should not be dismissed, due to the multi-system nature of these measures and mitochondrial disease (McFarland *et al.*, 2002; Turnbull *et al.*, 2010). Also the lack of change reported in the functional measures mirrored that reported in disease severity. Further studies are required using functional measures on a larger cohort of patients to establish if the relationship between changes in functional measures and disease burden is robust. The establishment of a battery of functional measures would be valuable in providing further methods of monitoring disease progression and the effectiveness of interventions in this multi-system disease in a meaningful way for both clinicians and patients.

6.7.3 Are disease burden, quality of life and cognition improved following an aerobic exercise intervention?

This study was unable to demonstrate improvements in disease burden, quality of life or cognition following an aerobic intervention. Previous exercise studies have attempted to measure quality of life (Cejudo *et al.*, 2005; Jeppesen *et al.*, 2006b; Taivassalo *et al.*, 2006; Jeppesen *et al.*, 2009) but none have measured changes in disease burden or cognitive function.

Quality of life has been reported to improve in previous exercise studies although this has not always been statistically significant (Cejudo *et al.*, 2005; Taivassalo *et al.*, 2006). The measurement of quality of life is notoriously difficult and numerous methods are available to measure this abstract concept (Wade, 2003). This study, due to the high level of participant burden chose the SF12 to measure quality of life, as this measure already formed part of the

disease severity scale (NMDAS). This questionnaire is a shortened version of the SF36 used by Taivassalo *et al,* who previously detected an improvement in quality of life in patients with mitochondrial disease following an exercise program (Taivassalo *et al.*, 2006), and has been shown to relate to disease burden (Orsucci *et al.*, 2012). The lack of improvement reported in this study may have been due to the reduced sensitivity of the SF12 and that the quality of life scores of our participants were higher than those previously reported. The development of a disease specific quality of life questionnaire in Newcastle may provide a more relevant and sensitive measure of quality of life (Elson *et al.*, 2013).

The ability of this study to measure the effect of an aerobic intervention on cognitive performance was prevented as only two participants presented with a cognitive impairment. This is in stark contrast to the incidence reported in previous studies (Kartsounis *et al.*, 1992; Finsterer, 2009). It is known that aerobic exercise can improve cognitive function in other populations such as those post stroke and the healthy elderly (Weuve *et al.*, 2004; Angevaren M, 2008; Quaney *et al.*, 2009; Kramer.Arthur F *et al.*, 1999), but the effect of exercise on cognition has not been investigated previously in mitochondrial disease. Unfortunately as only two participants at baseline demonstrated cognitive impairment the study was unable to comment on the effect of aerobic exercise on cognition of the group, although cognitive scores did not improve in these two participants. The relatively small number of participants with cognitive impairment in this study may be due to the fact that either this population represents a high functioning subgroup of patients, or that the ACE-R was not sufficiently sensitive to detect deficits in cognitive function. As the ACE-R was devised as a brief screening tool, and not to provide in depth

neuropsychological assessment, this is unremarkable (Eneida *et al.*, 2006). The NMDAS also includes a number of neuro-psychometric tests; the use of these more robust tests detected only 3 participants with cognitive deficits, which confirms the high level of cognitive function in the cohort of patients in this study. The intervention in this study required participants to exercise independently in a community setting and therefore seems to have limited recruitment to a group of patients with minimal cognitive impairment.

In summary, quality of life is a complicated construct (Wade, 2003) which is unlikely to be changed by improvement in one impairment (exercise capacity). In addition, slowly progressive conditions such as mitochondrial disease appear to lead to a re-setting of quality of life scores, this may explain the near normal quality of life scores reported in our cohort at baseline (Burns *et al.*, 2012).

6.8 **Limitations**

Although this is the first study to evaluate the effect of an exercise intervention on the functional capacity of patients with mitochondrial disease the numbers involved remain small due to the rarity of the condition and the limited number of patients eligible to participate. A power calculation was performed to ascertain the number of participants required to be able to demonstrate a change in exercise capacity; this number may not have been sufficient to show a change in functional tests or to compare changes between groups.

The two groups, although originally matched for age and sex, did not remain so, due to the withdrawal of two participants. These differences in the groups were further exacerbated by differences in physical activity, exercise and functional capacity at baseline. The differences reported were deemed to be acceptable, as studies have demonstrated that the differences in the physical and exercise capacity reported are typical in this disease population (Taivassalo *et al.*, 2003; Apabhai *et al.*, 2011).

The mitochondrial group showed a considerable heterogeneity, despite limiting the study to two genotypes, and unfortunately contained only two participants with the m.8344A>G mutation whose abilities were different to the m.3243A>G participants. The inclusion of both genotypes was felt to be appropriate as both have high levels of muscle involvement (Chinnery *et al.*, 1997) that has been shown to improve with exercise (Taivassalo *et al.*, 2001; Trenell *et al.*, 2006). The great variation in symptoms seen in this study group is typical of the mitochondrial disease cohort in Newcastle and was thought to represent a typical variation in the presentations of two genotypes. This study has highlighted the differences in performance of these two genotypes, suggesting

that future studies would benefit by restricting participants to one genotype. The rarity of mitochondrial disease will make this difficult and will require collaboration with other specialist centres within this country and around the world to achieve the number of participants necessary.

The study was a case controlled study using sedentary controls unaffected by mitochondrial disease; no comment can be made on the whether a group with mitochondrial disease would have remained unchanged without an exercise intervention as no disease control group was used. The likelihood of any change in abilities was, however, thought to be unlikely over the 4 months of the intervention as mitochondrial disease is a slowly progressive condition.

Participants had to be able to complete an unsupervised exercise intervention; therefore selection of a higher functioning group of mitochondrial patients may have occurred in this study thereby creating bias. Despite the patients in the study functioning at a high level, the level of disease burden was high (mutation load) and physical abilities were different to the control group.

No conclusions can be drawn about the exact mechanisms that resulted in the improved exercise capacity in this study as no muscle biopsies were performed as part of this trial. Previous studies have reported changes to exercise capacity similar to the changes reported in this trial; therefore it is reasonable to presume that the mechanism of change is improved oxygen extraction due to mitochondrial biogenesis or improved efficiency of the mitochondrial respiratory chain.

6.9 **Future work**

Further work is required to assess the effects of different exercise interventions on other mitochondrial genotypes to ensure that exercise is safe for all types of mitochondrial disease. The wide range of exercise ability reported in mitochondrial disease and the variable responses to exercise are still not fully understood. Further studies are needed to enable the prescription of individual exercise programmes at a level that is beneficial for all patients.

The assessment of the reliability, validity, and sensitivity, along with measures of minimal detectable change, of functional outcome measures is required within this population to enable measures to be more informative in future clinical trials. Greater use of functional measures in the clinical management of this disease group will allow clinicians and researchers to gain further information about how disease progression and interventions affect the activity and participation of patients with mitochondrial disease.

6.10 **Conclusion**

This study demonstrates that aerobic exercise is safe and well tolerated within another two mitochondrial genotypes. Exercise capacity improved in the mitochondrial and control groups to a similar level, which confirms that the improvements reported in this group of patients are likely to be due to reversal of deconditioning rather than alterations in the primary biological deficit.

The improvement in exercise capacity reported was not translated into improvements in the functional tasks measured. The functional measures used mirrored measures of disease burden and quality of life and therefore may have the potential to be used as proxy measures of disease burden. The lack of change in functional capacity in this study should encourage researchers to investigate other interventions with multi-faceted approaches. The inclusion of task orientated and balance interventions alongside aerobic and resistance exercises may lead to improvements in patients' abilities as they challenge all the systems potentially involved in mitochondrial disease and this therefore warrants further study.

Chapter 7 The effect of aerobic exercise on gait characteristics of patients with mitochondrial disease

7.1 **Introduction**

Exercise as an intervention for people with mitochondrial disease has previously been shown to be beneficial for muscle symptoms such as muscle fatigue, muscle weakness and reduced exercise capacity, alongside benefits in quality of life (Cejudo *et al.*, 2005; Jeppesen *et al.*, 2006b; Taivassalo *et al.*, 2006; Trenell *et al.*, 2006; Jeppesen *et al.*, 2009). Previous exercise studies in mitochondrial disease have assessed changes in muscle at a molecular and physiological level, but few have assessed whether these changes translated into improvement in activities of daily living such as walking. Although improvement in exercise capacity may be the primary goal of the researcher, translation into improved walking capacity is more likely to be of interest to the participant and if demonstrable may result in improved compliance with interventions.

Walking is critical to independent function and involves the integration of many neurological systems to produce a coordinated movement. Gait analysis provides a quantifiable, selective and sensitive measurement tool that is capable of monitoring disease progression, is associated with primary pathology and is vital for independent living (Baltadjieva *et al.*, 2006; Verghese *et al.*, 2007; Rochester *et al.*, 2012; Rochester *et al.*, 2014b).

Measurement of walking speed is commonly used in clinical and research settings, but only measures the endpoint of changes in gait and, while highly sensitive, lacks specificity. Individual characteristics of gait, however, have been used as markers of pathology and been shown to detect changes post an intervention (Rochester *et al.*, 2014b). The previous cross-sectional study of gait in mitochondrial disease demonstrated that impairments in selective gait

characteristics were linked to mitochondrial disease, genotype, and that they related to the pathological changes and also to the burden of disease (Galna *et al.*, 2013b).

To our knowledge this is the first case controlled study utilising gait characteristics as measures of change after an intervention in mitochondrial disease. The use of gait characteristics as a measure of disease progression and as a research tool to evaluate interventions will allow us to further our knowledge concerning which aspects of walking are most affected by disease and which are amenable to intervention.

7.2 **Study aims and hypotheses**

Aim

To determine whether changes in aerobic capacity following an aerobic exercise intervention translate into changes in gait characteristics of people with mitochondrial disease.

Primary hypothesis

Changes in exercise capacity will result in improvement in impaired gait characteristics.

Dual task performance will improve following an aerobic exercise intervention.

Improvements in exercise capacity following an aerobic exercise intervention will translate into improved gait efficiency over a prolonged walk.

7.3 Subjects and methods

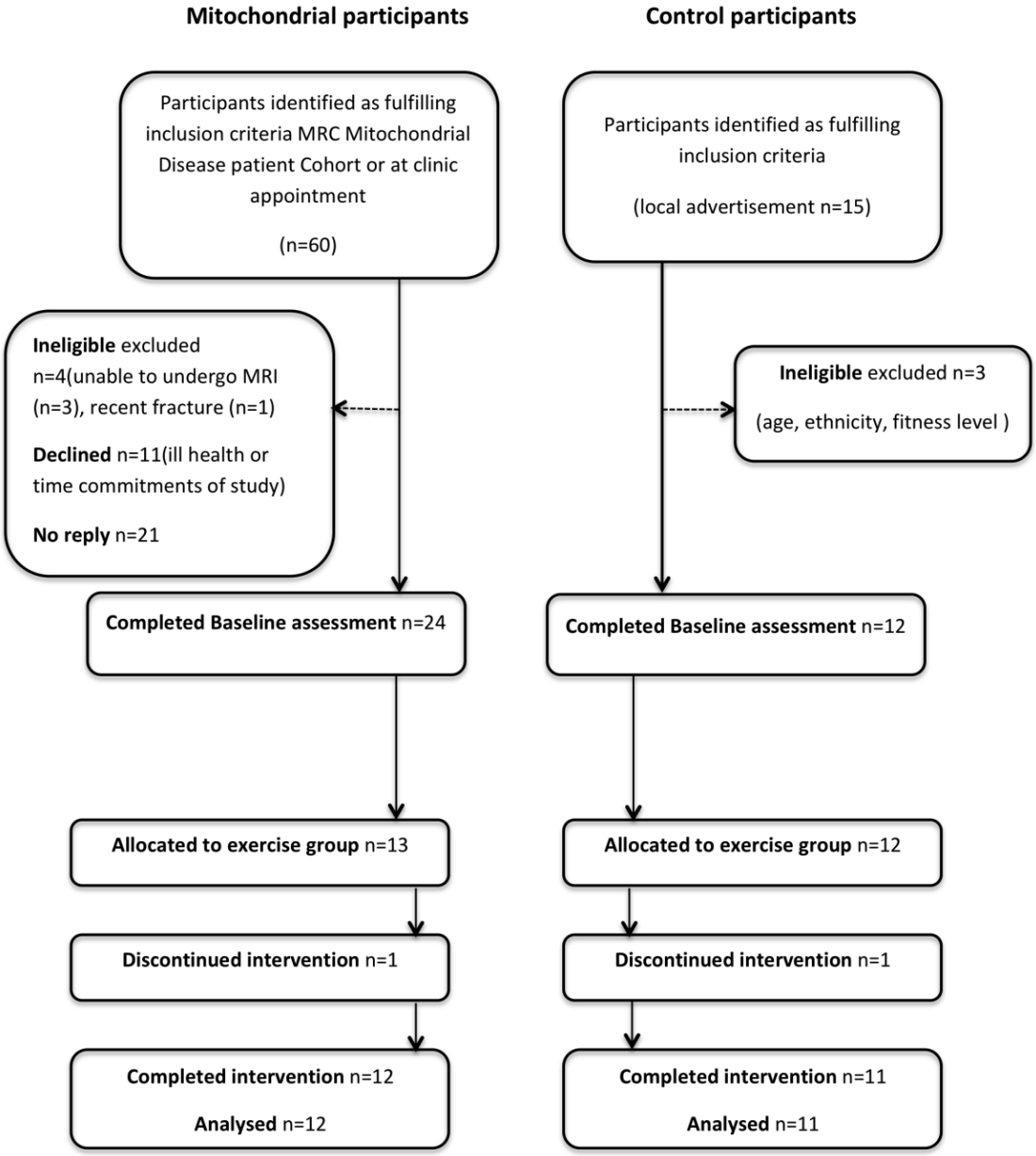
7.3.1 Subjects and study design

Sixty mitochondrial patients were invited to participate in the exercise study from consecutive attendees at a specialist outpatient clinic between August 2010 and July 2011. Patients were clinically identified as having the m.3243A>G or m.8344A>G mitochondrial DNA mutation Figure 7-1.

All eligible patients were invited to participate based on the following clinical inclusion criteria: (i) clinical stability for >6 months; (ii) ability to use an upright stationary bicycle ergometer; (iii) no current participation in regular physical activity (≤1 weekly session) and (iv) reported exercise intolerance and fatigue. Exclusion criteria were the presence of known cardiac involvement, co morbidities precluding exercise training, and contra-indications to Magnetic Resonance Imaging.

From the 36 participants in the previous observational study (chapter 3) a convenience sample of thirteen patients with mitochondrial disease and the 12 sedentary control participants continued onto an exercise intervention study. One participant from each group failed to return for final testing due to time commitments.

Figure 7-1: Consort diagram for the study of the effect of aerobic exercise on physiological, functional and gait measures in mitochondrial disease.

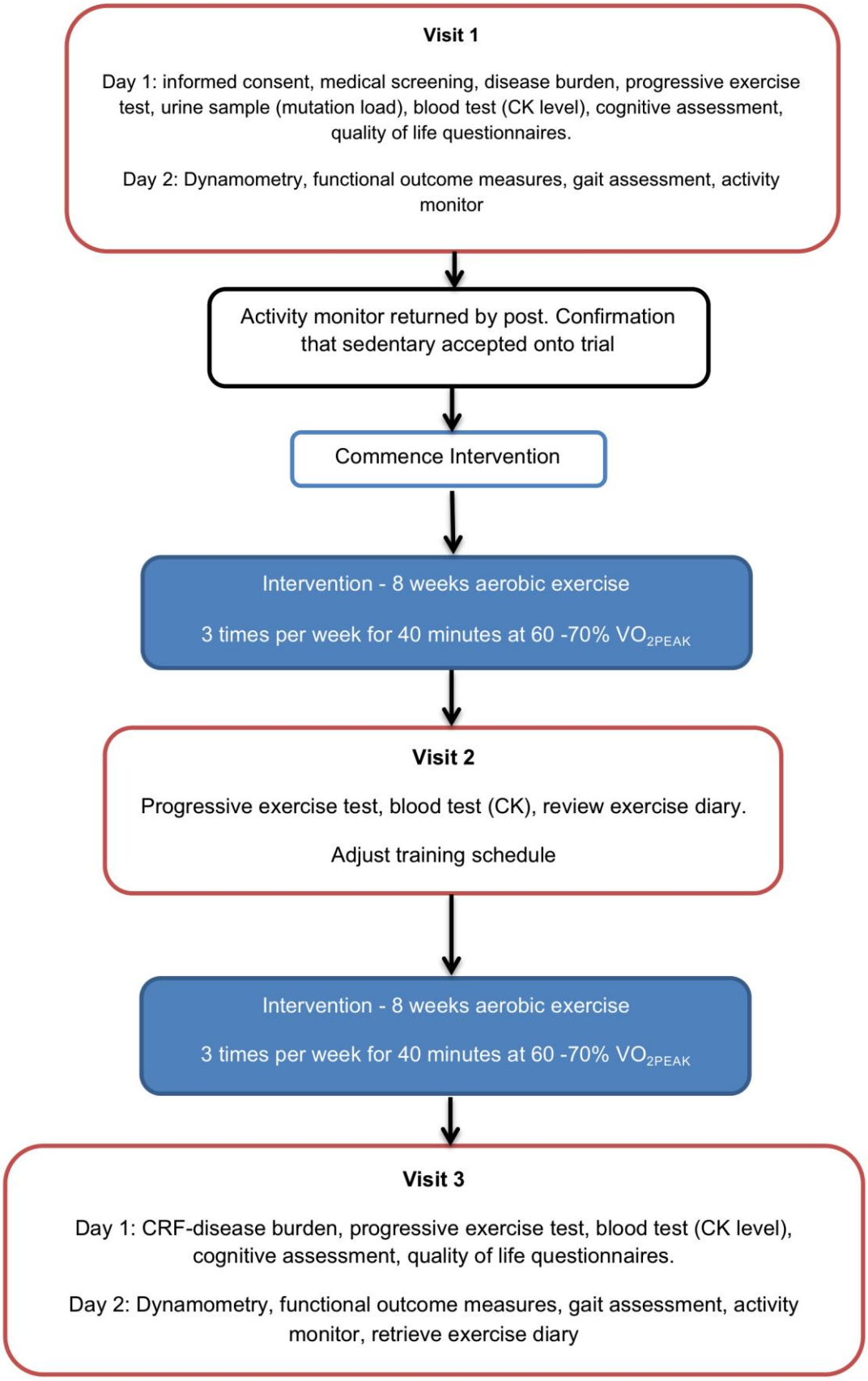


All visits were undertaken at the two centres listed in the methods section over a period of two days (Chapter 2) (Figure 7-2). Over the two days both disease and control groups underwent assessments of; baseline physical activity, anthropometry, resting metabolism, peak exercise capacity, proximal muscle strength and gait analysis. In addition the disease burden of the mitochondrial participants was assessed, whilst the sedentary control volunteers underwent a review of their medical history, physical examination and completed the Physical Activity Readiness Questionnaire.

After completion of a 16-week aerobic exercise intervention all initial assessments (except physical activity measurement) were repeated. An additional visit was performed midway through the intervention, to ensure that no adverse responses to exercise had occurred and to reassess the exercise capacity of participants.

The following section will include a brief explanation of the methods used in this study. (Full details can be found in Chapter 2).

Figure 7-2: Summary of study visits and intervention.



7.3.2 Disease Burden

All subjects underwent a physical examination by an experienced clinician. Disease severity was assessed and disease burden was established using the Newcastle Mitochondrial Disease Adult Scale (Schaefer *et al.*, 2006). Mutation load, an established marker of clinical outcome, was derived from urinary epithelial cells (Whittaker *et al.*, 2009).

7.3.3 Anthropometry

Body weight (kg) and standing height (m) were measured before and after the exercise intervention (Section 2.3.5). Whole body composition was determined using air displacement plethysmography (Section 2.3.6).

7.3.4 Physical Activity

Physical activity and energy expenditure were assessed objectively using a validated multi-sensor array (SenseWear Pro3, Bodymedia Inc, Pennsylvania, USA 2.5)(St-Onge *et al.*, 2007).

7.3.5 Progressive Exercise testing

A stepped incremental workload exercise test was performed using analysis of expired air gases and non-invasive bio reactance cardiac output, to elicit a symptom limited maximum oxygen uptake and heart rate response.

7.3.6 Muscle Strength

Proximal muscle power of the hip flexor and extensor muscles was measured using an isokinetic dynamometer (CSMI HUMAC®/NORM testing and rehabilitation system).

7.3.7 Quantitative gait assessment

Gait was assessed using a 7m long × 0.6m wide instrumented mat (Platinum model Gaitrite, software version 4.5, CIR systems, USA).

Participants were asked to perform 3 different walking tasks (single task, dual task and a continuous walk over 6 minutes). During the single task walks participants were instructed to perform three 12m walks at their “comfortable pace”. The mat was placed in the centre of the 12 m walkway to ensure that steady gait speed was measured. The concurrent task for the dual task walks consisted of a cognitive task (digit span recall).

The 6-minute walk involved participants walking continuously around a 25m oval circuit. Gait was repeatedly recorded as they walked over the mat placed on one side of circuit.

Gait was quantified using a predefined model hypothesized to reflect independent features of neural control and characterise the features of gait associated with mitochondrial disease and its genotypes (Lord *et al.*, 2013b).

7.4 **Aerobic training intervention**

Upon completion of baseline tests, participants undertook 16 weeks of aerobic exercise training. Exercise sessions were conducted at a local gym. Participants attended sessions 3 times a week for the 16 weeks (48 sessions in total). The intensity of these were tailored to the individual and increased progressively over the 16 weeks. Full details of the monitoring and progression of the exercise intervention can be found in section 6.4.

Midway through the training period participants attended the clinical research facility to repeat the progressive exercise test performed at baseline. Training heart rates were adjusted to maintain training at a heart rate ≈70% VO_{2PEAK} for the remainder of the exercise intervention.

7.5 **Statistical Analysis**

The small numbers and variability in some gait parameters required a conservative approach to statistical analysis to be undertaken. The failure of one m.8344A>G participant to complete all walking tests led to the remaining participant with the m.8344A>G mutation being reported separately, with the remaining mitochondrial participants only containing people with the m.3243A>G mutation. This decision was made due to the large difference in performance of the two genotypes noted in the cross-sectional study (Galna *et al.*, 2013b).

To compare within group differences, before and after the exercise intervention, Related-samples Wilcoxon Signed Rank Tests were performed.

Continuous data is presented as medians and interquartile ranges unless otherwise stated. Statistical significance was achieved with a p value of < 0.5, or < 0.1 where multiple comparisons were performed.

7.6 **Results**

All consented participants attended baseline testing (Mitochondrial group n=13, control group n=12). At baseline one mitochondrial patient was unable to complete dynamometry due to an adverse response to the peak exercise test and two mitochondrial participants were unable to complete walking with the addition of a dual task due to an increased risk of falling. One of these participants was unable to complete any of the walking tasks during the final visit due to a high risk of falling. The data of this participant was, therefore, excluded from the final analysis. One participant from each group failed to attend the final visit; therefore the final results are reported for two groups of 11 participants (MD group, m.3243A>G n=10, m.8344A>G n=1, control n=11).

All participants completed the exercise intervention, with all participants completing ≥ 80% of the exercise sessions and no adverse events were reported.

The following sections will report the baseline anthropological, clinical and gait characteristics of the participants and the responses of both groups to the exercise intervention. Results are presented comparing the control group with the m.3243A>G. The sole m.8344A>G participant's data is reported separately.

7.6.1 Characteristics of participants

The baseline clinical features and disease burden of the mitochondrial participants shown in Table 7-1 demonstrate the wide variety of clinical features reported in the mitochondrial group, with a high mitochondrial mutation load and disease burden seen in the participants with the m.3243A>G mutation (% heteroplasmy; median 74,quartiles 60, 82, NMDAS score; median 15, quartiles

11, 22). Only one participant with the m.3243A>G mutation demonstrated cognitive impairment. The m.8344A>G participant showed a much higher disease burden with a mutation load of 95% and a NMDAS score of 55.

Baseline demographics of all mitochondrial participants and controls are shown in Table 7-2. Differences at baseline between the m.3243A>G group and controls are clearly seen, with the m3243A<G group having a lower weight and baseline physical activity level.

Table 7-1: Baseline clinical features of exercise participant with mitochondrial diseases. PEO-Progressive external opthalmoplegia.

Genotype	Age	Sex	Heteroplasmy (%)	NMDAS	Principle clinical features
m.3243A>G	58	F	59	12	Hearing loss, ataxia, constipation, underweight, myopathy, myalgia, exercise intolerance
	39	M	80	17	Hearing loss, diabetes, migraine, fatigue, hypothyroidism, ataxia, constipation, exercise intolerance
	37	F	48	10	Hearing loss, diabetes, exercise intolerance, ataxia, dysarthria, asthma
	42	M	82	12	Hearing loss, diabetes, migraine, ataxia, depression, exercise intolerance
	47	M	63	28	Hearing loss, diabetes, fatigue, ataxia, myalgia, depression, myopathy, neuropathy, ptosis, PEO
	38	F	53	11	Hearing loss, migraine, constipation, myopathy, fatigue, exercise intolerance, asthma
	22	M	89	17	Hearing loss, migraine, epilepsy, ataxia, constipation, underweight, exercise intolerance, fatigue
	36	M	80	4	Migraine, exercise intolerance
	50	M	87	23	Hearing Loss, exercise intolerance, ataxia, myopathy, fatigue, depression, retinopathy, epilepsy, encephalopathy, cognitive decline, stroke-like episodes
	55	F	68	25	Hearing loss, diabetes, myopathy, exercise intolerance, ataxia, constipation, depression, retinopathy, PEO, ptosis, short stature, mild dysphagia, hypertension
m.8344A>G	28	M	95	55	Hearing loss, fatigue, epilepsy, ataxia, retinopathy, constipation, dysarthria, myopathy, myoclonus, neuropathy, exercise intolerance, underweight

Table 7-2: Baseline demographics and activity levels of control and mitochondrial participants.
*All data are reported as medians and (quartiles). Differences between control and m3243A<G groups are shown as * p<0.05, ** p<0.01.*
IPAQ=International Physical Activity Questionnaire

Variable	**Control**	**Mitochondrial participants**		**p value**
	(n=11)	**(m.3243A>G) (n=10)**	**(m.8344A>G) (n=1)**	**m.3243A>G v control**
Age (years)	37 (28, 45)	41 (38, 49)	28	.349
Height (cm)	170 (166, 177)	173 (164, 178)	173	.918
Mass (kg)	81.1 (71.9, 84.7)	61.6 (52.6, 70.4)	51.7	.013*
BMI (Kg/m2)	25 (24, 29)	20 (19, 24)	17	.006**
Fat Free Mass (kg)	57.5 (46.8, 60.5)	45.6 (36.6, 58.6)	40.3	.173
% Fat	29 (23, 38)	27 (18, 29)	22	.387
Energy Expenditure (calories per day)	2520 (2219, 2795)*	1928 (1789, 2340)	1745	.043*
Steps taken per day	8754 (7728, 10,536)*	6385 (4280, 7076)	1465	.013*

7.6.2 Gait characteristics of participant's pre and post exercise intervention.

Gait characteristics during single task walking.

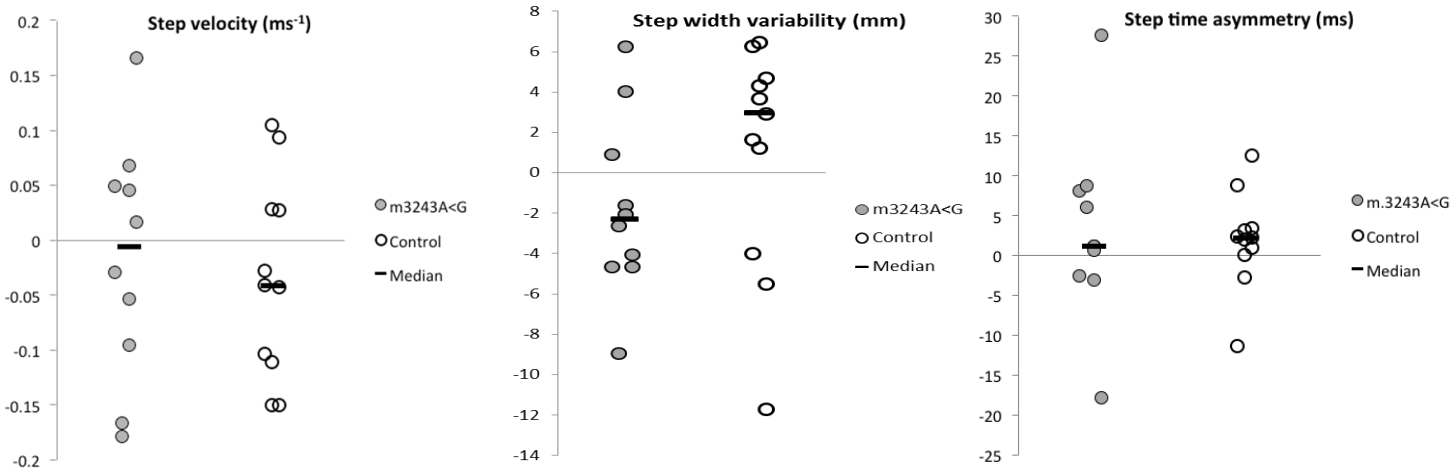
Gait variables during a single task walk pre and post intervention are reported for patients with the m.3243A>G mutation and sedentary controls in Table 7-3. Step width variability was the only gait variable to demonstrate a difference between the m.3243A>G and control group at baseline with gait speed being within normal limits.

Gait variables during a single task walk demonstrated no significant changes post the exercise intervention in either group, except for step length variability in the control group. To demonstrate the variable responses of both groups' scatterplots are shown below for 3 gait variables (Figure 7-3).

Table 7-3: Gait characteristics during single task walking pre and post exercise intervention.
** denotes a significant difference within group over time, † =.002 denoting a significant difference between groups at baseline.*

Gait variable	Mitochondrial patients (m.3243A>G) (n=10)		Median change score	% change	*p* value	Controls (n=11)		Median change score	% change	*p* value
	Baseline	Post intervention				Baseline	Post intervention			
Step velocity (ms^{-1})	1.43 (1.33, 1.64)	1.50 (1.38, 1.60)	-0.006	-1	.575	1.55 (1.49, 1.64)	1.51 (1.46, 1.59)	-0.04	-2	.155
Step length (m)	0.74 (0.69, 0.86)	0.74 (0.68, 0.87)	-0.005	-1	.575	0.77 (0.76, 0.80)	0.78 (0.73, 0.81)	0.002	<1	.477
Cadence ($steps.min^{-1}$)	116 (109, 123)	116 (109, 120)	-1	-1	.959	119 (115, 123)	118 (111, 122)	-1	-1	.213
Step time asymmetry (ms)	8 (4, 16)	10 (4, 16)	1	52	.333	9 (5, 11)	11 (9, 12)	2	24	.131
Step length variability (mm)	22 (18, 26)	17 (14, 25)	1	4	.959	18 (16, 21)	16 (13, 18)	-3	-16	**.021***
Step time variability (ms)	15 (13, 18)	12 (11, 16)	-2	-14	.386	14 (10, 16)	12 (9, 12)	-3	-24	.091
Step width (cm)	9.4 (7.7, 10.7)	9.8 (7.5, 0.106)	0.1	1	.721	9.3 (8.5, 10.4)	9.6 (7.9, 11.4)	0.3	<1	.790
Step width variability (mm)	27 (24, 34)†	27 (20, 32)	-2	-7	.203	17 (16, 21)	19 (17, 21)	3	14	.424
Step length asymmetry (mm)	11 (7, 14)	13 (7, 17)	2	20	.721	17 (9, 26)	12 (11, 23)	-1	-3	.534

Figure 7-3: Scatterplots of median change scores of mitochondrial disease and control participants during single task walking.



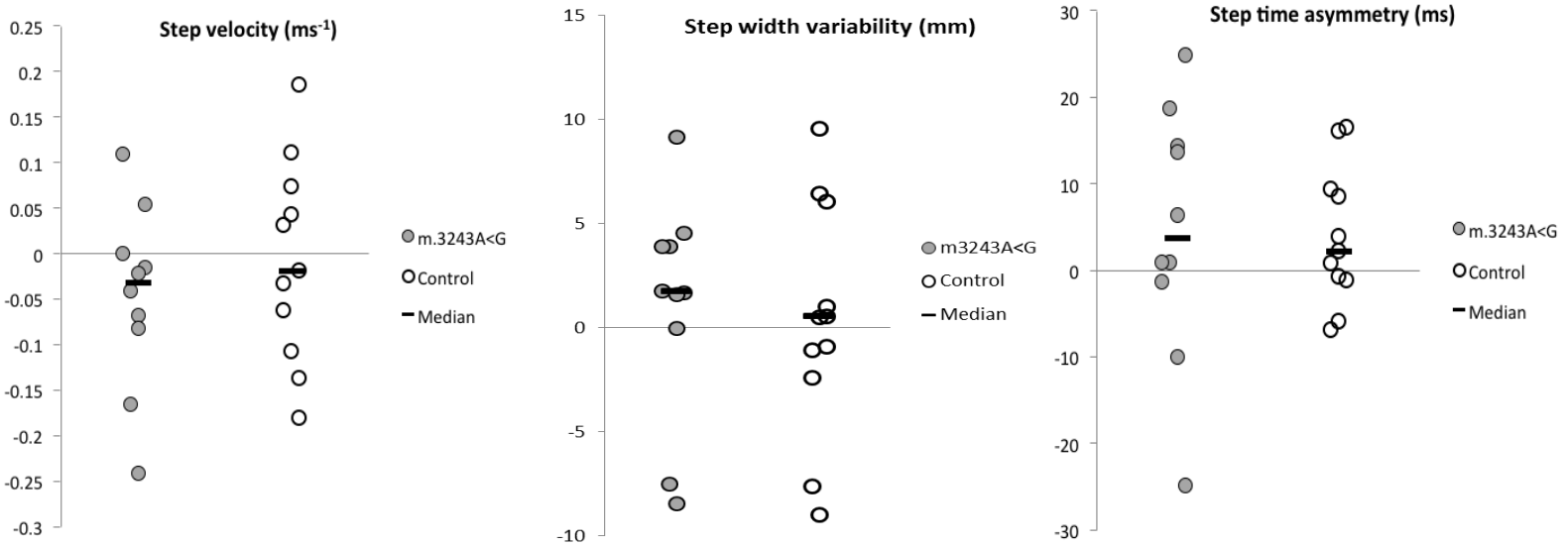
Gait characteristics during a dual task walk.

Dual task walking at baseline demonstrated a difference in the same variable as reported during single task walking; step width variability. No significant changes were demonstrated post intervention in the either group (Table 7-4). Scatterplots of a selective group of variables are shown (Figure 7-4) to demonstrate the variety in change scores. These show a mixed picture, with gait velocity reduced post intervention in all but two mitochondrial participants, but step width variability and time symmetry increased.

Table 7-4: Dual task walking performance pre and post exercise intervention.
† = .02 significant difference between groups at baseline.

Gait variable	Mitochondrial patients (m.3243A>G) (n=10)		Median change score	% change	*p* value	Controls (n=11)		Median change score	% change	*p* value
	Baseline	Post intervention				Baseline	Post intervention			
Step velocity (ms^{-1})	1.46 (1.22, 1.52)	1.39 (1.20, 1.53)	-0.03	-2	.169	1.50 (1.42, 1.59)	1.46 (1.39, 1.60)	-0.18	-1	.859
Step length (m)	0.74 (0.67, 0.75)	0.71 (0.67, 0.79)	-0.007	-1	.241	0.75 (0.73, 0.77)	0.76 (0.70, 0.78)	-0.004	-1	.929
Cadence ($steps.min^{-1}$)	117 (108, 121)	112(106,120)	-1	-1	.169	121 (114, 123)	119 (111, 122)	-1	<-1	.675
Step time asymmetry (ms)	8 (3, 10)	10 (7, 15)	4	125	.285	5 (3, 6)	9 (5, 14)	2	34	.155
Step length variability (mm)	22 (19, 28)	26 (21,29)	2	7	.285	18 (16, 22)	20 (16, 27)	2	10	.534
Step time variability (ms)	16 (15, 18)	16 (14.17)	-2	-11	.093	14 (12, 15)	12 (11, 15)	-1	-9	.657
Step width (cm)	9.0 (7.6, 11.7)	9.1 (7.3, 10.6)	-0.5	-4	.386	9.0 (8.7, 10.5)	8.7 (8.3, 12.4)	-0.2	-2	1.00
Step width variability (mm)	25 (22, 28) †	27 (22, 31)	2	8	.333	19 (16, 22)	18 (14, 23)	1	3	1.00
Step length asymmetry (mm)	13 (5, 21)	5 (2,15)	-2	-16	.721	17 (5, 26)	15 (12, 28)	4	32	.131

Figure 7-4: Scatterplots of median change scores of mitochondrial disease and control participants during dual task walking.



Gait characteristics during a continuous, prolonged walk.

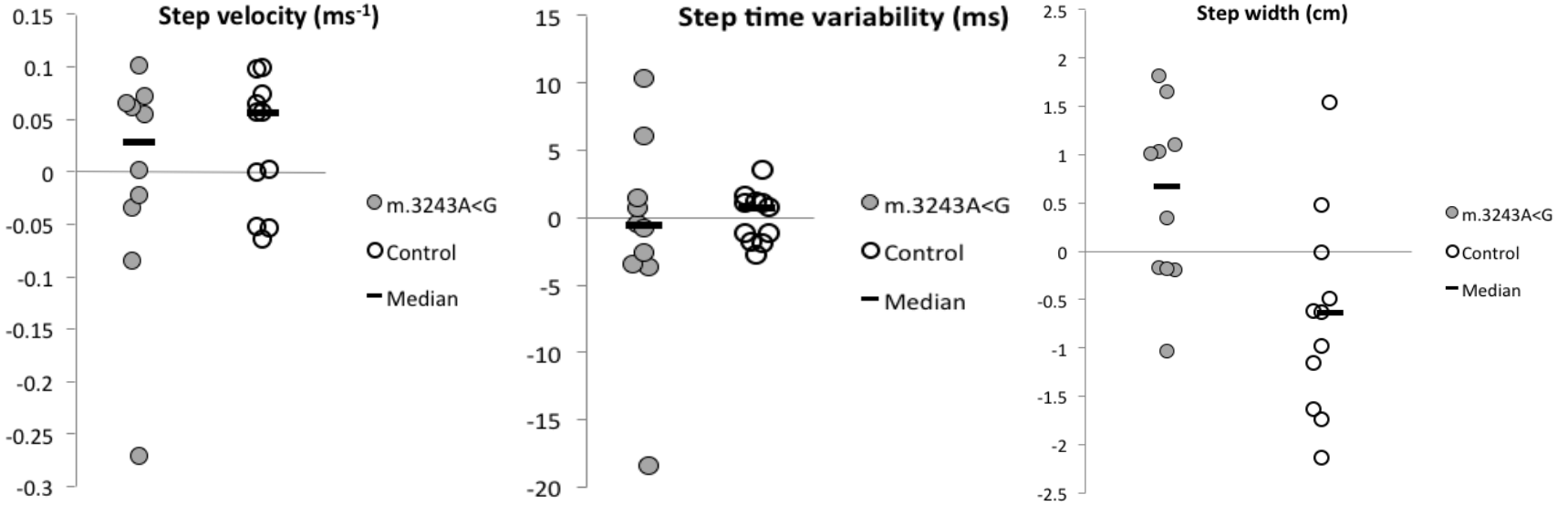
A different gait characteristic (step time variability) demonstrated a difference at baseline between the groups during a continuous walk. One gait characteristic demonstrated a change post intervention (step width). This was, however, only within the control group, with the majority of participants in this group having a narrower gait post intervention, whereas the mitochondrial response remained varied (Table 7-5)(Figure 7-5).

These results combined demonstrate that both mitochondrial and control groups were similar in all aspects of gait except variability at baseline, with the mitochondrial group demonstrating a more variable gait. Gait characteristics of the groups remained essentially unchanged over all three walking tests post exercise intervention. Investigation of individual change scores showed a varied response, the small numbers of participant's involved prevents further interpretation of these results.

Table 7-5: Pre and post intervention gait characteristics over a 6 minute walk.
†denotes p=.002 between groups at baseline time point. significant difference within group pre and post intervention.*

Gait variable	Mitochondrial patients (m.3243A>G) (n=10)		Median change score	% change	*p* value	Controls (n=11)		Median change score	% change	*p* value
	Baseline	Post intervention				Baseline	Post intervention			
Step velocity (ms^{-1})	1.29 (1.22, 1.57)	1.31 (1.24, 1.52)	0.03	2	.646	1.47 (1.35, 1.51)	1.49 (1.38, 1.56)	0.06	4	.110
Step length (m)	0.73 (0.65, 0.81)	070 (0.66,0.81)	0.003	<1	.878	0.74 (0.71, 0.77)	0.75 (0.71, 0.80)	0.02	2	.248
Cadence ($steps.min^{-1}$)	115 (108, 119)	111(106, 119)	<1	<1	.799	114 (111, 118)	117 (111, 122)	2	2	.424
Step time asymmetry (ms)	8 (5, 17)	9 (5, 15)	2	13	.575	10 (5, 13)	10 (7, 15)	0.4	15	.374
Step length variability (mm)	20 (19, 23)	21 (18, 24)	-1	-3	.721	17 (16, 22)	18 (16, 22)	0.2	1	.722
Step time variability (ms)	17 (13, 18) †	14 (12, 22)	-1	-5	.721	11 (10, 12)	12 (10, 13)	1	7	.790
Step width (cm)	10.4(8.3, 11.3)	10.5(9.5, 12.5)	0.7	5	.114	10.9 (9.0, 12.2)	9.2 (8.5, 11.3)	-0.6	-8	.041*
Step width variability (mm)	28 (24, 30)	28 (24, 32)	1	3	.333	23 (19, 26)	22 (18, 27)	-1	-3	.374
Step length asymmetry (mm)	11 (10, 14)	13 (5, 21)	1	11	.721	12 (9, 21)	12 (2, 18)	-4	-27	.182

Figure 7-5: Scatterplots of median change scores of mitochondrial disease and control participants during a prolonged walk



Dual task interference on gait characteristics

Dual task interference was calculated using equation shown below (Rochester *et al.*, 2014a):

$$Dual\ task\ interference = \frac{dual\ task - single\ task}{single\ task}$$

Table 7-6 demonstrates that dual task interference was similar between the two groups at baseline and no change was reported within the mitochondrial disease group post intervention. A difference in interference was reported in step length variability within the control group.

Table 7-6: Dual task interference pre and post exercise intervention.

Gait variable	Mitochondrial patients (m.3243A>G)		Median change score	*p* value	Controls		Median change score	*p* value
	Baseline	Post intervention			Baseline	Post intervention		
Step velocity (ms^{-1})	-0.06 (-0.12, 0.04)	-0.08 (-0.13, -0.04)	<0.001	.386	-0.06 (-0.07, -0.03)	-0.04 (-0.06,-0.002)	0.008	.534
Step length (m)	-0.04 (-0.1, -0.002)	-0.07 (-0.1, -0.03)	-0.007	.575	-0.04 (-0.06, -0.02)	-0.03 (-0.05,-0.01)	0.003	.657
Cadence ($steps.min^{-1}$)	-0.01 (-0.03, 0.03)	-0.02 (-0.04, -0.002)	-0.01	.241	-0.02 (-0.03,0.09)	0.002 (-0.01, 0.02)	0.007	.534
Step time asymmetry (ms)	-0.50 (-0.59, 0.12)	0.37 (-0.39, 2.07)	0.1	.508	-0.24 (-0.60, 0.84)	0.26 (-0.4, 0.4)	0.3	.477
Step length variability (mm)	0.11 (-0.14, 0.29)	0.27 (0.03, 0.53)	0.1	.333	-0.03 (-0.06, 0.11)	0.35 (0.04, 0.72)	0.5	.033*
Step time variability (ms)	-0.003 (-0.12, 0.24)	0.11 (-0.1, 0.3)	0.1	.878	-0.03 (-0.19, 0.32)	0.18 (-0.17, 0.53)	0.05	.534
Step width (m)	0.05 (-0.1, 0.15)	-0.04 (-0.08, 0.05)	0.004	.799	-0.008 (-0.04, 0.08)	0.08 (0.01, 0.13)	0.05	.647
Step width variability (mm)	-0.12 (-0.24,0.03)	0.10 (-0.14, 0.14)	0.31	.241	-0.02 (-0.07,0.12)	-0.08 (-0.28, 0.16)	-0.2	.722
Step length asymmetry (mm)	-0.33 (-0.48, 0.39)	-0.37 (-0.71, 0.02)	0.03	.455	-0.13 (-0.58, 0.71)	0.14 (-0.08, 1.38)	0.2	155

Changes in gait characteristics patients with mitochondrial disease during a continuous 6-minute walk.

This study investigated whether an aerobic exercise intervention reduced deterioration in gait characteristics over a prolonged walk of 6 minutes. Comparisons were made between the gait characteristics during the first two and last two minutes of the 6-minute walk. At baseline no differences were reported between the gait characteristics between the first and last two minutes of a 6-minute walk ($p > .05$). Comparisons of median change scores pre and post intervention revealed no differences in gait variables.

Table 7-7: Gait variables at the beginning and end of a 6 minute walk in participants with mitochondrial disease, pre and post intervention. Median (Quartiles). p value= comparison of median change scores pre and post intervention.

Gait variable	**6 minute walk pre intervention**		**Median change score**	**6 minute walk post intervention**		**Median change score**	***p* value**
	Minute 1&2	**Minute 5&6**		**Minute 1&2**	**Minute 5&6**		
Step velocity (ms^{-1})	1.37 (1.27, 1.55)	1.27 (1.17, 1.59)	-0.04	1.35 (1.34, 1.53)	1.27(1.19, 1.52)	-0.03	.878
Step length (m)	0.72 (0.67, 0.81)	0.74 (0.64, 0.81)	-0.01	0.71 (0.67, 0.82)	0.69 (0.65, 0.80)	-0.01	.721
Cadence ($steps.min^{-1}$)	115 (110,119)	115 (107,118)	-2	114 (109,120)	111 (105,118)	-1	.721
Step time asymmetry (ms)	9.5 (5.6,15.1)	9.2 (1.5, 19.3)	-2	8.8 (6.4, 15.5)	9.5 (7.2, 12.2)	0.4	.721
Step length variability (mm)	21.1 (18.5, 26.8)	19.3 (17.3,23.0)	-0.4	21.8 (18.0, 24.4)	23.2 (17.2, 25.2)	0.2	.333
Step time variability (ms)	17.9 (14.5, 20.2)	15.7 (13.6, 19.3)	-1	16.2 (13.6, 18.8)	14.9 (13.4, 20.0)	0.04	.575
Step width (cm)	9.4 (8.8, 10.8)	10.9 (8.0, 12.2)	4	10.6 (8.6, 12.0)	10.7 (9.7, 12.2)	0.004	.508
Step width variability (mm)	28.4 (23.3, 30.7)	24.2(22.3,28.5)	-3	27.2 (22.8, 35.3)	27.6 (24.3, 28.9)	-1	.878
Step length asymmetry (mm)	7.7 (4.1, 14.4)	11.7 (6.3,16.4)	-0.1	10.4 (7.3, 19.6)	11.3 (6.7, 22.2)	< 0.1	.799

7.6.3 The effect of an aerobic exercise intervention on demographics, exercise parameters and gait characteristics.

Demographic features remained unchanged after the exercise intervention except for mass and BMI within the mitochondrial group (mass; 61.6 (52.6, 70.4) vs. 62.1 (53.4, 70.9), p= .047). This represents less than a 1% change in the median. The cognitive performance remained the same (94 (92, 97) vs. 95 (92, 98) (p= *.833*), as did the disease severity (NMDAS) (15 (11, 22) vs. 13 (10, 21) (p=. 631).

As reported in the previous chapter the exercise parameters and hip strength improved in both mitochondrial and control groups post an aerobic exercise intervention in both the genotypes assessed (Table 6-4,Table 6-5).

7.6.4 The effect of an exercise intervention on demographics, exercise and gait characteristics of the m.8344A>G participant

Only one participant with the m.8344A>G mutation was able to complete the gait analysis under single task conditions. The m.8344A>G participant's gait was shown to be more globally affected than the m.3243A>G group and therefore their results are presented separately (Table 7-8).

The baseline demographics show an increase in weight, fat free mass, and percentage fat, post exercise intervention (weight (kg); 52-55; fat free mass (kg) 40-41; % fat 22-26). Improvements are observed in peak exercise capacity (peak heart rate; 165-144, peak exercise capacity (ml/kg/min); 13.2-15.3; anaerobic threshold (ml/kg/min); 8.1-11.3; peak workload (watts/kg); 0.8-0.7, arterial venous O_2 difference; 5.9-6.7). Proximal hip strength demonstrated a mixed response with an improvement in hip extensor (0.63-0.72 nm/kg) and a reduction in hip flexor (0.25-0.15 nm/kg). A small reduction was observed in disease severity (NMDAS score 55-52).

The following table demonstrates an improvement in all aspects of gait for the sole participant with the m.8344A>G mutation, with improvements observed in velocity, step length and step width alongside a reduction in variability and asymmetry.

Table 7-8: Changes in gait characteristics of the participant with the m.8344A>G mutation.

Gait variable	Baseline	Post exercise
Step velocity (ms^{-1})	0.71	1.07
Step length (m)	0.53	0.66
Cadence ($steps.min^{-1}$)	80	98
Step time asymmetry (ms)	51.7	19.6
Step length variability (mm)	56.7	37.9
Step time variability (ms)	87.98	28.64
Step width (m)	0.17	0.11
Step width variability (mm)	60.6	35.8
Step length asymmetry (mm)	41.1	20.9

7.7 **Discussion**

Mitochondrial diseases result in a wide variety of symptoms that have the potential to affect gait. The previous cross-sectional study demonstrated that patients with mitochondrial disease present with selective gait deficits. These deficits relate to the pathological consequences of mitochondrial disease, and clinically measured disease burden, in a selective manner (Galna *et al.*, 2013b).

Exercise intervention studies within mitochondrial populations have been shown to improve exercise capacity, muscle strength, fatigue and function (Cejudo *et al.*, 2005; Taivassalo *et al.*, 2006; Trenell *et al.*, 2006; Mancuso *et al.*, 2012). Efficient gait is essential for independent living and functional activities (Studenski *et al.*, 2003). Measurement of gait has been used previously to identify pre-clinical symptoms, monitor disease progression (Lord *et al.*, 2013a; Rochester *et al.*, 2014b) and evaluate exercise interventions within neurological and elderly populations (Newman *et al.*, 2007; King and Horak, 2009; VanSwearingen *et al.*, 2011; Reisman *et al.*, 2013). This case controlled intervention study investigated the changes in gait characteristics post an aerobic exercise intervention as a marker of disease progression, fatigue and cognitive function.

The key findings of the study were that gait characteristics remained unchanged following an aerobic exercise intervention in the group of patients with the m.3243A>G mutation. Improvements were detected in participant with the m.8344A>G mutation with more globally affected gait, but these results are difficult to extrapolate to a wider group. Gait characteristics of the mitochondrial group with the m.3243A>G mutation mirrored measures of disease burden rather than exercise capacity.

The following sections will discuss the effects of the exercise intervention in this population and elucidate a possible explanation for the limited effects on gait of the exercise intervention used in this study.

7.7.1 The effects of an aerobic exercise intervention on the exercise performance of patients with mitochondrial disease

As previously discussed (chapter 6), all aspects of exercise performance improved following the exercise intervention in the mitochondrial group and the sub set of patients with the m.3243A>G mutation. The improvements in exercise characteristics were in agreement with previous studies and were probably due to peripheral adaptation of muscle tissue to exercise. Despite changes in exercise capacity, disease burden and cognition remained unchanged.

7.7.2 The effects of an aerobic exercise intervention on the gait characteristics of patients with mitochondrial disease

In this study gait characteristics during all three walks (single, dual task and continuous) remained unchanged following an exercise intervention in participants with the m.3243A>G mutation, despite improvements in exercise capacity and hip strength. These findings were not replicated in the one participant with the m.8344A>G mutation demonstrating a more globally impaired gait. In this participant improvements were reported in step velocity with increased cadence and step length, without increases in variability, asymmetry and step width. These improvements are likely to be due to improvements in secondary compensations to disease rather than primary pathology.

This study is the first study to use gait characteristics as a method of evaluating an intervention in mitochondrial disease. It therefore remains unclear if the results of this study are typical of this population, or a result of the type of intervention or gait measures used. To evaluate the results of this study, it is necessary to review the baseline characteristics of the study population and the use of gait measures within this and other populations following an exercise intervention.

The mitochondrial population participating in this exercise study was a subset of the previous cross sectional study. Fewer gait impairments were reported compared to the larger cross sectional study possibly due to the population being higher functioning and smaller in number. Gait speed was within normal limits, with only the gait variables of step time and width variability demonstrating differences compared to the sedentary control group. It is likely that having such a high functioning population at baseline meant that gait measures were unlikely to demonstrate a change due to ceiling effects within the measures despite an improvement in endurance.

Although this is the first study to use specific gait measures in this population, gait has previously been used to evaluate exercise studies in other neurological and elderly populations(Macko *et al.*, 2005; Herman *et al.*, 2007; Bello *et al.*, 2008; Lee *et al.*, 2008; van Eijkeren *et al.*, 2008; Frazzitta *et al.*, 2009; VanSwearingen *et al.*, 2011). Results from these studies are difficult to compare due to the inconsistent use of gait measures and the propensity to use gait speed, rather than characteristics that may link to specific pathological changes (Macko *et al.*, 2005; Herman *et al.*, 2007; van Eijkeren *et al.*, 2008; Cadore *et al.*, 2013). Of the studies that did include multiple gait characteristics in the evaluation of exercise (Frenkel-Toledo *et al.*, 2005; Newman *et al.*, 2007; Bello

et al., 2008; Frazzitta *et al.*, 2009), all involved treadmill training or walking as an intervention and therefore it is uncertain whether changes were due to aerobic training or the task specific nature of the intervention (King and Horak, 2009). Despite the differences in populations, and the gait measures and exercise interventions used, some aspects of these studies may warrant discussion in relation to this study.

Cycling as an exercise intervention has previously demonstrated improvement in walking in a number of neurological populations, such as Parkinson disease (Lauhoff P *et al.*, 2013), Multiple Sclerosis (Rampello *et al.*, 2007) and stroke (Lennon *et al.*, 2008). In this study, as in others, cycling enabled participation of a wider ability group, as participants could still perform the intervention confidently despite mild balance impairments. The inclusion of a wider inclusion group is important in rare diseases such as mitochondrial disease where numbers are limited. In previous studies using cycling as an intervention, improvements have been reported in muscle strength (Lovell DI *et al.*, 2010), gait efficiency (Lauhoff P *et al.*, 2013), balance and walking distance (Kileff and Ashburn, 2005), some of which were reported in the previous study in this thesis (chapter 6). Dibble *et al* reported that any activity is beneficial in participants that are deconditioned and the type of intervention did not dictate that improvement (Dibble *et al.*, 2009). This result supports the findings of improved gait characteristics reported in the most affected participant of this study (m.8344A>G mutation), where improvement was substantial in all aspects of gait. These findings warrant further investigation despite the likely reason for improvements being the reduction in the secondary consequences of a sedentary lifestyle such as improved exercise tolerance (Rampello *et al.*, 2007), rather than primary pathology. The effects of aerobic exercise have been shown

to extend beyond improvement in cardiovascular fitness to include improvements in cognition (Yaffe K *et al.*, 2001;; van Gelder *et al.*, 2004), motor relearning and neuroplasticity (Fisher and Sullivan, 2001; Herman *et al.*, 2007; Bello *et al.*, 2008), it is therefore possible that disease specific benefits may have occurred in this participant over and above the maintenance of fitness. As only one participant reported this improvement these results should be viewed with caution.

Cycling has been shown to be useful in deconditioned subjects where walking may be limited. However, multi component exercise programs which include task orientated interventions such as Nordic walking and treadmill training have been shown to be potentially more beneficial in many neurological and elderly populations at improving walking than cycling alone (Buchner *et al.*, 1997 ; Sabapathy *et al.*, 2011; VanSwearingen *et al.*, 2011). Also interventions that result in the improvement of specific impairments such as strength and exercise do not necessarily improve function (Lee *et al.*, 2008), with improvements in strength only leading to improved walking where weakness is profound (Cup *et al.*, 2007). It can also be expected that to improve walking participants need to walk (Buchner *et al.*, 1997). A recent Cochrane review of the treatment of balance impairments in elderly also reported that there was no evidence that general physical activity such as cycling improved balance (Howe *et al.*, 2011). It is therefore likely that a high functioning group of mitochondrial patients, as in this study will require a more specific intervention that targets changes in the nervous system by challenging balance and gait performance.

Interventions such as treadmill training have been shown to alter gait variability, asymmetry and speed in neurological conditions such as Parkinson's Disease (Frenkel-Toledo *et al.*, 2005) and Stroke (Moore *et al.*, 2010; Reisman *et al.*,

2013). Treadmill training and walking programmes in patients with Parkinson's disease are thought to act as an external cue for patients and result in reduced variability in gait, or have an effect on motor relearning as patients concentrate on walking and override deficits in basal ganglia (van Eijkeren *et al.*, 2008). Although the length of time these improvements remain post training is still under debate (Mehrholz *et al.*, 2010). These task specific exercises may be of greater benefit in high functioning groups (van Eijkeren *et al.*, 2008) due to the specific nature of training and the greater challenge placed on balance systems and may warrant further investigation in a population of mitochondrial patients.

In summary the gait characteristics mirrored disease severity not exercise capacity following an aerobic exercise intervention. The near normal gait of the study population and the type of intervention may have restricted the ability of this study to demonstrate that gait characteristics have potential to measure change during intervention studies in mitochondrial disease.

7.7.3 Can changes in fatigue and cognition following an aerobic exercise intervention be demonstrated by changes in gait characteristics?

Originally this study aimed to demonstrate that gait characteristics would be able to predict changes seen in cognition and fatigue following an aerobic intervention in the mitochondrial participants. Unfortunately only one participant with the m.3243A>G mutation demonstrated a cognitive deficit at baseline and no change was detected in the cognitive function of the mitochondrial group after the exercise intervention using a clinical measure of cognition. Dual task interference also remained unchanged post intervention, so we are consequently unable to report that dual task walking reflects cognitive function in patients with mitochondrial disease.

The lack of cognitive impairment within this group is surprising as previous studies have reported that cognitive impairment is a common manifestation of mitochondrial disease (Kartsounis *et al.*, 1992; Finsterer, 2009). Aerobic exercise has been shown to improve cognitive function in other disease populations (Weuve *et al.*, 2004; Quaney *et al.*, 2009). Deterioration in dual task performance has been linked to cognitive decline, attention and executive function (Sheridan *et al.*, 2003; Yogev *et al.*, 2005). The lack of cognitive impairment in the study population limited the ability to investigate the effects of an exercise intervention on changes in cognitive function and the relationship with gait performance.

In summary this study was unable to draw any firm conclusions on the use of aerobic exercise as an intervention to reduce cognitive decline in this population. Further work is required to ascertain the mechanisms of gait impairment in mitochondrial disease and whether cognitive impairment is a contributor to gait impairments in this group of patients.

The use of gait characteristics to measure fatigue is a novel one, in the previous cross sectional study it was hypothesised that gait characteristics would deteriorate over time as the participant's muscles became fatigued. The performance of an aerobic intervention was hypothesised to improve muscle function and therefore reduce the deterioration in gait characteristics over time.

Gait characteristics over a 6-minute walk remained unchanged and remained the same post exercise intervention. These results could be due to the length of walk not resulting in muscle fatigue or the fact that fatigue is not the primary cause of the gait changes seen in these patients. The repeated testing pre and post intervention over a time period of 4 months demonstrated that cycling

neither improved nor worsened gait characteristics over a 6-minute walk. As no disease control group was included, this study was unable to report that gait would have remained unchanged without the exercise intervention. Although this is thought to be unlikely as participants included in the study had been stable for 6 months and mitochondrial disease is slowly progressive. Again, the likely causes for the lack of change in gait were that, the mitochondrial population used in the study were only mildly affected and therefore the 6-minute timed walk was unable to measure fatigue. These limitations have already been discussed in the cross-sectional study looking at gait characteristics in the larger cohort of patients.

Fatigue and exercise intolerance are common symptoms, with fatigue present in 35% of patients with a m.3243A>G mutation and reported as the 4th most common clinical manifestation of disease (Mancuso *et al.*, 2012), with 62% of a Newcastle cohort of mitochondrial patients reporting a score over 40 in the Fatigue Impact Scale (Gorman *et al.*, 2015). The 6-minute walk is commonly used as a clinical measure of physical fitness and mobility in numerous populations (Lord and Menz, 2002; Duncan *et al.*, 2003). The study sought to evaluate whether, the selective nature of gait characteristics would provide insights into the potential mechanisms of an exercise intervention to reduce fatigue and whether gait could be used as a surrogate measure of fatigue.

Currently the mechanisms of fatigue in mitochondrial disease are unclear, with the focus of previous work relating it to exercise intolerance (Mancuso *et al.*, 2012), although recent work appears to also indicate that fatigue in mitochondrial disease may also have more central components (Gorman *et al.*, 2015). Gait characteristics such as cadence, hip extension and step width are reported to relate to the energy cost of walking (Wert *et al.*, 2010). Impairments

in gait characteristics in mitochondrial disease may result in an inefficient gait pattern which may lead to increased fatigue and hence reduced activity levels. Gait analysis in this population as a measure of fatigue needs to be investigated in a patient group with higher fatigue and disease burden. Investigation within a more severely affected group may provide different results to those reported in this study.

Further work investigating gait over greater time periods and within patients with higher levels of fatigue may provide more insight into the effect of fatigue on gait. Measurement in the community my provide this. The introduction of technologies that allow gait measurement in real life situations mean that gait should still be considered as a selective, sensitive, reliable and unobtrusive means of evaluating interventions in mitochondrial disease.

7.8 **Limitations**

Despite this study being the first investigation of gait characteristics post an intervention the numbers involved remain very small, with the m.8344A>G group being reduced to one. The numbers included were based on a power calculation devised to detect a “within group” change in exercise capacity; this may not have been large enough to detect changes in gait. To overcome the small numbers a conservative approach was adopted for statistical analysis and the m.8344A>G participant was reported separately, thereby increasing the homogeneity of the mitochondrial group. As the m.8344A>G group only included one participant little can be deduced about the effects of exercise on gait in this genotype, although improvements were noted in this individual.

Due to the rarity of these conditions randomisation was not thought to be appropriate as it may have limited recruitment and hence self-selection was used to provide the exercise participants. This may well have resulted in the participants volunteering for the study, coming from a very high functioning population that was mobile and without cognitive impairment.

As the mitochondrial population presented with minimal impairments this study was unable to fully explore the use of dual task walking as a measure of changes in cognitive function and the potential use of gait analysis to measure fatigue.

7.9 **Future work**

Longitudinal studies are required to quantify the natural progression of gait impairments in mitochondrial disease and to assess the value of gait as a sensitive marker of disease progression.

The measurement of gait variability is a novel technique and hence no clear figures are available to ascertain what would be constituted as a meaningful change? The more widespread use of gait variables as measures of disease burden and progression could prove valuable in detecting small changes in function over time and post interventions.

Other types of exercise intervention and specific gait rehabilitation require investigation in patients with mitochondrial disease to establish the potential for rehabilitation of gait in this population.

7.10 **Conclusion**

In summary, this study demonstrated that improvements in muscle function due to an aerobic exercise intervention were not translated into improved gait performance in a group of high functioning patients with the m.32423A>G mutation. The lack of change in gait is likely to be due to a number of reasons, with the most obvious ones being the lack of demonstrable gait impairments prior to the intervention and the small numbers included in the study.

As the gait impairments reported reflect neurological pathological changes, while the intervention improved muscle function, the lack of resulting improvement in gait is unsurprising. Interventions, which target both neurological and muscular impairments, may more effective at maintaining and improving gait efficiency in this group and should be considered for future studies.

Chapter 8 Discussion

This research has demonstrated that a discrete population with mitochondrial disease was sedentary, that they had lower levels of exercise capacity and that this reduced exercise capacity could be safely ameliorated with aerobic exercise. However a link between improved exercise capacity and improved functional ability has not been clearly demonstrated. The novel findings of this thesis are that; i) functional outcomes have the potential to be used in future research studies and clinical practice to demonstrate impairment in activities and; ii) that gait analysis is capable of providing selective measurement of these impairments with the capacity to relate to pathology.

This discussion will explore the use of measures in mitochondrial disease and their potential use in future clinical practice and research. Finally it will review the benefits and limitations of aerobic exercise as a therapy in mitochondrial disease and explore other therapies that could be of value in this population.

The multi systemic presentation of mitochondrial disease with its great range of symptoms, severity and the limited relationship between genotype and phenotype (Chinnery *et al.*, 1997; McFarland and Turnbull, 2009; Nesbitt *et al.*, 2013), has meant that monitoring disease progression and evaluating interventions has been problematic (Chinnery *et al.*, 2006; Pfeffer *et al.*, 2012). These difficulties have been further exacerbated by the rarity of patients and the small numbers participating in clinical trials. The commencement of the UK MRC Mitochondrial disease patient cohort study has partly addressed this issue by enabling the collection of data over a prolonged period of time in a large number of mitochondrial disorders and acting as a cohort of patients willing to participate in clinical trials (Nesbitt *et al.*, 2013).

Despite great strides being made in understanding the causes of mitochondrial disease and methods to limit transmission, the measurement of patient's abilities has thus far been limited to a disease rating scale (NMDAS) (Schaefer *et al.*, 2006) and assessment of exercise capacity (Jeppesen *et al.*, 2006b; Taivassalo *et al.*, 2006; Trenell *et al.*, 2006). The use of the NMDAS has proven successful in providing an indication of disease severity, progression and quality of life, but the total score fails to identify which aspects of disease are most affected or changing and provides little assistance in identifying possible interventions that may be of benefit. So far the use of exercise capacity as a measure of mitochondrial function has been limited to the small numbers of patients partaking in exercise trials due to the cost and time required for testing. The use of exercise capacity as a measure in mitochondrial disease has clearly demonstrated impairment and has led to the development of exercise as a potential treatment in these conditions (Taivassalo *et al.*, 2006; Jeppesen *et al.*, 2009). Despite its invaluable contribution as a measure, especially in studies including exercise as an intervention, it is still unclear if improved exercise capacity is beneficial in increasing activity or participation (World Health Organization, 2001).

The methods of measurement used in this thesis (functional outcome measures and gait analysis) have demonstrated that they are capable of identifying people with mitochondrial disease, discriminate between genotypes and mirror changes in disease severity. The future use of these measures is exciting as their ease of use will enable clinicians and researchers to collect further data on a much larger, more diverse group of patients with mitochondrial disease as part of the clinical review of patients. Also it should be remembered that changes in measures such as exercise capacity, although exciting to a

researcher, are often of little interest to a patient, unless they result in improvement in function or quality of life. With this in mind the development of measurements within the domains of activity and participation are vital to clinicians and patients alike in understanding the effects of this disease.

Despite functional measures providing a method of assessing numerous impairments via a single functional task it should not be assumed that one measure will fit all. Unfortunately this appears to have happened in other areas of neuromuscular research where the use of the 6MWD seems to have become extremely prevalent. The studies in this thesis have demonstrated that more than one measure may be required to enable detection of change in disease burden in patients with mitochondrial disease. As with any other measure, the researcher should always bear in mind what it is they want to measure, and what they expect to change, when choosing a functional outcome. Due to the variety of symptoms and abilities of patients with mitochondrial disease, one measure will never fit all. Therefore it is likely that a battery of outcomes will be required to cover all the possible presentations reported in this population. This thesis was limited to investigation of lower limb function; therefore further work will be required to include upper limb and non-ambulant function.

Although the recent Cochrane review (Pfeffer *et al.*, 2012) advised a rationalisation of primary outcomes in future studies, hopefully the studies contained in this thesis have demonstrated that although many inter-relationships occur between different measures, each has properties of value in measuring a different aspect of disease. In fact, to evaluate a multivariate construct such as function, where decline is observed as patterns of change occurring in strength, balance, endurance and other impairments, at different rates, it is essential to measure impairments and function simultaneously.

The use of multiple functional measures as suggested above still has limitations. Functional outcomes are simple, crude tests and represent the end results of impairments and therefore do not shed light into the potential mechanisms of change in performance. The use of gait analysis appears to give researchers and clinicians a way forward in detecting early changes in function and potential mechanisms for these changes.

Gait impairment is already recognised as one of the most common problems in neurological conditions (Nutt *et al.*, 2011). Further evaluation of gait in mitochondrial disease will provide researchers with numerous avenues for research. If linked with other neurological investigations such as MRI, it has the potential to confirm the impact of neurological pathology on walking in mitochondrial disease. In addition gait analysis provides clinicians the ability to quantify changes in walking not visible to the naked eye whilst gait speed may still be normal. This creates the potential to enable clinicians to intervene much earlier with treatment before secondary compensations become entrenched and therefore intervention is more likely to be successful.

Until now methods of measuring function have been limited to measurement of tasks within a laboratory environment and therefore do not accurately reflect function in the community. With the development of new technologies the measurement of functional tasks and gait in the real world is now possible. The use of these new techniques will provide much greater insight into how mitochondrial disease affects patients, as measurement can be performed over an extended time period and represents total activity/ambulation. This is seen as a gold standard (Busse *et al.*, 2004).

The intervention of aerobic exercise in this thesis was shown to be beneficial in another discrete group of mitochondrial patients. Due to the specific response of the body to different types of exercise and the multivariate construct of function it was unsurprising that improvement in exercise capacity, did not translate to improvement in other activities. So has the benefit of exercise and its potential as a therapy in mitochondrial disease been over stated? Although exercise capacity in this study didn't improve to the extent necessary to improve function, this does not mean that it would not be effective in a more severely affected group of patients. Also the known benefits of physical exercise in the able bodied for health maintenance and disease prevention is just as, if not more vital in patients with mitochondrial disease where cardiac and metabolic dysfunction and sedentary behaviour are prevalent. Therefore the current practice of recommending physical activity to patients should be continued.

As aerobic training alone appeared to be unhelpful in changing function it is imperative that future studies investigate other methods of training that may be beneficial in this group. The functional and gait measures that were used in this thesis demonstrated difficulties with getting in and out of a chair and walking. When examining walking more closely by gait analysis, impairments were detected in gait variability. Therefore other interventions that challenge postural stability and cerebellar dysfunction (Martin *et al.*, 2009) should be considered. Essentially this population requires a multifaceted approach that specifically tackles impairments that have been exposed with these improved measurement tools. These types of programmes have already resulted in improvements in other populations, where strength, balance and task related exercises have been performed alongside aerobic training (Tiedemann *et al.*, 2011). These interventions are more likely to result in improvement, as they are able to

produce dynamic changes in the central nervous system by active participation in specific tasks at a level of difficulty and intensity individual to the patient (Fisher and Sullivan, 2001; Ip *et al.*, 2013).

As in all populations, continued participation in exercise is challenging. To maximise adherence it is necessary for exercise provision to be individual to that person and change with their condition. To further ensure participation exercise needs to be part of everyday life, accessible and enjoyable. The advent of computer based, activity related games provides another method of delivering exercise to a group who may not have the confidence to perform exercise in the community (Phillips *et al.*, 2009). Exploring the use of these games that often use large movements in a goal-orientated manner may provide a readily available exercise tool with 50 % of homes now containing a game console (Deloitte Media Consumer Survey 2013).

In summary these studies have clarified the value of cycling as a form of aerobic exercise in mitochondrial disease. The alternative methods of measurement used here, have highlighted that investigation of other, more specific, interventions would be beneficial in mitochondrial disease.

8.1 Future work

This thesis can only be seen as a starting point for the use of functional outcome measures and gait analysis in mitochondrial disease, with the results revealing many further questions to be investigated.

Despite the measures used in this thesis being shown to discriminate between disease and genotype, further work is required to ensure their reliability, reproducibility and the level of change that is deemed clinically relevant in this population. Measures also need to be investigated that cover the whole spectrum of mitochondrial disease. The investigation of upper limb function and the measurement of aspects of disease within a non–ambulant population are also still necessary.

This preliminary work in gait analysis has provided interesting insights into early gait impairments in this disease. Further work now needs to be performed in a larger cohort, over a longer time period to identify whether this method of measurement changes with disease progression and whether this will provide clues for the use of different type of interventions. The investigation of different types of aerobic exercise is essential in this disease group, as treatments such as treadmill training may provide the best of both worlds, improving mitochondrial function in both muscular and neurological systems and has been shown to arrest neuro-degeneration in animal and human models (Tillerson *et al.*, 2003; Fisher *et al.*, 2004; Voss *et al.*, 2010).

The small numbers of some genotypes and the great variety of symptoms reported in mitochondrial disorders continue to prevent large-scale trials. Researchers will need to collaborate more in the future to produce the participants required for such trials. However, the value of publishing small

scale or single case reports in these disorders should not be forgotten and have provided the starting point for interventions previously in these diseases (Taivassalo *et al.*, 1999b) and in fact may be the way forward in research in this disease.

8.2 **Conclusion**

Exercise and rehabilitation interventions need to be specific, tailored to each patient and constantly adapted to address the changes reported by patients as disease progression occurs. To evaluate these interventions and map disease progression measurements must be accurate, relevant and reproducible. Multi system diseases such as mitochondrial disease will always require numerous measurements to monitor disease progression and evaluate interventions. It is important that measures include all aspects of disease from impairment of bodily structures to activities and participation. Only by linking theses areas of measurement will we understand how mechanisms of disease result in loss of function and the effect they have on our patient's daily lives. With this knowledge researchers have the potential to develop more relevant and successful interventions.

Appendices

Appendix A Information Sheets

REC reference number:
Committee:

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Participant Information Sheet

Effect of aerobic exercise on cardiac function and movement in people with mitochondrial disease (exercise control group)

You are being invited to participate in this research project. Please take time to read the following information carefully. It explains why the research is being carried out and what it involves. If you have any questions about the information, you are very welcome to ask for a further explanation.

- Part 1 tells you about the purpose of this study and what will happen during the study.
- Part 2 gives more detailed information about the conduct of the study.

Discuss with others if you wish and take time to decide regarding your participation.

Thank you for reading this

Part 1

What is the purpose of the research project?
The aim of this research is to demonstrate that aerobic exercise training is an effective approach to therapy in certain patients with mitochondrial disease. Based on our previous research studies, we believe that such training will improve muscle strength, mitochondrial function, exercise tolerance and overall quality of life. In this project we will be looking at the effects exercise has on the hearts of people with mitochondrial disease, and a group of people without the condition so we can make a comparison between people with a mitochondrial disease and those who don't have the condition.

Why have I been chosen?
You have been chosen as you are between the ages of 18 and 60 years and are considered to be fit and healthy, do not do any exercise and do not suffer from a mitochondrial disease.

Do I have to take part?

Your participation is purely voluntary. If you decide to take part, you are still free to withdraw at any time without giving reasons and without any current or future care being affected in any way. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form indicating your willingness to participate in the research.

What will this research project involve?
You will be asked to attend the Newcastle Magnetic Resonance Centre at Newcastle General Hospital, or Royal Victoria Infirmary on 4 occasions over 4 months to have a metabolism examination. You will also be asked to attend exercise sessions 3 times a week for 16 weeks.

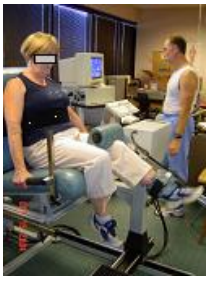
Visit 1:You will be asked to sign the consent form saying that you would like to take part in this research study and then complete two screening questionnaires. You will then do a cycling test. During this test you will cycle at the same pace but how hard you are cycling will increase every minute. You will keep on cycling until you decide to stop or until pedalling becomes difficult. Whilst you are cycling you will be asked to wear a mouthpiece and a heart rate monitor. The exercise test will last between 10 - 15 minutes. At the end of the test you will feel tired but will recover quickly.
Total Visit Time: 1hour

Visit 2: A blood sample will be taken.

Magnetic resonance imaging (MRI) will be used to measure the function of your heart. This exam takes 1 hour in total and is non-invasive (which means there will be no harm to you). However if you have any steel pins or a pacemaker inside your body, or are pregnant, you will not undergo this exam.

You will then be asked to lie quietly on a bed whilst your resting metabolism is measured. After this you will repeat a cycle test similar to the previous day.

This will be followed by some walking tests in the gait laboratory, where you will be asked to:
a) Walk short distances while we will measure your speed and step length
b) Walk for 6 minutes so we can measure the distance you have walked in this time.



c) Perform a repeated sit to stand movement while being timed.

The maximal strength of your thigh muscles will be measured on weight machines, as shown here in the picture.

You will also be asked to complete some questionnaires regarding fatigue, quality of life and cognition

Total visit time: 6 hours

Following this you will be assigned an exercise programme.

You will be asked to attend 3 exercise classes per week over 16 weeks. Each exercise session will involve you doing endurance training on a stationary bicycle at moderate intensity (70-80% of your maximal heart rate) 3 times per week for a total of 30 minutes per session increasing as you are able. If you are unable to tolerate 30 minutes of continuous exercise, you will do exercise intervals (for example, 3 x 10 minutes with rest periods in between).
You will also be asked to attend the centre at 4 weeks for the following procedures:

Visit 3: A blood sample will be taken.

We will discuss with you how your exercise has been going

We will repeat the cycle and strength tests done at visit 2

Total visit time: 2hours

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After 16 weeks, the metabolic evaluations performed at Visits 2 will be repeated, making a maximum of 4 study visits in total..

Expenses and payments

Throughout the exercise studies, you will be provided with:

1. Membership to gym near your home;
2. Heart rate monitor to measure the intensity of cycling exercise during trial
3. Overnight accommodation will be provided for visits to assessments where necessary. All travel expenses to and from Newcastle will be provided by the trial funds.

What do I have to do?

It will be important that you attend over 90% of the exercise sessions. You will be asked not to drink alcohol or exercise the day before the three test days. Each of the metabolic assessments will be in the morning before breakfast – you should not eat before you come to the centre. Food will be provided for you after the examination.

What are the side effects of treatment received when taking part?

This study involves exercise so there are no side effects (such as may occur with drugs).

Are there any other possible disadvantages of taking part?

Giving up time to participate has to be considered.

What are the possible benefits of taking part?

Being more physically active may be beneficial to your heart function and if sustained after the study, may help in preventing other complications such as heart disease and diabetes. You will have supervised exercise sessions (like a personal trainer) who will teach you about your body, show you how to exercise correctly and help you become more physically fit.

What happens at the end of the research project?

At the end of the project, we shall be able to inform you of how the exercise affected your heart.

What if there is a problem?

If you have any concern or complaint about any aspect of the study this will be dealt with immediately by Dr Trenell. Contact details are given at the end of Part 1.

Will my taking part in the project be kept confidential?

All information obtained during the course of the research project will be kept strictly confidential. Your own GP will be informed of your participation in the project.

What will happen to the results of the research study?

The results of the project will be presented in national and international neuromuscular meetings and will be published in one of the neurology journals. You will not be identified in any report or publication. You will be welcome to have a copy of the results once they are published.

Who has reviewed the study?

Ethical review of the study has been conducted by Sunderland Research Ethics Committee.

Who are the contacts for further information?

Further information can be obtained from:

Dr Mike Trenell
Diabetes UK RD Lawrence Fellow
Newcastle Magnetic Resonance Centre
Campus for Ageing and Vitality
Newcastle University
NE3 5JB

Tel: 0191 2411158
M.I.Trenell@ncl.ac.uk

Or

Mr G Bell
Clinical Trial Co-ordinataor
MRC Centre for neuromuscular research
Institute of Human Genetics
Newcastle upon Tyne
NE1 3BZ

Tel: 0191 241 8649
Geoffrey.bell@ncl.ac.uk

Thank you.

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Part 2

What if relevant new information becomes available?

If new information is published during the course of a study this can sometimes change how the research should go forward. However, for this study it is most unlikely that this would occur. The study design is unique, and there are very few research groups worldwide able to carry out magnetic research of this kind.

What will happen if I don't want to carry on with the study?

You would be able to withdraw from the study at any time. Measurements already made would still be used if you were to agree to this.

What if there is a problem?

a) Complaints
If you have any concern or complaint about any aspect of this study you should contact Dr Trenell by phone on 0191 241 1158, or write to him at the address at the end of Part 1 of this document. If you remain unhappy you can contact Dr Lesley Hall, Research Governace Manager,Newcastle upon Tyne Hospitals NHS Foundation Trust, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP; Tel 0191 233 6161
b) Harm
In the unlikely event that something does go wrong and you suffer in any way the arrangements are as follows. If negligence of staff led to harm, then this would be covered by the Newcastle upon Tyne Hospitals Trust clinical negligence scheme. You may have to meet legal costs. If any harm was non-negligent then the hospital trust may consider a discretionary payment.

Will my taking part in the project be kept confidential?

All information obtained during the course of the research project will be kept strictly confidential. This will be achieved by storing information in password-protected computer files, and appointment information in locked filing systems within the Magnetic Resonance Centre. No individually identifiable information will be stored outside the Centre.

Analysis of the detailed results of the research will be done by Dr Trenell, Jane Newman, Dr Hollingsworth, Professor Turnbull and Dr Bates. At this stage no personal information is part of the dataset. Results will be sent to participants, presented at scientific meetings and published in scientific journals without personal identification of any volunteer although thanks to the volunteers will be recorded.

Your own General Practitioner will be informed of your help with this study, should you agree to this. The detailed results of the research tests will not be sent to anybody outside the Magnetic Resonance Centre.

What will happen to blood samples?

The samples will be tested for CK, liver function, insulin, sugar fat, and other food derived substances. We will also be performing a test called a 'BNP assay' – this would allow us to detect any changes in your heart function during the period of the study. Samples will be stored until it is certain that the test results are accurate, and then they will be disposed of. During storage, samples are identified only by a code number, not your name. No genetic or other tests will be carried out on the samples.

What will happen to results of the research?

The results will be presented at scientific meetings for discussion by other experts in this field. They will be written up in the form of a scientific paper and this will be intended to be published in a suitable scientific journal. As soon as the results are fully analysed after the end of the entire study you will receive a letter describing what we have found.

Who is organising and funding the research?

This project is funded from a project grant from the Medical Research Council. The design and organisation of the study is the responsibility of Dr Trenell and Professor Turnbull who are internationally recognised as experts in this field.

There is no payment to any of the researchers involved in this study. They are employed by Newcastle University to work in the NHS, to teach and to research and have no financial link with the study.

Who has reviewed the study?

Ethical review of the study has been conducted by Sunderland Research Ethic Committee.

Design of this information sheet

This document is written in accordance with the requirements of the European Clinical Trials Directive 2001/20/EC, the ICH Good Clinical Practice guidelines and the UK Medicines for Human Use (Clinical Trials) Regulation 2004.

NEWCASTLE MITOCHONDRIAL NCG DIAGNOSTIC SERVICE

Rare Mitochondrial Disorders Service for Adults and Children
Clinical
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Dr Robert McFarland MA MBBS PhD MRCP MRCPCH
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Patient Information Sheet

Effect of aerobic exercise on cardiac function and movement in people with mitochondrial disease

You are invited to participate in this medical research project. Please take time to read the following information carefully. It explains why the research is being done and what it involves. If you have any questions about the information, you are very welcome to ask for further explanation. Thank you for reading this.

- Part 1 tells you about the purpose of this study and what will happen during the study.
- Part 2 gives more detailed information about the conduct of the study.

Discuss with others if you wish and take time to decide regarding your participation.

Part 1

What is the purpose of the research project?
To demonstrate that aerobic exercise training is an effective approach to therapy in certain patients with mitochondrial myopathy. Based on our previous research studies, we believe that such training will improve muscle strength, mitochondrial function, exercise tolerance and overall quality of life. In this

project we will be looking at the effects exercise has on the hearts of people with Mitochondrial disease.

Why have I been chosen?
Because you are between the ages of 18 and 70 years and have had a previous a muscle biopsy showing a defect in skeletal muscle mitochondrial DNA.

Do I have to take part?

Your participation is purely voluntary. If you decide to take part, you are still free to withdraw at any time without giving reasons and without any bad feelings. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form.

What will the research project involve?
You will be asked to attend the Newcastle Magnetic Resonance Centre at Newcastle General Hospital, or Royal Victoria Infirmary on 4 occasions over 4 months to have a metabolism examination. If you take part in the exercise group you will also be asked to attend exercise sessions 3 times a week for 16 weeks.

Visit 1:Day 1.

You will be asked to sign the consent form saying that you would like to take part in this research study and then complete two screening questionnaires. You will then do a cycling test. During this test you will cycle at the same pace but how hard you are cycling will increase every minute. You will keep on cycling until you decide to stop or until pedalling becomes difficult. Whilst you are cycling you will be asked to wear a mouthpiece and a heart rate monitor. The exercise test will last between 10 - 15 minutes. At the end of the test you will feel tired but will recover quickly. A disease scale will be performed involving some questionnaires. A brief heart scan using ultrasound (echocardiogram) will be performed.
Total Visit Time: 1.5hour

Visit 1: Day 2.

A blood sample will be taken. This will be sent to the laboratory and checked for routine levels, as well as a test called a BNP assay – this is an analysis of your blood that could help us detect any changes in your heart function. We will repeat this check at the end of the study.

Magnetic resonance imaging (MRI) will be used to measure the function of your heart. This exam takes 1.5 hours in total, and is very safe (no harm to you), however if you have any steel pins or pacemaker inside your body, or are pregnant or have significant kidney disease, you will not undergo this exam. A small drip (cannula) will be placed in a vein in your arm so that we can use a type of dye during the scan. The cannula will be

placed by an experienced doctor or nurse and will be removed after the scan.
You will then be asked to lie quietly on a bed whilst your resting metabolism is measured. After this you will repeat a cycle test similar to the previous day.

This will be followed by some walking tests in the gait laboratory, where you will have to:
a) Walk short distances and we will measure your speed and step length
b) Walk for 6 minutes and we will measure the distance walked
c) Be timed doing a repeated sit to stand movement

The maximal strength of your thigh muscles will be measured on weight machines as shown here in the picture.

You will be asked to complete some questionnaires regarding fatigue, quality of life and cognition.

You will be provided with an activity monitor to wear for 7 days whilst performing your normal daily activities.

Total visit time: 6 hours

You will be assigned to one of two groups.

The *first group* will attend 3 exercise classes per week over 16 weeks. Each exercise session will involve you doing endurance training on a stationary bicycle at moderate intensity (70-80% of your maximal heart rate) 3 times per week for a total of 30 minutes per session increasing as able to 40 minutes. If you are unable to tolerate 30 minutes of continuous exercise, you will do exercise intervals (for example, 3 x 10 minutes with rest periods in between). This group will also attend the centre at 4 weeks.

Visit 2 :A blood sample will be taken.
We will discuss with you how your exercise has been going.
We will repeat the cycle and strength tests done on visit 2

Total visit time: 2hours

The *second group* will not attend the exercise classes or be required to undertake any exercise over the 16 weeks.

After 16 weeks, the metabolic evaluations performed at Visits 2 will be repeated, making 4 study visits in total (for the exercise group).

If you are placed in the group which does not attend any exercise classes, at the end of the study you will be given the opportunity to participate in exercise sessions, though this attendance is not compulsory. No further tests will be undertaken if you choose to take part in these exercise sessions.

Expenses and payments

Throughout the exercise studies, you will be provided with:

4. Membership to gym near your home;
5. Heart rate monitor to measure the intensity of cycling exercise during trial
6. Overnight accommodation will be provided for visits to assessments where necessary. All travel expenses to and from Newcastle will be provided by the trial funds.

What do I have to do?

You will continue with your usual treatment(s) during the project. It will be important that you attend over 90% of the exercise sessions. You will be asked not to drink alcohol or exercise the day before the three test days.
Each of the metabolic assessments will be in the morning before breakfast – you should not eat before you come to the centre. Food will be provided for you after the examination.

What are the side effects of treatment received when taking part?

This study involves exercise so there are no side effects (such as may occur with drugs).

Are there any other possible disadvantages of taking part?

Giving up time to participate has to be considered.

What are the possible benefits of taking part?

Being more physically active may be beneficial to your heart ,mitochondrial function and if sustained after the study, may help in preventing other complications such as heart disease and diabetes. You will have supervised exercise sessions (like a personal trainer) which will teach you about your body, show you how to exercise correctly and help you become more physically fit.

What happens at the end of the research project?

At the end of the project, we shall be able to inform you of how the exercise affected your heart and mitochondrial function

What if there is a problem?

If you have any concern or complaint about any aspect of the study this will be dealt with immediately by Dr Trenell. Contact details are given at the end of Part 1.

Will my taking part in the project be kept confidential?

All information obtained during the course of the research project will be kept strictly confidential. Your own GP and the Mitochondrial Disorders Service will be informed of your participation in the project.

What will happen to the results of the research study?

The results of the project will be presented in national and international neuromuscular meetings and will be published in one of the neurology journals. You will not be identified in any report or publication. You will be welcome to have a copy of the results once they are published.

Who has reviewed the study?
Ethical review of the study has been conducted by the Sunderland Research Ethic Committee.

Who are the contacts for further information?

Further information can be obtained from:

Dr Mike Trenell
Diabetes UK RD Lawrence Fellow

MRC Muscle Performance and Training Laboratory
Newcastle General Hospital,
Westgate Road,
Newcastle upon Tyne
Tel 0191 248 1150

Thank you.

****

Part 2

What if relevant new information becomes available?

If new information is published during the course of a study this can sometimes change how the research should go forward. However, for this study it is most unlikely that this would occur. The study design is unique, and there are very few research groups worldwide able to carry out magnetic research of this kind.

What will happen if I don't want to carry on with the study?

You would be able to withdraw from the study at any time. Measurements already made would still be used if you were to agree to this.

What if there is a problem?

c) Complaints
If you have any concern or complaint about any aspect of this study you should contact Dr M Trenell by phone on 0191 248 1150, or write to him at the address at the end of Part 1 of this document. If you remain unhappy you can contact the Research Governace Framework Manager of the Newcastle upon Tyne Hospitals NHS Foundation Trust (Dr L Hall, Joint Research Office, Level 6, Leazes Wing, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne NE1 4LP; Tel 0191 233 6161).

d) Harm
In the unlikely event that something does go wrong and you suffer in any way the arrangements are as follows. If negligence of staff led to harm, then this would be covered by the Newcastle upon Tyne Hospitals NHS Foundation Trust clinical negligence scheme. You may have to meet legal costs. If any harm was non-negligent then the hospital trust may consider a discretionary payment.

Will my taking part in the project be kept confidential?

All information obtained during the course of the research project will be kept strictly confidential. This will be achieved by storing information in password-protected computer files, and appointment information in locked filing systems within the Magnetic Resonance Centre. No individually identifiable information will be stored outside the Centre.

Analysis of the detailed results of the research will be done by Dr Trenell, Jane Newman, Dr Hollingsworth, Professor Turnbull and Dr Bates . At this stage no personal information is part of the dataset. Results will be sent to participants, presented at scientific meetings and published in scientific journals without personal identification of any volunteer although thanks to the volunteers will be recorded.

Your own General Practitioner will be informed of your help with this study, and this is normal practice. Your specialist liver consultant will also be aware. The

detailed results of the research tests will not be sent to anybody outside the Magnetic Resonance Centre.

What will happen to blood samples?

The samples will be tested for CK, liver function, insulin, sugar fat, and other food derived substances. We will also be performing a test called a 'BNP assay' – this would allow us to detect any changes in your heart function during the period of the study. Samples will be stored until it is certain that the test results are accurate, and then they will be disposed of. During storage samples are identified only by a code number, not your name. No genetic or other tests will be carried out on the samples.

What will happen to results of the research?

The results will be presented at scientific meetings for discussion by other experts in this field. They will be written up in the form of a scientific paper and this will be intended to be published in a suitable scientific journal. As soon as the results are fully analysed after the end of the entire study you will receive a letter describing what we have found, and what implications it has for people with mitochondrial disorders

Who is organising and funding the research?

This project is funded from a project grant from the Newcastle Hospitals Trust Joint Research Committee. The design and organisation of the study is the responsibility of Dr Trenell, Professor Turnbull who are internationally recognised as experts in this field.

There is no payment to any of the researchers involved in this study. They are employed by Newcastle University to work in the NHS, to teach and to research and have no financial link with the study.

Who has reviewed the study?

Ethical review of the study has been conducted by the Newcastle and North Tyneside Research Ethic Committee No. 1.

Design of this information sheet

This document is written in accordance with the requirements of the European Clinical Trials Directive 2001/20/EC, the ICH Good Clinical Practice guidelines and the UK Medicines for Human Use (Clinical Trials) Regulation 2004.

Appendix B Consent forms

REC reference number:
Committee:

CONSENT FORM for participants

Title of Study: The effect of aerobic exercise on cardiac function and movement in people with Mitochondrial disease(exercise control group)

Name of Researcher:
Dr M I Trenell PhD, MSc, BSc (hon)
Diabetes UK RD Lawrence Fellow
Newcastle Magnetic Resonance Centre
Campus for Ageing and Vitality
Newcastle University
NE3 5JB

Tel: 0191 2411158
M.I.Trenell@newcastle.ac.uk

Please write your initials in the box

1. I confirm that I have read and understand the information sheet dated **May 2010 (Version 2.0)** 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 my medical care or legal rights being affected. ☐
3. I agree to sections of my medical records being viewed by members of the research team for this study ☐
4. I agree to my GP being informed to my participation in the study ☐
5. I agree to take part in the above study. ☐

_____
Name of Patient

_____ _____
_____

	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

REC reference number:
Committee:

CONSENT FORM for patients

Title of Study: The effect of aerobic exercise on cardiac function and movement in people with

Mitochondrial disease

Name of Researcher:
Dr M I Trenell PhD, MSc, BSc (hon)
Diabetes UK RD Lawrence Fellow
Newcastle Magnetic Resonance Centre
Campus for Ageing and Vitality
Newcastle University
NE3 5JB

Tel: 0191 2411158
M.I.Trenell@newcastle.ac.uk

Please write your initials in the box

1. I confirm that I have read and understand the information sheet dated May 2010 (Version 3.0) 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 my medical care or legal rights being affected. ☐
3. I agree to sections of my medical records being viewed by members of the research team for this study ☐
4. I agree to my GP being informed to my participation in the study ☐
5. I agree to take part in the above study. ☐

_____
Name of Patient

_____ _____
_____
Date Signature

_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Appendix C PARQ

Physical Activity Readiness Questionnaire

Name: _____

Date of Birth: _____

		Please choose	
1	Has your doctor ever said that you have a *heart condition* and that you should only do physical activity recommended by a doctor?	YES	NO
2	Do you ever feel *pain* in your chest when you do physical activity?	YES	NO
3	Have you ever had chest pain when you are not doing physical activity?	YES	NO
4	Do you ever feel *faint or have spells of dizziness?*	YES	NO
5	Do you have a *joint problem* (also back problem) that could be made worse by exercise?	YES	NO
6	Have you ever been told that you have high blood pressure?	YES	NO
7	Are you currently taking any *medication*? If so, what? _____ Reason _____	YES	NO
8	Are you pregnant, have you had a baby in the last 6 months, or do you plan to have a baby this year?	YES	NO
9	Is there any other reason why you should not participate in physical activity? If so, what? _____	YES	NO

Appendix D IPAQ

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

Appendix E Order of gait and functional measures

Counterbalanced condition order list

Subject	Task 1	Task 2	Task 3	Task 4	Task 5	Task 6
GLMEC 001 P	S T S	Dual walk	Single walk	Dual + 1 walk	TuG	6m walk
GLMEC 002 P	Single walk	Dual walk	Dual + 1 walk	S T S	TuG	6m walk
GLMEC 003 P	TuG	Single walk	Dual walk	Dual + 1 walk	S T S	6m walk
GLMEC 004 P	S T S	TuG	Dual walk	Single walk	Dual + 1 walk	6m walk
GLMEC 005 P	S T S	Single walk	Dual walk	Dual + 1 walk	TuG	6m walk
GLMEC 006 P	TuG	S T S	Single walk	Dual walk	Dual + 1 walk	6m walk
GLMEC 007 P	Single walk	Dual walk	Dual + 1 walk	TuG	S T S	6m walk
GLMEC 008 P	S T S	TuG	Single walk	Dual walk	Dual + 1 walk	6m walk
GLMEC 009 P	Dual walk	Single walk	Dual + 1 walk	TuG	S T S	6m walk
GLMEC 010 P	TuG	S T S	Dual walk	Single walk	Dual + 1 walk	6m walk
GLMEC 011 P	Dual walk	Single walk	Dual + 1 walk	S T S	TuG	6m walk
GLMEC 012 P	TuG	Dual walk	Single walk	Dual + 1 walk	S T S	6m walk
GLMEC 001 PC	Dual walk	Single walk	Dual + 1 walk	S T S	TuG	6m walk
GLMEC 002 PC	TuG	Dual walk	Single walk	Dual + 1 walk	S T S	6m walk
GLMEC 003 PC	Single walk	Dual walk	Dual + 1 walk	S T S	TuG	6m walk
GLMEC 004 PC	TuG	S T S	Single walk	Dual walk	Dual + 1 walk	6m walk
GLMEC 005 PC	S T S	Single walk	Dual walk	Dual + 1 walk	TuG	6m walk
GLMEC 006 PC	Single walk	Dual walk	Dual + 1 walk	TuG	S T S	6m walk
GLMEC 007 PC	TuG	Single walk	Dual walk	Dual + 1 walk	S T S	6m walk
GLMEC 008 PC	Dual walk	Single walk	Dual + 1 walk	TuG	S T S	6m walk
GLMEC 009 PC	S T S	TuG	Single walk	Dual walk	Dual + 1 walk	6m walk
GLMEC 010 PC	S T S	TuG	Dual walk	Single walk	Dual + 1 walk	6m walk
GLMEC 011 PC	S T S	Dual walk	Single walk	Dual + 1 walk	TuG	6m walk
GLMEC 012 PC	TuG	S T S	Dual walk	Single walk	Dual + 1 walk	6m walk
GLMEC 001 C	Dual walk	Single walk	Dual + 1 walk	S T S	TuG	6m walk
GLMEC 002 C	Dual walk	Single walk	Dual + 1 walk	TuG	S T S	6m walk
GLMEC 003 C	S T S	TuG	Single walk	Dual walk	Dual + 1 walk	6m walk
GLMEC 004 C	TuG	S T S	Single walk	Dual walk	Dual + 1 walk	6m walk
GLMEC 005 C	Single walk	Dual walk	Dual + 1 walk	TuG	S T S	6m walk
GLMEC 006 C	TuG	S T S	Dual walk	Single walk	Dual + 1 walk	6m walk
GLMEC 007 C	S T S	TuG	Dual walk	Single walk	Dual + 1 walk	6m walk
GLMEC 008 C	Single walk	Dual walk	Dual + 1 walk	S T S	TuG	6m walk
GLMEC 009 C	TuG	Dual walk	Single walk	Dual + 1 walk	S T S	6m walk
GLMEC 010 C	S T S	Dual walk	Single walk	Dual + 1 walk	TuG	6m walk
GLMEC 011 C	S T S	Single walk	Dual walk	Dual + 1 walk	TuG	6m walk
GLMEC 012 C	TuG	Single walk	Dual walk	Dual + 1 walk	S T S	6m walk

Appendix F ACE-R

ADDENBROOKE'S COGNITIVE EXAMINATION - ACE-R

Final Revised Version A (2005)

Name :
Date of birth :
Hospital no. :

Addressograph

Date of testing: ..... /..... /.....
Tester's name: .....
Age at leaving full-time education: .....
Occupation: .....
Handedness: .....

ORIENTATION

➢ Ask: What is the	Day	Date	Month	Year	Season	[Score 0-5]
➢ Ask: Which	Building	Floor	Town	County	Country	[Score 0-5]

REGISTRATION

➢ Tell: 'I'm going to give you three words and i'd like you to repeat after me: lemon, key and ball'. After subject repeats, say 'Try to remember them because i'm going to ask you later'. Score only the first trial (repeat 3 times if necessary).

Register number of trials .....

[Score 0-3]

ATTENTION & CONCENTRATION

➢ Ask the subject: ' could you take 7 away from a 100? After the subject responds, ask him or her to take away another 7 to a total of 5 subtractions. If subject make a mistake, carry on and check the subsequent answer (i.e. 93, 84, 77, 70, 63 -score 4)

Stop after five subtractions (93, 86, 79, 72, 65). ....

[Score 0-5]
(for the best performed task)

➢ Ask: 'could you please spell **WORLD** for me? Then ask him/her to spell it backwards:

....

MEMORY - Recall

➢ Ask: 'Which 3 words did I ask you to repeat and remember?'

....

[Score 0-3]

MEMORY - Anterograde Memory

➢ Tell: ' I'm going to give you a name and address and I'd like you to repeat after me. We'll be doing that 3 times, so you have a chance to learn it. I'll be asking you later'

Score only the third trial

[Score 0-7]

	1st Trial	2nd Trial	3rd Trial
Harry Barnes	....	....	....
73 Orchard Close	....	....	....
Kingsbridge	.....	.....	.....
Devon	.....	.....	.....

MEMORY - Retrograde Memory

[Score 0 -4]

➢ Name of current Prime Minister .....
➢ Name of the woman who was Prime Minister .....
➢ Name of the USA president .....
➢ Name of the USA president who was assassinated in the 1960's .....

ATTENTION&ORIENTATION MEMORY

VERBAL FLUENCY - Letter 'P' and animals

➢ **Letters**

Say: 'I'm going to give you a letter of the alphabet and I'd like you to generate as many words as you can beginning with that letter, but not names of people or places. Are you ready? You've got a minute and the letter is P'

[Score 0 - 7] ☐

				>17	7
				14-17	6
				11-13	5
				8-10	4
				6-7	3
				4-5	2
				2-3	1
				<2	0
				total	correct

➢ **Animals**

Say: 'Now can you name as many animals as possible, beginning with any letter?

[Score 0 - 7] ☐

				>21	7
				17-21	6
				14-16	5
				11-13	4
				9-10	3
				7-8	2
				5-6	1
				<5	0
				total	correct

LANGUAGE - Comprehension

➢ Show written instruction:

[Score 0-1] ☐ ☐

Close your eyes

➢ 3 stage command:
'Take the paper in your right hand. Fold the paper in half. Put the paper on the floor'

[Score 0-3] ☐ ☐

LANGUAGE - Writing

➢ Ask the subject to make up a sentence and write it in the space below:
Score 1 if sentence contains a subject and a verb (see guide for examples)

[Score 0-1] ☐ ☐

FLUENCY LANGUAGE

LANGUAGE - Repetition

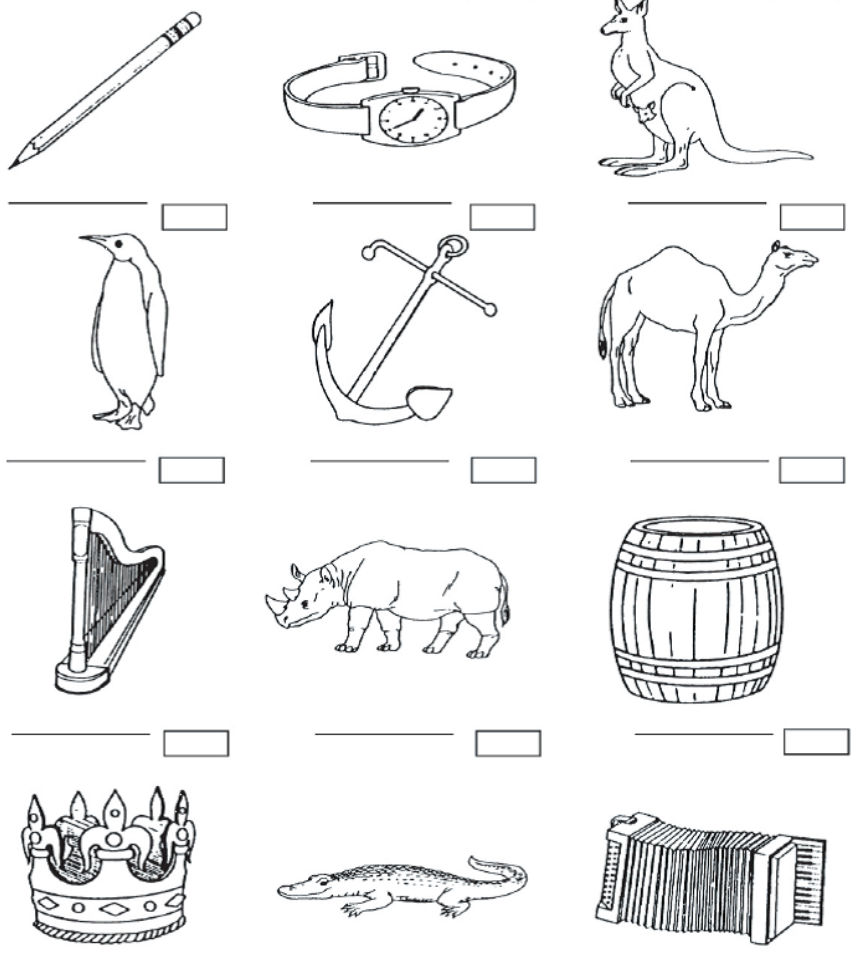
➢ Ask the subject to repeat:**' hippopotamus'; 'eccentricity; 'unintelligible'; 'statistician'**
Score 2 if all correct; 1 if 3 correct; 0 if 2 or less. [Score 0-2] ☐

➢ Ask the subject to repeat: **'Above, beyond and below'** [Score 0-1] ☐

➢ Ask the subject to repeat: **'No ifs, ands or buts'** [Score 0-1] ☐

LANGUAGE - Naming

➢ Ask the subject to name the following pictures: [Score 0-2] pencil + watch ☐☐



[Score 0-10] ☐

LANGUAGE - Comprehension

➢ Using the pictures above, ask the subject to: [Score 0-4] ☐

- Point to the one which is associated with the monarchy _____
- Point to the one which is a marsupial _____
- Point to the one which is found in the Antarctic _____
- Point to the one which has a nautical connection _____

LANGUAGE

LANGUAGE - Reading

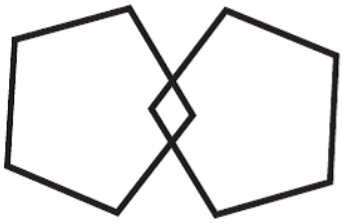
➢ Ask the subject to read the following words: [Score 1 only if all correct] [Score 0-1] ☐

sew
pint
soot
dough
height

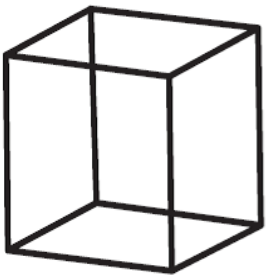
LANGUAGE

VISUOSPATIAL ABILITIES

➢ Overlapping pentagons: Ask the subject to copy this diagram: [Score 0-1] ☐ ☐



➢ Wire cube : Ask the subject to copy this drawing (for scoring, see instructions guide) [Score 0-2] ☐



➢ Clock: Ask the subject to draw a clock face with numbers and the hands at ten past five. (for scoring see instruction guide: circle = 1, numbers = 2, hands = 2 if all correct) [Score 0-5] ☐

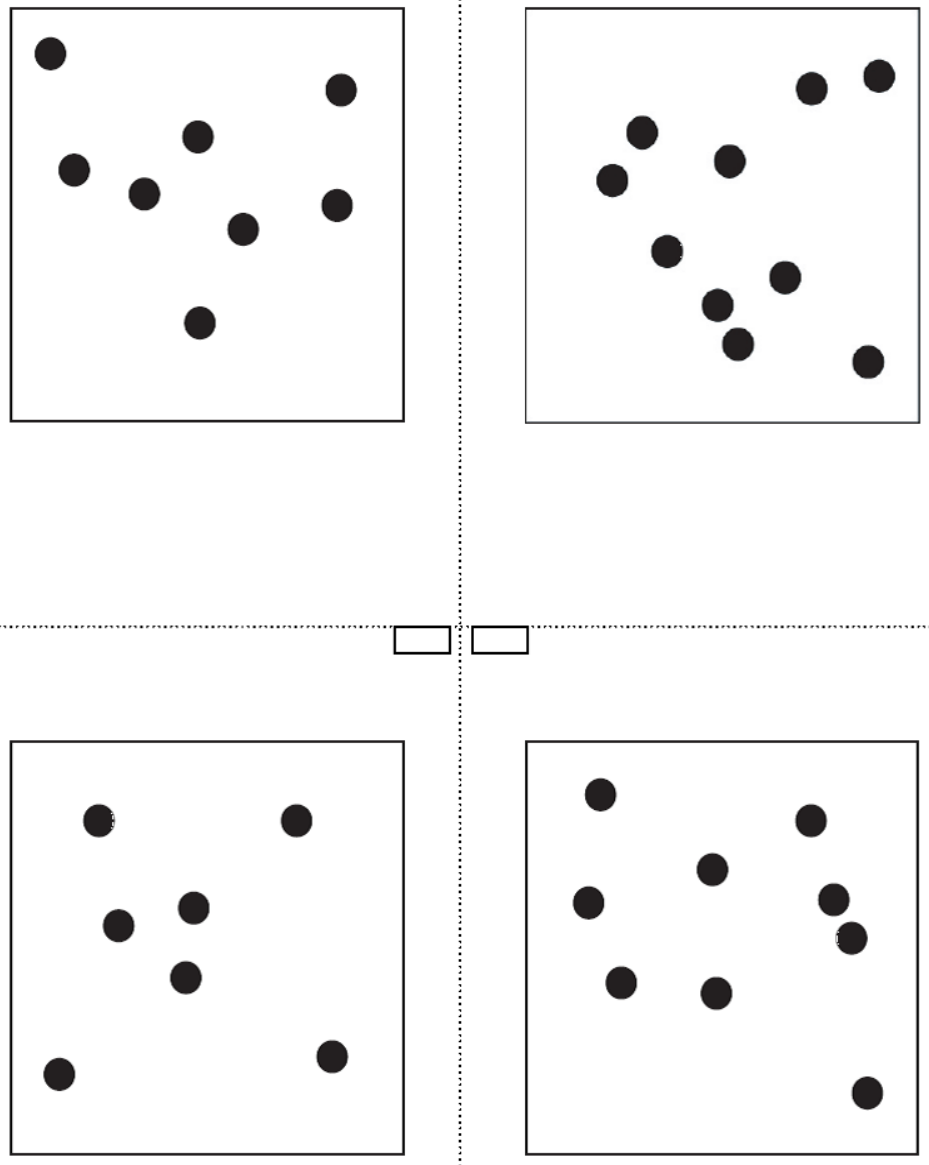
VISUOSPATIAL

PERCEPTUAL ABILITIES

VISUOSPATIAL

➢ Ask the subject to count the dots without pointing them

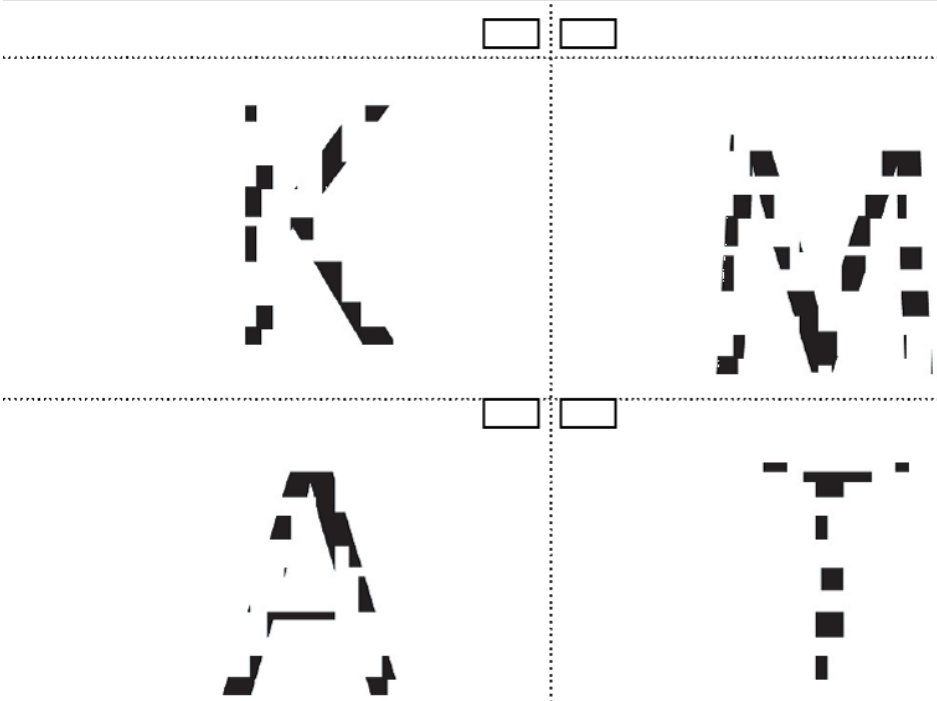
[Score 0-4]



ADDENBROOKE'S COGNITIVE EXAMINATION - ACE-R *Final Revised Version A (2005)*

PERCEPTUAL ABILITIES

➢ Ask the subject to identify the letters [Score 0-4]



RECALL

➢ Ask "Now tell me what you remember of that name and address we were repeating at the beginning"

[Score 0-7]

Harry	Barnes	....
73	Orchard	Close ....
	Kingsbridge	.....
	Devon	.....

RECOGNITION

➢ This test should be done if subject failed to recall one or more items. If all items were recalled, skip the test and score 5. If only part is recalled start by ticking items recalled in the shadowed column on the right hand side. Then test not recalled items by telling "ok, I'll give you some hints: was the name X, Y or Z?" and so on. Each recognised item scores one point which is added to the point gained by recalling.

[Score 0-5]

Jerry Barnes		Harry Barnes		Harry Bradford		recalled	
37		73		76		recalled	
Orchard Place		Oak Close		Orchard Close		recalled	
Oakhampton		Kingsbridge		Dartington		recalled	
Devon		Dorset		Somerset		recalled	

General Scores

MMSE	/30
ACE-R	/100

Subscores

Attention and Orientation	/18
Memory	/26
Fluency	/14
Language	/26
Visuospatial	/16

VISUOSPATIAL MEMORY SCORE

Appendix G Relationships between gait variables and NMDAS

	Spearman correlations (*p* value)								
	Pace		**Rhythm**	**Asymmetry**	**Variability**		**Postural control**		
NMDAS component	*Step Velocity*	*Step Length*	*Cadence*	*Step time asymmetry*	*Step length variability*	*Step time variability*	*Step width*	*Step width variability*	*Step length asymmetry*
Vision	-.398 (-0.054)	-.460(.024)	-.198 (.353)	.345 (.099)	.253 (.233)	.032 (.883)	.446 (.029)	.272 (.198)	.378 (.068)
Hearing	-.130 (.546)	-.064 (.765)	.046 (.832)	-.071 (.741)	-.214 (.315)	-.144 (.502)	.256 (.227)	.259 (.221)	.309 (.141)
Speech	-.469 (.021)	-.431 (.035)	-.423 (.039)	.382 (.066)	.307 (.145)	.240 (.259)	.352 (.092)	.377 (.069)	.333 (.112)
Swallowing	-.291 (.167)	-.239 (.260)	-.117 (.585)	.030 (.888)	.120 (.578)	.048 (.825)	.309 (.142)	.450 (.027)	.503 (.012)
Handwriting	**-.581 (.003)**	-.472 (.020)	-.449 (.028)	.379 (.068)	**.521 (.009)**	.408 (.048)	.388 (.061)	.511 (.011)	.394 (.057)
Cutting	**-.526 (.008)**	-.539 (.007)	-.338 (.107)	.344 (.100)	**.633 (.001)**	**.599 (.002)**	.247 (.244)	.420 (.041)	.354 (.090)
Dressing	-.513 (.010)	-.494 (.014)	-.459 (.024)	.513 (.010)	**.570 (.004)**	**.532 (.007)**	.267 (.207)	**.551 (.005)**	.358 (.086)
Hygiene	**-.518 (.010)**	-.500 (.013)	-.465 (.022)	**.518 (.010)**	**.572 (.003)**	**.535 (.007)**	.291 (.167)	**.555 (.005)**	.346 (.098)
Exercise	**-.662 (.000)**	**-.699 (.000)**	-.348 (.096)	.485 (.016)	.513 (.010)	.504 (.012)	.357 (.087)	.367 (.078)	.440 (.032)
Gait	**-.546 (.006)**	**-.570 (.004)**	-.408 (.048)	.446 (.029)	.459 (.024)	.331 (.114)	.437 (.033)	**.601 (.002)**	.287 (.174)
Psychiatric	-.389 (.060)	-.419 (.041)	-.052 (.811)	.151 (.481)	.167 (.434)	.027 (.901)	.163 (.447)	.065 (.764)	**.684 (.000)**
Migraine	.248 (.243)	.214 (.316)	.019 (.929)	.089 (.680)	-.202 (.344)	-.450 (.027)	-.453 (.026)	-.041 (.849)	.209 (.327)
Seizures	-.432 (.035)	-.435 (.034)	-.362 (.082)	**.610 (.002)**	.417 (.043)	.146 (.496)	-.015 (.944)	.416 (.043)	.397 (.055)
Stroke like episodes	.009 (.966)	-.014 (.950)	.140 (.515)	.131 (.540)	-.345 (.098)	-.372 (.074)	-.143 (.504)	.005 (.983)	.121 (.575)
Encephalopathic	.022 (.920)	0.000 (1.000)	.152 (.477)	.131 (.543)	-.348 (.095)	-.370 (.075)	-.152 (.477)	0.000 (1.000)	.131 (.543)
Gastrointestinal	.408 (.048)	.326 (.120)	.255 (.230)	-.196 (.359)	-.463 (.023)	**-.597 (.002)**	-.260 (.219)	-.212 (.321)	.006 (.979)
Diabetes	-.024 (.911)	.141 (.512)	.045 (.833)	-.496 (.014)	.022 (.918)	.201 (.347)	.234 (.271)	-.022 (.920)	-.112 (.603)
Respiratory	.138 (.521)	.265 (.212)	-.013 (.950)	.152 (.480)	.126 (.557)	.011 (.959)	-.336 (.108)	-.061 (.777)	-.139 (.516)
Cardiovascular	-.433 (.034)	-.315 (.133)	**-.529 (.008)**	.389 (.060)	.364 (.080)	.174 (.417)	.214 (.316)	.484 (.017)	.132 (.537)
Visuals	-.419 (.041)	-.455 (.026)	-.096 (.656)	.137 (.524)	.320 (.127)	.165 (.440)	.465 (.022)	.321 (.126)	**.528 (.008)**
Ptosis	.122 (.569)	.054 (.801)	.412 (.046)	-.073 (.736)	-.277 (.189)	-.190 (.373)	-.189 (.378)	.095 (.658)	.347 (.096)

	Pace		**Rhythm**	**Asymmetry**	**Variability**		**Postural control**		
NMDAS component	*Step Velocity*	*Step Length*	*Cadence*	*Step time asymmetry*	*Step length variability*	*Step time variability*	*Step width*	*Step width variability*	*Step length asymmetry*
CPEO	-.410 (.047)	-.464 (.022)	-.209 (.326)	.282 (.182)	.337 (.108)	.355 (.089)	.373 (.073)	**.519 (.009)**	.264 (.213)
Dysphonia/Dysarthria	-.423 (.039)	-.379 (.068)	-.405 (.050)	.267 (.207)	.296 (.160)	.274 (.196)	.323 (.123)	.318 (.130)	.209 (.326)
Cerebellar	-.424 (.039)	-.379 (.068)	-.296 (.160)	.434 (.034)	**.518 (.009)**	.513 (.010)	.423 (.039)	**.634 (.001)**	.446 (.029)
Neuropathy	-.494 (.014)	-.391 (.059)	-.448 (.028)	.479 (.018)	.222 (.298)	.196 (.360)	.228 (.284)	.450 (.027)	.321 (.126)
Pyramidal	-.087 (.686)	-.087 (.686)	0.000 (1.000)	.305 (.147)	.044 (.840)	.087 (.686)	.065 (.762)	.196 (.359)	.109 (.613)
Extrapyramidal	-.346 (.097)	-.346 (.097)	-.316 (.132)	.346 (.097)	.346 (.097)	.346 (.097)	.346 (.097)	.346 (.097)	-.105 (.624)
Cognition	-.464 (.022)	-.461 (.023)	-.345 (.099)	.339 (.105)	.160 (.456)	.056 (.796)	.087 (.685)	.245 (.248)	**.615 (.001)**
Total	**-.645 (.001)**	**-.588 (.003)**	-.418 (.042)	.459 (.024)	.334 (.110)	.143 (.506)	.364 (.081)	**.600 (.002)**	**.615 (.001)**

Statistical significant results ($p<.01$) are shown in bold.

References

Abresch, R.T., Han, J.J. and Carter, G.T. (2009) 'Rehabilitation Management of Neuromuscular Disease: The Role of Exercise Training.[Review]', *Journal of Clinical Neuromuscular Disease*, September(1), pp. 7-21.

ACSM (2006) *ACSM's Resource Manual for Guidelines for Exercise Testing and Prescription*. Fifth Edition edn. Lippincott Williams & Wilkins.

Al-Yahya, E., Dawes, H., Smith, L., Dennis, A., Howells, K. and Cockburn, J. (2011) 'Cognitive motor interference while walking: A systematic review and meta-analysis', *Neuroscience & Biobehavioral Reviews*, 35(3), pp. 715-728.

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. (2004) 'Essential Cell Biology ', .

American Thoracic Society Statement (2002) 'Guidelines for the Six-Minute Walk Test', *Am. J. Respir. Crit. Care Med.*, 166(1), pp. 111-117.

Andersson, C., Asztalos, L. and Mattsson, E. (2006) 'Six-minute walk test in adults with cerebral palsy. A study of reliability', *Clinical Rehabilitation*, 20(6), pp. 488-495.

Angevaren M, A.G., Verhaar HJJ, Aleman A, Vanhees L. (2008) 'Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive impairment. ', *Cochrane Database of Systematic Reviews*, (3).

Apabhai, S., Gorman, G.S., Sutton, L., Elson, J.L., Plötz, T., Turnbull, D.M. and Trenell, M.I. (2011) 'Habitual Physical Activity in Mitochondrial Disease', *PLoS One*, 6(7), p. e22294.

Atkinson, H.H., Rosano, C., Simonsick, E.M., Williamson, J.D., Davis, C., Ambrosius, W.T., Rapp, S.R., Cesari, M., Newman, A.B., Harris, T.B., Rubin, S.M., Yaffe, K., Satterfield, S., Kritchevsky, S.B. and for the Health, A.B.C.s. (2007) 'Cognitive Function, Gait Speed Decline, and Comorbidities: The Health, Aging and Body Composition Study', *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 62(8), pp. 844-850.

Baltadjieva, R., Giladi, N., Gruendlinger, L., Peretz, C. and Hausdorff, J.M. (2006) 'Marked alterations in the gait timing and rhythmicity of patients with de novo Parkinson's disease', *European Journal of Neuroscience*, 24(6), pp. 1815-1820.

Baltzopoulos, V. and Brodie, D.A. (1989) 'Isokinetic Dynamometry', *Sports Medicine*, 8(2), pp. 101-116.

Bates, M.G.D.a.N., Jane H., Jakovljevic, D.G., Hollingsworth, K.G., Alston, C.L., Zalewski, P., Klawe, J.J., Blamire, A.M., MacGowan, G.A., Keavney, B.D., Bourke, J.P., Schaefer, A., McFarland, R., Newton, J.L., Turnbull, D.M., Taylor, R.W., Trenell, M.I. and Gorman, G.S. (2013) 'Defining cardiac adaptations and safety of endurance training in patients with m.3243A>G-related mitochondrial disease', *International Journal of Cardiology*, 168(4), pp. 3599-3608.

Bello, O., Sanchez, J.A. and Fernandez-del-Olmo, M. (2008) 'Treadmill walking in Parkinson's disease patients: Adaptation and generalization effect', *Movement Disorders*, 23(9), pp. 1243-1249.

Bérard, C., Payan, C., Hodgkinson, I. and Fermanian, J. (2005) 'A motor function measure for neuromuscular diseases. Construction and validation study.', *Neuromuscul Disord*, 15(7), pp. 463-70.

Berger, R.A., Riley, P.O., Mann, R.W. and Hodge, W.A. (1988) 'Total body dynamics in ascending stairs and rising from a chair following total knee arthroplasty.', *Trans Orthop Res Soc.* , 13(542).

Biaggi, R.R., Vollman, M.W., Nies, M.A., Brener, C.E., Flakoll, P.J., Levenhagen, D.K., Sun, M., Karabulut, Z. and Chen, K.Y. (1999) 'Comparison of air-displacement plethysmography with hydrostatic weighing and bioelectrical impedance analysis for the assessment of body composition in healthy adults', *The American Journal of Clinical Nutrition*, 69(5), pp. 898-903.

Bilney, B., Morris, M. and Webster, K. (2003) 'Concurrent related validity of the GAITRite® walkway system for quantification of the spatial and temporal parameters of gait', *Gait & Posture*, 17(1), pp. 68-74.

Bjørnstad, H., Storstein, L., Dyre Meen, H. and Hals, O. (1994) 'Ambulatory Electrocardiographic Findings in Top Athletes, Athletic Students and Control Subjects', *Cardiology*, 84(1), pp. 42-50.

Bogenhagen, D. and Clayton, D.A. (1977) 'Mouse l cell mitochondrial DNA molecules are selected randomly for replication throughout the cell cycle', *Cell*, 11(4), pp. 719-727.

Bohannon, R.W. (1989) 'Objective Measures', *PHYS THER*, 69(7), pp. 590-593.

Bohannon, R.W. (1995) 'Sit to stand test for measuring performance of lower extremity muscles', *Perceptual and Motor Skills*, 80(1), pp. 163-166.

Bohannon, R.W. (1997) 'Comfortable and maximum walking speed of adults aged 20—79 years: reference values and determinants', *Age and Ageing*, 26(1), pp. 15-19.

Bohannon, R.W. (1998) 'Alternatives for measuring knee extension strength of the elderly at home', *Clinical Rehabilitation*, 12(5), pp. 434-440.

Bohannon, R.W. (2007) 'Six-Minute Walk Test: A Meta-Analysis of Data From Apparently Healthy Elders', *Topics in Geriatric Rehabilitation*, 23(2), pp. 155-160 10.1097/01.TGR.0000270184.98402.ef.

Bohannon, R.W., Shove, M.E., Barreca, S.R., Masters, L.M. and Sigouin, C.S. (2007) 'Five-repetition sit-to-stand test performance by community-dwelling adults: A preliminary investigation of times, determinants, and relationship with self-reported physical performance', *Isokinetics and Exercise Science*, 15(2), pp. 77-81.

Bohannon, R.W. and Williams Andrews, A. (2011) 'Normal walking speed: a descriptive meta-analysis', *Physiotherapy*, 97(3), pp. 182-189.

Boling, M.C., Padua, D.A. and Creighton, R.A. (2009) 'Concentric and Eccentric Torque of the Hip Musculature in Individuals With and Without Patellofemoral Pain', *Journal of Athletic Training*, 44(1), pp. 7-13.

Bouchard, C. and Rankinen, T. (2001) 'Individual differences in response to regular physical activity', *Medicine and science in sports and exercise*, 33(6 Suppl), pp. S446-51; discussion S452-3.

Brach, J.S., Berlin, J.E., VanSwearingen, J.M., Newman, A.B. and Studenski, S.A. (2005) 'Too much or too little step width variability is associated with a fall history in older persons who walk at or near normal gait speed', *Journal of NeuroEngineering and Rehabilitation* 2 (21).

Brusse, K.J., Zimdars, S., Zalewski, K.R. and Steffen, T.M. (2005) 'Testing Functional Performance in People With Parkinson Disease', *Physical Therapy*, 85(2), pp. 134-141.

Buchner, D.M., Cress, M.E., de Lateur, B.J., Esselman, P.C., Margherita, A.J., Price, R. and Wagner, E.H. (1997) 'A comparison of the effects of three types of endurance training on balance and other fall risk factors in older adults.', *Aging* Feb-Apr(9(1-2)), pp. 112-9.

Burnett, C.N., Betts, E.F. and King, W.M. (1990) 'Reliability of Isokinetic Measurements of Hip Muscle Torque in Young Boys', *PHYS THER*, 70(4), pp. 244-249.

Burns, T.M., Graham, C.D., Rose, M.R. and Simmons, Z. (2012) 'Quality of life and measures of quality of life in patients with neuromuscular disorders', *Muscle & Nerve*, 46(1), pp. 9-25.

Busse, M.E., Pearson, O.R., Van Deursen, R. and Wiles, C.M. (2004) 'Quantified measurement of activity provides insight into motor function and recovery in neurological disease', *Journal of Neurology, Neurosurgery & Psychiatry*, 75(6), pp. 884-888.

Busse, M.E., Wiles, C.M. and van Deursen, R.W.M. (2006) 'Community walking activity in neurological disorders with leg weakness', *J Neurol Neurosurg Psychiatry*, 77(3), pp. 359-362.

C G Blomqvist, a. and Bengt, S. (1983) 'Cardiovascular Adaptations to Physical Training', *Annual Review of Physiology*, 45(1), pp. 169-189.

Cadore, E.L., Rodríguez-Mañas, L., Sinclair, A. and Izquierdo, M. (2013) 'Effects of Different Exercise Interventions on Risk of Falls, Gait Ability, and

Balance in Physically Frail Older Adults: A Systematic Review', *Rejuvenation Research*, 16(2), pp. 105-114.

Cahalan, T.D., Johnson, M.E., Liu, S. and Chao, E.Y.S. (1989) 'Quantitative Measurements of Hip Strength in Different Age Groups', *Clinical Orthopaedics and Related Research*, 246, pp. 136-145.

Cejudo, P., Bautista, J., Montemayor, T., Villagómez, R., Jiménez, L., Ortega, F., Campos, Y., Sánchez, H. and Arenas, J. (2005) 'Exercise training in mitochondrial myopathy: A randomized controlled trial', *Muscle & Nerve*, 32(3), pp. 342-350.

Chan, D.C. (2006a) 'Dissecting Mitochondrial Fusion', *Developmental Cell*, 11(5), pp. 592-594.

Chan, D.C. (2006b) 'Mitochondrial Fusion and Fission in Mammals', *Annual Review of Cell and Developmental Biology*, 22(1), pp. 79-99.

Chen, H. and Chan, D.C. (2010) 'Physiological functions of mitochondrial fusion', *Annals of the New York Academy of Sciences*, 1201(1), pp. 21-25.

Chinnery, P.F., Howel, D., Turnbull, D.M. and Johnson, M.A. (2003) 'Clinical progression of mitochondrial myopathy is associated with the random accumulation of cytochrome c oxidase negative skeletal muscle fibres', *Journal of the Neurological Sciences*, 211(1–2), pp. 63-66.

Chinnery, P.F., Howell, N., Lightowlers, R.N. and Turnbull, D.M. (1997) 'Molecular pathology of MELAS and MERRF. The relationship between mutation load and clinical phenotypes', *Brain*, 120(10), pp. 1713-1721.

Chinnery, P.F., Majamaa, K., Turnbull, D. and Thorburn, D. (2006) 'Treatment for mitochondrial disorders', *Cochrane Database of Systematic Reviews*, (Issue 1).

Claiborne, T.L., Timmons, M.K. and Pincivero, D.M. (2009) 'Test–retest reliability of cardinal plane isokinetic hip torque and EMG', *Journal of Electromyography and Kinesiology*, 19(5), pp. e345-e352.

Collett, J., Dawes, H., Meaney, A., Sackley, C., Barker, K., Wade, D., Izardi, H., Bateman, J., Duda, J. and Buckingham, E. (2011) 'Exercise for multiple sclerosis: a single-blind randomized trial comparing three exercise intensities', *Multiple Sclerosis Journal*, 17(5), pp. 594-603.

Cooper, C.B. and Storer, T.W. (2001) *Exercise testing and interpretation; A practical approach .* press syndicate of the university of Cambridge Cambridge University press.

Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF and P., O. (2003) 'International physical activity questionnaire: 12-country reliability and validity.', *Med Sci Sports Exerc.*, 8(35), pp. 1381-95.

Cup, E.H., Pieterse, A.J., ten Broek-Pastoor, J.M., Munneke, M., van Engelen, B.G., Hendricks, H.T., van der Wilt, G.J. and Oostendorp, R.A. (2007) 'Exercise Therapy and Other Types of Physical Therapy for Patients With Neuromuscular Diseases: A Systematic Review', *Archives of Physical Medicine and Rehabilitation*, 88(11), pp. 1452-1464.

Dandurand, R.J., Matthews, P.M., Arnold, D.L. and Eidelman, D.H. (1995) 'Mitochondrial disease : Pulmonary function, exercise performance, and blood lactate levels', *Chest*, 108(1), pp. 182-189.

Daussin, F.N., Zoll, J., Ponsot, E., Dufour, S.P., Doutreleau, S., Lonsdorfer, E., Ventura-Clapier, R., Mettauer, B., Piquard, F., Geny, B. and Richard, R. (2008) 'Training at high exercise intensity promotes qualitative adaptations of mitochondrial function in human skeletal muscle', *Journal of Applied Physiology*, 104(5), pp. 1436-1441.

Davis, J.A., Vodak, P., Wilmore, J.H., Vodak, J. and Kurtz, P. (1976) 'Anaerobic threshold and maximal aerobic power for three modes of exercise', *Journal of Applied Physiology*, 41(4), pp. 544-550.

Dawes, H., Collett, J., Ramsbottom, R., Howells, K., Sackley, C. and Wade, D. (2004) 'Measuring Oxygen Cost During Level Walking in Individuals with Acquired Brain Injury in the Clinical Setting', *Journal of Sports Science & Medicine*, 3(2), pp. 76-82.

De Rekeneire, N., Visser, M., Peila, R., Nevitt, M.C., Cauley, J.A., Tylavsky, F.A., Simonsick, E.M. and Harris, T.B. (2003) 'Is a Fall Just a Fall: Correlates of Falling in Healthy Older Persons. The Health, Aging and Body Composition Study', *Journal of the American Geriatrics Society*, 51(6), pp. 841-846.

Dibble, L.E., Addison, O. and Papa, E. (2009) 'The Effects of Exercise on Balance in Persons with Parkinson's Disease: A Systematic Review Across the Disability Spectrum', *Journal of Neurologic Physical Therapy*, 33(1), pp. 14-26.

Diedrichsen, J., Criscimagna-Hemminger, S.E. and Shadmehr, R. (2007) 'Dissociating Timing and Coordination as Functions of the Cerebellum', *The Journal of Neuroscience,* , June(27(23):), pp. 6291-6301

DiMauro, S. and Schon, E.A. (2003) 'Mitochondrial Respiratory-Chain Diseases', *New England Journal of Medicine*, 348(26), pp. 2656-2668.

Duncan, P., Studenski, S., Richards, L., Gollub, S., Lai, S.M., Reker, D., Perera, S., Yates, J., Koch, V., Rigler, S. and Johnson, D. (2003) 'Randomized Clinical Trial of Therapeutic Exercise in Subacute Stroke', *Stroke*, 34(9), pp. 2173-2180.

Durham, S.E., Bonilla, E., Samuels, D.C., DiMauro, S. and Chinnery, P.F. (2005) 'Mitochondrial DNA copy number threshold in mtDNA depletion myopathy', *Neurology*, 65(3), pp. 453-455.

Dvir, Z. (2004) *Isokinetics Muscle Testing ,Interpretation and Clinical Applications* Second edn. Churchill Livingstone

Dysgaard Jeppesen, T., Olsen, D. and Vissing, J. (2003) 'Cycle ergometry is not a sensitive diagnostic test for mitochondrial myopathy', *Journal of Neurology*, 250(3), pp. 293-299.

El Mhandi, L. and Bethoux, F. (2013) 'Isokinetic Testing in Patients with Neuromuscular Diseases: A Focused Review', *American Journal of Physical Medicine & Rehabilitation*, 92(2), pp. 163-178.

Elliott, H.R., Samuels, D.C., Eden, J.A., L., R.C. and Chinnery, P.F. (2008) 'Pathogenic Mitochondrial DNA Mutations Are Common in the General Population', *The American Journal of Human Genetics* 83(8), pp. 254-260.

Elson, J.L., Cadogan, M., Apabhai, S., Whittaker, R.G., Phillips, A., Trennell, M.I., Horvath, R., Taylor, R.W., McFarland, R., McColl, E., Turnbull, D.M. and

Gorman, G.S. (2013) 'Initial development and validation of a mitochondrial disease quality of life scale', *Neuromuscular Disorders*, 23(4), pp. 324-329.

Eneida, M., Kate, D., Joanna, M., Robert, A. and John, R.H. (2006) 'The Addenbrooke's Cognitive Examination Revised (ACE-R): a brief cognitive test battery for dementia screening', *International Journal of Geriatric Psychiatry*, 21(11), pp. 1078-1085.

Favier, F., Benoit, H. and Freyssenet, D. (2008) 'Cellular and molecular events controlling skeletal muscle mass in response to altered use', *Pflügers Archiv European Journal of Physiology*, 456(3), pp. 587-600.

Fawcett, D.W. (1994) *A Textbook of Histology,*. 12th edition edn. Chapman and Hall.

Ferretti, G. (2014) 'Maximal oxygen consumption in healthy humans: theories and facts', *European Journal of Applied Physiology*, 114(10), pp. 2007-2036.

Fields DA, Higgins PB and D., R. (2005) 'Air-displacement plethysmography: here to stay', *Curr Opin Clin Nutr Metab Care.* , Nov;(8(6)), pp. 624-9.

Finsterer, J. (2009) 'Mitochondrial disorders, cognitive impairment and dementia', *Journal of the Neurological Sciences*, 283(1-2), p. 143.

Fisher, B.E., Petzinger, G.M., Nixon, K., Hogg, E., Bremmer, S., Meshul, C.K. and Jakowec, M.W. (2004) 'Exercise-induced behavioral recovery and neuroplasticity in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse basal ganglia', *Journal of Neuroscience Research*, 77(3), pp. 378-390.

Fisher, B.E. and Sullivan, K.J. (2001) 'Activity-Dependent Factors Affecting Poststroke Functional Outcomes', *Topics in Stroke Rehabilitation*, 8(3), pp. 31-44.

Flansbjer, U.B., Holmbäck, A.M., Downham, D., Patten, C. and Lexell, J. (2005) 'Reliability of gait performance tests in men and women with hemiparesis after stroke', *Journal of rehabilitation medicine*, 37(2), pp. 75-82.

Fletcher, G. (1999) 'Physical inactivity as a risk factor for cardiovascular disease', *The American Journal of Medicine*, 107(2, Supplement 1), pp. 10-11.

Florence, J.M., van der Ploeg, A., Clemens, P.R., Escolar, D.M., Laforet, P., Rosenbloom, B., Wasserstein, M., Skrinar, A., Pestronk, A. and Mayhew, J.E. (2008) 'Use of the 6 min walk test as an endpoint in clinical trials for neuromuscular diseases', *Neuromuscular Disorders*, 18(9–10), pp. 738-739.

Flück, M. (2006) 'Functional, structural and molecular plasticity of mammalian skeletal muscle in response to exercise stimuli', *Journal of Experimental Biology*, 209(12), pp. 2239-2248.

Frayn, K.N. (1983) 'Calculation of substrate oxidation rates in vivo from gaseous exchange', *J Appl Physiol*, 55(2), pp. 628-634.

Frazzitta, G., Maestri, R., Uccellini, D., Bertotti, G. and Abelli, P. (2009) 'Rehabilitation treatment of gait in patients with Parkinson's disease with freezing: A comparison between two physical therapy protocols using visual and auditory cues with or without treadmill training', *Movement Disorders*, 24(8), pp. 1139-1143.

Frenkel-Toledo, S., Giladi, N., Peretz, C., Herman, T., Gruendlinger, L. and Hausdorff, J.M. (2005) 'Treadmill walking as an external pacemaker to improve gait rhythm and stability in Parkinson's disease', *Movement Disorders*, 20(9), pp. 1109-1114.

Frese, E., Brown, M. and Norton, B.J. (1987) 'Clinical Reliability of Manual Muscle Testing: Middle Trapezius and Gluteus Medius Muscles', *Physical Therapy*, 67(7), pp. 1072-1076.

Fritz, S. and Lusardi, M. (2009) '"Walking Speed: the Sixth Vital Sign"', *J Geriatr Phys Ther*, 32, pp. 2-5.

Galna, B., Lord, S., Burn, D.J. and Rochester, L. (2014) 'Progression of gait dysfunction in incident Parkinson's disease: Impact of medication and phenotype', *Movement Disorders*, pp.1-9.

Galna, B., Lord, S. and Rochester, L. (2013a) 'Is gait variability reliable in older adults and Parkinson's disease? Towards an optimal testing protocol', *Gait & Posture*, 37(4), pp. 580-585.

Galna, B., Newman, J., Jakovljevic, D., Bates, M., Schaefer, A., McFarland, R., Turnbull, D., Trenell, M., Gorman, G. and Rochester, L. (2013b) 'Discrete gait characteristics are associated with m.3243A>G and m.8344A>G variants of mitochondrial disease and its pathological consequences', *Journal of Neurology*, pp. 1-10.

Gorman, G.S., Elson, J.L., Newman, J., Payne, B., McFarland, R., Newton, J.L. and Turnbull, D.M. (2015) 'Perceived fatigue is highly prevalent and debilitating in patients with mitochondrial disease', *Neuromuscular Disorders*, 25(7), pp. 563-566.

Goto, Y., Nonaka, I. and Horai, S. (1990) 'A mutation in the tRNALeu(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies', *NATURE*, 348(6302), pp. 651-653.

Graf, W.D., Sumi, S.M., Copass, M.K., Ojemann, L.M., Longstreth, W.T., Shanske, S., Lombes, A. and DiMauro, S. (1993) 'Phenotypic heterogeneity in families with the myoclonic epilepsy and ragged-red fiber disease point mutation in mitochondrial DNA', *Annals of Neurology*, 33(6), pp. 640-645.

Graham, J.E., Ostir, G.V., Fisher, S.R. and Ottenbacher, K.J. (2008) 'Assessing walking speed in clinical research: a systematic review', *Journal of Evaluation in Clinical Practice*, 14(4), pp. 552-562.

Gross, M.M., Stevenson, P.J., Charette, S.L., Pyka, G. and Marcus, R. (1998) 'Effect of muscle strength and movement speed on the biomechanics of rising from a chair in healthy elderly and young women', *Gait & Posture*, 8(3), pp. 175-185.

Guette, M., Gondin, J. and Martin, A. (2005) 'Time-of-Day Effect on the Torque and Neuromuscular Properties of Dominant and Non-Dominant Quadriceps Femoris', *Chronobiology International*, 22(3), pp. 541-558.

Guralnik, J.M., Ferrucci, L., Pieper, C.F., Leveille, S.G., Markides, K.S., Ostir, G.V., Studenski, S., Berkman, L.F. and Wallace, R.B. (2000) 'Lower Extremity Function and Subsequent Disability: Consistency Across Studies, Predictive Models, and Value of Gait Speed Alone Compared With the Short Physical Performance Battery', *J Gerontol A Biol Sci Med Sci*, 55(4), pp. M221-231.

Hagströmer, M., Oja, P. and M., S. (2006) 'The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity.', *Public Health Nutrition*, 9(06), pp. 755-62.

Haller, R.G., Lewis, S.F., Estabrook, R.W., Dimauro, S., Servidei, S. and Foster, D.W. (1989) 'Exercise intolerance, lactic-acidosis, and abnormal cardiopulmonary regulation in exercise associated with adult skeletal-muscle cytochrome-c oxidase deficiency', *Journal of Clinical Investigation*, 84(1), pp. 155-161.

Haller, R.G., Wyrick, P., Taivassalo, T. and Vissing, J. (2006) 'Aerobic conditioning: An effective therapy in McArdle's disease', *Annals of Neurology*, 59(6), pp. 922-928.

Hammans, S.R., Sweeney, M.G., Brockington, M., Lennox, G.G., Lawton, N.F., Kennedy, C.R., Morgan-Hughes, J.A. and Harding, A.E. (1993) 'The mitochondrial DNA transfer RNALysA→G[8344] mutation and the syndrome of myoclonic epilepsy with ragged red fibres [MERRF]', *Brain*, 116(3), pp. 617-632.

Hammans, S.R., Sweeney, M.G., Hanna, M.G., Brockington, M., Morgan-Hughes, J.A. and Harding, A.E. (1995) 'The mitochondrial DNA transfer RNA Leu(UUR) A→G(3243) mutation: A clinical and genetic study', *Brain*, 118(3), pp. 721-734.

Hashizume, A., Katsuno, M., Banno, H., Suzuki, K., Suga, N., Mano, T., Atsuta, N., Oe, H., Watanabe, H., Tanaka, F. and Sobue, G. (2012) 'Longitudinal changes of outcome measures in spinal and bulbar muscular atrophy', *Brain*, 135(9), pp. 2838-2848.

Haskell, W.L., Lee, I.M., Pate, R.R., Powell, K.E. and Blair, S.N. (2007) 'Physical Activity and Public Health', *Circulation*, 116(9), pp. 1081-1093.

Hausdorff, J.M. (2007) 'Gait dynamics, fractals and falls: Finding meaning in the stride-to-stride fluctuations of human walking', *Human Movement Science*, 26(4), pp. 555-589.

Hausdorff, J.M., Balash, J. and Giladi, N. (2003) 'Effects of Cognitive Challenge on Gait Variability in Patients with Parkinson's Disease', *Journal of Geriatric Psychiatry and Neurology*, 16(1), pp. 53-58.

Hausdorff, J.M., Cudkowicz, M.E., Firtion, R., Wei, J.Y. and Goldberger, A.L. (1998) 'Gait variability and basal ganglia disorders: Stride-to-stride variations of gait cycle timing in parkinson's disease and Huntington's disease', *Movement Disorders*, 13(3), pp. 428-437.

Hengartner, M.O. (2000) 'The biochemistry of apoptosis', *NATURE*, 407(6805), pp. 770-776.

Herman, T., Giladi, N., Gruendlinger, L. and Hausdorff, J.M. (2007) 'Six Weeks of Intensive Treadmill Training Improves Gait and Quality of Life in Patients With Parkinson's Disease: A Pilot Study', *Archives of Physical Medicine and Rehabilitation*, 88(9), pp. 1154-1158.

Holland, A., O'Connor, R.J., Thompson, A.J., Playford, E.D. and Hobart, J.C. (2006) 'Talking the talk on walking the walk', *Journal of Neurology*, 253(12), pp. 1594-1602.

Holloszy, J.O. (1967) 'Biochemical Adaptations in Muscle', *Journal of Biological Chemistry*, 242(9), pp. 2278-2282.

Holloszy, J.O. and Coyle, E.F. (1984) 'Adaptations of skeletal muscle to endurance exercise and their metabolic consequences', *Journal of Applied Physiology*, 56(4), pp. 831-838.

Holt, I.J., Harding, A.E. and Morgan-Hughes, J.A. (1988) 'Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies', *NATURE*, 331(6158), pp. 717-719.

Hood, D. (2009) 'Mechanisms of exercise-induced mitochondrial biogenesis in skeletal muscle.', *Appl Physiol Nutr Metab*, June;34(3), pp. 465-72.

Hood, D.A., Irrcher, I., Ljubicic, V. and Joseph, A.-M. (2006) 'Coordination of metabolic plasticity in skeletal muscle', *J Exp Biol*, 209(12), pp. 2265-2275.

Horvath, R., Gorman, G. and Chinnery, P.F. (2008) 'How Can We Treat Mitochondrial Encephalomyopathies? Approaches to Therapy', *Neurotherapeutics*, 5(4), pp. 558-568.

Howe, T.E., Rochester, L., Neil, F., Skelton, D.A. and Ballinger, C. (2011) 'Exercise for improving balance in older people', *Cochrane Database of Systematic Reviews*, 11.

Ilg, W., Golla, H., Thier, P. and Giese, M.A. (2007) 'Specific influences of cerebellar dysfunctions on gait', *Brain*, 130(3), pp. 786-798.

Inkster, L.M., Eng, J.J., MacIntyre, D.L. and Stoessl, A.J. (2003) 'Leg muscle strength is reduced in Parkinson's disease and relates to the ability to rise from a chair', *Movement Disorders*, 18(2), pp. 157-162.

Innes, J.A., De Cort, S.C., Evans, P.J. and Guz, A. (1992) 'Central command influences cardiorespiratory response to dynamic exercise in humans with unilateral weakness', *The Journal of Physiology*, 448(1), pp. 551-563.

Ip, E.H., Church, T., Marshall, S.A., Zhang, Q., Marsh, A.P., Guralnik, J., King, A.C., Rejeski, W.J. and Investigators, L.-P.S. (2013) 'Physical Activity Increases Gains in and Prevents Loss of Physical Function: Results From the Lifestyle

Interventions and Independence for Elders Pilot Study', *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 68(4), pp. 426-432.

Jahn, K., Zwergal, A. and Schniepp, R. (2010) 'Gait Disturbances in Old Age—Classification, Diagnosis, and Treatment From a Neurological Perspective', *Dtsch Arztebl International*, 107(17), pp. 306-16.

Jakovljevic, D.G., Moore, S., Hallsworth, K., Fattakhova, G., Thoma, C. and Trenell, M.I. (2012,) 'Comparison of cardiac output determined by bioimpedance and bioreactance methods at rest and during exercise', *Journal of Clinical Monitoring and Computing*, 26,(2,), pp. 63-68.

Jeppesen, T.D., Dunø, M., Schwartz, M., Krag, T., Rafiq, J., Wibrand, F. and Vissing, J. (2009) 'Short- and long-term effects of endurance training in patients with mitochondrial myopathy', *European Journal of Neurology*, 16(12), pp. 1336-1339.

Jeppesen, T.D., Schwartz, M., Frederiksen, A.L., Wibrand, F., Olsen, D.B. and Vissing, J. (2006a) 'Muscle Phenotype and Mutation Load in 51 Persons With the 3243A>G Mitochondrial DNA Mutation', *Arch Neurol*, 63(12), pp. 1701-1706.

Jeppesen, T.D., Schwartz, M., Olsen, D.B. and Vissing, J. (2003) 'Oxidative capacity correlates with muscle mutation load in mitochondrial myopathy', *Annals of Neurology*, 54(1), pp. 86-92.

Jeppesen, T.D., Schwartz, M., Olsen, D.B., Wibrand, F., Krag, T., Duno, M., Hauerslev, S. and Vissing, J. (2006b) 'Aerobic training is safe and improves exercise capacity in patients with mitochondrial myopathy', *Brain*, 129(12), pp. 3402-3412.

Jones, C.J., Rikli, R.E. and Beam, W.C. (1999) 'A 30-s Chair-Stand Test as a Measure of Lower Body Strength in Community-Residing Older Adults', *Research Quarterly for Exercise and Sport*, 70(2), pp. 113-119.

Jones, T.W., Houghton, D., Cassidy, S., MacGowan, G.A., Trenell, M.I. and Jakovljevic, D.G. (2015) 'Bioreactance is a reliable method for estimating cardiac output at rest and during exercise', *British Journal of Anaesthesia*.

Julia, M., Dupeyron, A., Laffont, I., Parisaux, J.M., Lemoine, F., Bousquet, P.J. and Hérisson, C. (2010) 'Reproducibility of isokinetic peak torque assessments of the hip flexor and extensor muscles', *Annals of Physical and Rehabilitation Medicine*, 53(5), pp. 293-305.

Kärppä, M., Herva, R., Moslemi, A.-R., Oldfors, A., Kakko, S. and Majamaa, K. (2005) 'Spectrum of myopathic findings in 50 patients with the 3243A>G mutation in mitochondrial DNA', *Brain*, 128(8), pp. 1861-1869.

Kartsounis, L.D., Troung, D.D., Morgan-Hughes, J.A. and Harding, A.E. (1992) 'The neuropsychological features of mitochondrial myopathies and encephalomyopathies', *Archives of Neurology*, 49(2), pp. 158-160.

Katz-Leurer, M., Rotem, H., Lewitus, H., Keren, O. and Meyer, S. (2008) 'Functional Balance Tests for Children with Traumatic Brain Injury: Within-Session Reliability', *Pediatric Physical Therapy*, 20(3), pp. 254-258

Kelly, V., Janke, A. and Shumway-Cook, A. (2010) 'Effects of instructed focus and task difficulty on concurrent walking and cognitive task performance in healthy young adults', *Experimental Brain Research*, 207(1-2), pp. 65-73.

Kelly, V.E., Eusterbrock, A.J. and Shumway-Cook, A. (2012a) 'The Effects of Instructions on Dual-Task Walking and Cognitive Task Performance in People with Parkinson's Disease', *Parkinson's Disease*, 2012, p. 9.

Kelly, V.E., Eusterbrock, A.J. and Shumway-Cook, A. (2012b) 'A Review of Dual-Task Walking Deficits in People with Parkinsons Disease: Motor and Cognitive Contributions, Mechanisms, and Clinical Implications', *Parkinson's Disease*, 2012, p. 14.

Keysor, J.J. and Jette, A.M. (2001) 'Have We Oversold the Benefit of Late-Life Exercise?', *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 56(7), pp. M412-M423.

Kileff, J. and Ashburn, A. (2005) 'A pilot study of the effect of aerobic exercise on people with moderate disability multiple sclerosis', *Clinical Rehabilitation*, 19(2), pp. 165-169.

Kilmer, D.D. (2002a) 'Response to Aerobic Exercise Training in Humans with Neuromuscular Disease', *American Journal of Physical Medicine & Rehabilitation*, 81(11,November), pp. S148-S150.

Kilmer, D.D. (2002b) 'Response to Resistive Strengthening Exercise Training in Humans with Neuromuscular Disease', *American Journal of Physical Medicine & Rehabilitation*, 81(11), pp. S121-S126.

Kilmer DD (2002) 'Response to training in humans with Neuromuscular disease', *Am J Phys Med Rehabil*, 81, pp. 148-150.

King, L.A. and Horak, F.B. (2009) 'Delaying Mobility Disability in People With Parkinson Disease Using a Sensorimotor Agility Exercise Program', *Physical Therapy*, 89(4), pp. 384-393.

Kodama, S., Saito, K., Tanaka, S. and et al. (2009) 'Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: A meta-analysis', *JAMA*, 301(19), pp. 2024-2035.

Kramer.Arthur F, Sowon Hahn*, Neal J. Cohen*, Marie T. Banich*, Edward McAuley*, Catherine R. Harrison*, Julie Chason*, Eli Vakil†, Lynn Bardell*, Richard A. Boileau* and Colcombe, A. (1999) 'Ageing, fitness and neurocognitive function', *NATURE*, 400(29 JULY), pp. 418-419.

Kuys, S.S., Brauer, S.G. and Ada, L. (2011) 'Test-retest reliability of the GAITRite system in people with stroke undergoing rehabilitation', *Disability and Rehabilitation*, 33(19-20), pp. 1848-1853.

Larsson, N.G. and Clayton, D.A. (1995) 'Molecular Genetic Aspects of Human Mitochondrial Disorders', *Annu Rev Genetics*, 29, pp. 151-78.

Lauhoff P, Murphy N, Doherty C and NF., H. (2013) 'A controlled clinical trial investigating the effects of cycle ergometry training on exercise tolerance, balance and quality of life in patients with Parkinson’s disease', *Disability and Rehabilitation*, 35(5), pp. 382-387.

Lauhoff, P., Murphy, N., Doherty, C. and Horgan, N.F. (2013) 'A controlled clinical trial investigating the effects of cycle ergometry training on exercise tolerance, balance and quality of life in patients with Parkinson’s disease', *Disability and Rehabilitation*, 35(5), pp. 382-387.

Lee, I.M., Shiroma, E.J., Lobelo, F., Puska, P., Blair, S.N. and Katzmarzyk, P.T. (2012) 'Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy', *The Lancet*, 380(9838), pp. 219-229.

Lee, M.J., Kilbreath, S.L., Singh, M.F., Zeman, B., Lord, S.R., Raymond, J. and Davis, G.M. (2008) 'Comparison of Effect of Aerobic Cycle Training and Progressive Resistance Training on Walking Ability After Stroke: A Randomized Sham Exercise–Controlled Study', *Journal of the American Geriatrics Society*, 56(6), pp. 976-985.

Lennon, O., Carey, A., Gaffney, N., Stephenson, J. and Blake, C. (2008) 'A pilot randomized controlled trial to evaluate the benefit of the cardiac rehabilitation paradigm for the non-acute ischaemic stroke population', *Clinical Rehabilitation*, 22(2), pp. 125-133.

Lerario, A., Bonfiglio, S., Sormani, M., Tettamanti, A., Marktel, S., Napolitano, S., Previtali, S., Scarlato, M., Natali-Sora, M., Mercuri, E., Bresolin, N., Mongini, T., Comi, G., Gatti, R., Ciceri, F., Cossu, G. and Torrente, Y. (2012) 'Quantitative muscle strength assessment in duchenne muscular dystrophy: longitudinal study and correlation with functional measures', *BMC Neurology*, 12(1), p. 91.

Lexell, J. (2000) 'Muscle Structure and Function in Chronic Neurological Disorders: The Potential of Exercise to Improve Activities of Daily Living', *Exercise and Sport Sciences Reviews*, 28(2), pp. 80-84.

Lim, L.I.I.K., van Wegen, E.E.H., de Goede, C.J.T., Jones, D., Rochester, L., Hetherington, V., Nieuwboer, A., Willems, A.M. and Kwakkel, G. (2005) 'Measuring gait and gait-related activities in Parkinson's patients own home environment: a reliability, responsiveness and feasibility study', *Parkinsonism & Related Disorders*, 11(1), pp. 19-24.

Lindemann, U., Najafi, B., Zijlstra, W., Hauer, K., Muche, R., Becker, C. and Aminian, K. (2008) 'Distance to achieve steady state walking speed in frail elderly persons', *Gait and Posture*, 27(1), pp. 91-96.

Loewen, S.C. and Anderson, B.A. (1988) 'Reliability of the Modified Motor Assessment Scale and the Barthel Index', *Physical Therapy*, 68(7), pp. 1077-1081.

Lord, S., Galna, B. and Rochester, L. (2013a) 'Moving forward on gait measurement: Toward a more refined approach', *Movement Disorders*, 28(11), pp. 1534-1543.

Lord, S., Galna, B., Verghese, J., Coleman, S., Burn, D. and Rochester, L. (2013b) 'Independent Domains of Gait in Older Adults and Associated Motor and Nonmotor Attributes: Validation of a Factor Analysis Approach', *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 68(7), pp. 820-827.

Lord, S., Howe, T., Greenland, J., Simpson, L. and Rochester, L. (2011) 'Gait variability in older adults: A structured review of testing protocol and clinimetric properties', *Gait & Posture*, 34(4), pp. 443-450.

Lord, S.E. and Rochester, L. (2005) 'Measurement of Community Ambulation After Stroke: Current Status and Future Developments', *Stroke*, 36(7), pp. 1457-1461.

Lord, S.R. and Menz, H.B. (2002) 'Physiologic, psychologic, and health predictors of 6-minute walk performance in older people', *Archives of Physical Medicine and Rehabilitation*, 83(7), pp. 907-911.

Lord, S.R., Murray, S.M., Chapman, K., Munro, B. and Tiedemann, A. (2002) 'Sit-to-Stand Performance Depends on Sensation, Speed, Balance, and Psychological Status in Addition to Strength in Older People', *J Gerontol A Biol Sci Med Sci*, 57(8), pp. M539-543.

Lovell DI, Cuneo R and GC, G. (2010) 'Can aerobic training improve muscle strength and power in older men?', *J Aging Phys Act.* , Jan(18), pp. 14-26.

Macko, R.F., Ivey, F.M., Forrester, L.W., Hanley, D., Sorkin, J.D., Katzel, L.I., Silver, K.H. and Goldberg, A.P. (2005) 'Treadmill exercise rehabilitation improves ambulatory function and cardiovascular fitness in patients with chronic stroke: a randomized, controlled trial', *Stroke*, 36(10), pp. 2206 - 2211.

Maffiuletti, N.A. (2010) 'Assessment of Hip and Knee Muscle Function in Orthopaedic Practice and Research', *J Bone Joint Surg Am*, 92(1), pp. 220-229.

Mancuso, M., Angelini, C., Bertini, E., Carelli, V., Comi, G.P., Minetti, C., Moggio, M., Mongini, T., Servidei, S., Tonin, P., Toscano, A., Uziel, G., Zeviani, M. and Siciliano, G. (2012) 'Fatigue and exercise intolerance in mitochondrial diseases. Literature revision and experience of the Italian Network of mitochondrial diseases', *Neuromuscular Disorders*, 22, Supplement 3(0), pp. S226-S229.

Mancuso, M., Orsucci, D., Angelini, C., Bertini, E., Carelli, V., Comi, G., Donati, A., Minetti, C., Moggio, M., Mongini, T., Servidei, S., Tonin, P., Toscano, A., Uziel, G., Bruno, C., Ienco, E., Filosto, M., Lamperti, C., Catteruccia, M., Moroni, I., Musumeci, O., Pegoraro, E., Ronchi, D., Santorelli, F., Sauchelli, D., Scarpelli, M., Sciacco, M., Valentino, M., Vercelli, L., Zeviani, M. and Siciliano, G. (2013) 'Phenotypic heterogeneity of the 8344A>G mtDNA "MERRF" mutation.', *Neurology.*, May 28(80(22)), pp. 2049-54.

Mandavilli, B.S., Santos, J.H. and Van Houten, B. (2002) 'Mitochondrial DNA repair and aging', *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 509(1-2), pp. 127-151.

Manwaring, N., Jones, M.M., Wang, J.J., Rochtchina, E., Howard, C., Mitchell, P. and Sue, C.M. (2007) 'Population prevalence of the MELAS A3243G mutation', *Mitochondrion*, 7(3), pp. 230-233.

Margineantu, D.H., Gregory Cox, W., Sundell, L., Sherwood, S.W., Beechem, J.M. and Capaldi, R.A. (2002) 'Cell cycle dependent morphology changes and associated mitochondrial DNA redistribution in mitochondria of human cell lines', *Mitochondrion*, 1(5), pp. 425-435.

Martin, C.L., Tan, D., Bragge, P. and Bialocerkowski, A. (2009) 'Effectiveness of physiotherapy for adults with cerebellar dysfunction: a systematic review', *Clinical Rehabilitation*, 23(1), pp. 15-26.

Mayo, N.E., MacKay-Lyons, M.J., Scott, S.C., Moriello, C. and Brophy, J. (2013) 'A randomized trial of two home-based exercise programmes to improve functional walking post-stroke', *Clinical Rehabilitation*, 27(7), pp. 659-671.

Mcardle, W.D., Katch, F.I. and Katch, V.L. (2007) *Exercise Physiology: Nutrition, Energy, and Human Performance*. Sixth Edition edn. Lippincott Williams & Wilkins.

McDonald, C. (2002) 'Physical activity, health impairments, and disability in neuromuscular disease.', *Am J Phys Med Rehabil*, Nov(81), pp. 108-20.

McDonald, C.M., Henricson, E.K., Abresch, R.T., Han, J.J., Escolar, D.M., Florence, J.M., Duong, T., Arrieta, A., Clemens, P.R., Hoffman, E.P., Cnaan, A. and the Cinrg, I. (2013) 'The cooperative international neuromuscular research group duchenne natural history study—a longitudinal investigation in the era of glucocorticoid therapy: Design of protocol and the methods used', *Muscle & Nerve*, 48(1), pp. 32-54.

McDonald, C.M., Henricson, E.K., Han, J.J., Abresch, R.T., Nicorici, A., Elfring, G.L., Atkinson, L., Reha, A., Hirawat, S. and Miller, L.L. (2010) 'The 6-minute walk test as a new outcome measure in Duchenne muscular dystrophy', *Muscle & Nerve*, 41(4), pp. 500-510.

McDonnell, M.T., Schaefer, A.M., Blakely, E.L., McFarland, R., Chinnery, P.F., Turnbull, D.M. and Taylor, R.W. (2004) 'Noninvasive diagnosis of the 3243A>G mitochondrial DNA mutation using urinary epithelial cells', *Eur J Hum Genet*, 12(9), pp. 778-781.

McFarland, R., Taylor, R.W. and Turnbull, D.M. (2002) 'A neurological perspective on mitochondrial disease', *The Lancet Neurology*, 9(8), pp. 829-840.

McFarland, R. and Turnbull, D.M. (2009) 'Batteries not included: diagnosis and management of mitochondrial disease', *Journal of Internal Medicine*, 265(2), pp. 210-228.

Mehrholz, J., Friis, R., Kugler, J., Twork, S., Storch, A. and Pohl, M. (2010) 'Treadmill training for patients with Parkinson's disease', *Cochrane Database of Systematic Reviews*.

Menz, H.B., Latt, M.D., Tiedemann, A., Mun San Kwan, M. and Lord, S.R. (2004) 'Reliability of the GAITRite® walkway system for the quantification of temporo-spatial parameters of gait in young and older people', *Gait & Posture*, 20(1), pp. 20-25.

Merlini, L., Bertini, E., Minetti, C., Mongini, T., Morandi, L., Angelini, C. and Vita, G. (2004) 'Motor function–muscle strength relationship in spinal muscular atrophy', *Muscle & Nerve*, 29(4), pp. 548-552.

Mirelman, A., Gurevich, T., Giladi, N., Bar-Shira, A., Orr-Urtreger, A. and Hausdorff, J.M. (2011) 'Gait alterations in healthy carriers of the LRRK2 G2019S mutation', *Annals of Neurology*, 69(1), pp. 193-197.

Mitchell, C. (2006) 'Pedestrian Mobility and Safety: A Key to Independence for Older People', *Topics in Geriatric Rehabilitation*, 22(1), pp. 45-52.

MITOMAP (2009) *A human mitochondrial genome database* Available at: http://www.mitomap.org.

Montes, J., McDermott, M.P., Martens, W.B., Dunaway, S., Glanzman, A.M., Riley, S., Quigley, J., Montgomery, M.J., Sproule, D., Tawil, R., Chung, W.K., Darras, B.T., De Vivo, D.C., Kaufmann, P., Finkel, R.S., For the Muscle Study, G. and the Pediatric Neuromuscular Clinical Research, N. (2010) 'Six-Minute Walk Test demonstrates motor fatigue in spinal muscular atrophy', *Neurology*, 74(10), pp. 833-838.

Moore, J.L., Roth, E.J., Killian, C. and Hornby, T.G. (2010) 'Locomotor Training Improves Daily Stepping Activity and Gait Efficiency in Individuals Poststroke Who Have Reached a “Plateau” in Recovery', *Stroke*, 41(1), pp. 129-135.

Moore, S.A., Hallsworth, K., Bluck, L.J.C., Ford, G.A., Rochester, L. and Trenell, M.I. (2012) 'Measuring Energy Expenditure After Stroke: Validation of a Portable Device', *Stroke*, 43(6), pp. 1660-1662.

Moraes, C.T., Sciacco, M., Ricci, E., Tengan, C.H., Hao, H., Bonilla, E., Schon, E.A. and DiMauro, S. (1995) 'Phenotype–genotype correlations in skeletal muscle of patients with mtDNA deletions', *Muscle & Nerve*, 18(S14), pp. S150-S153.

Morris, M., Iansek, R., Matyas, T. and Summers, J. (1998) 'Abnormalities in the stride length-cadence relation in parkinsonian gait', *Movement Disorders*, 13(1), pp. 61-69.

Morris, M.G., Dawes, H., Howells, K., Scott, O.M. and Cramp, M. (2008) 'Relationships Between Muscle Fatigue Characteristics and Markers of Endurance Performance', *Journal of Sports Science & Medicine*, 7(4), pp. 431-436.

Morton, S.M. and Bastian, A.J. (2004) 'Cerebellar Control of Balance and Locomotion', *The Neuroscientist*, 10(3), pp. 247-259.

Murphy, J.L., Blakely, E.L., Schaefer, A.M., He, L., Wyrick, P., Haller, R.G., Taylor, R.W., Turnbull, D.M. and Taivassalo, T. (2008) 'Resistance training in patients with single, large-scale deletions of mitochondrial DNA', *Brain*, 131(11), pp. 2832-2840.

Murphy, J.L., Ratnaike, T.E., Shang, E., Falkous, G., Blakely, E.L., Alston, C.L., Taivassalo, T., Haller, R.G., Taylor, R.W. and Turnbull, D.M. (2012) 'Cytochrome c oxidase-intermediate fibres: Importance in understanding the pathogenesis and treatment of mitochondrial myopathy', *Neuromuscular Disorders*, 22(8), pp. 690-698.

Nesbitt, V., Pitceathly, R.D.S., Turnbull, D.M., Taylor, R.W., Sweeney, M.G., Mudanohwo, E.E., Rahman, S., Hanna, M.G. and McFarland, R. (2013) 'The UK MRC Mitochondrial Disease Patient Cohort Study: clinical phenotypes associated with the m.3243A>G mutation—implications for diagnosis and management', *Journal of Neurology, Neurosurgery & Psychiatry*, 84(8), pp. 936-938.

Newman, M.A., Dawes, H., van den Berg, M., Wade, D.T., Burridge, J. and Izadi, H. (2007) 'Can aerobic treadmill training reduce the effort of walking and fatigue in people with multiple sclerosis: a pilot study', *Multiple Sclerosis*, 13(1), pp. 113-119.

Nitz, J., Burns, Y. and Jackson, R. (1997) 'Sit-to-stand and Walking Ability in Patients with Neuromuscular Conditions', *Physiotherapy*, 83(5), pp. 223-227.

Nóbrega, A.C., Williamson, J.W., Friedman, D.B., Araújo, C.G. and Mitchell, J.H. (1994) 'Cardiovascular responses to active and passive cycling movements.', *Med Sci Sports Exerc.*, Jun 6(26), pp. 709-14.

Nutt, J.G., Horak, F.B. and Bloem, B.R. (2011) 'Milestones in gait, balance, and falling', *Movement Disorders*, 26(6), pp. 1166-1174.

Ørngreen, M.C., Olsen, D.B. and Vissing, J. (2005) 'Aerobic training in patients with myotonic dystrophy type 1', *Annals of Neurology*, 57(5), pp. 754-757.

Orsucci, D., Calsolaro, V., Siciliano, G. and Mancuso, M. (2012) 'Quality of Life in Adult Patients with Mitochondrial Myopathy', *Neuroepidemiology*, 38(3), pp. 194-195.

Ostir, G.V., Kuo, Y.F., Berges, I.M., Markides, K.S. and Ottenbacher, K.J. (2007) 'Measures of Lower Body Function and Risk of Mortality over 7 Years of Follow-up', *American Journal of Epidemiology*, 166(5), pp. 599-605.

Paterson, K.L., Lythgo, N.D. and Hill, K.D. (2009) 'Gait variability in younger and older adult women is altered by overground walking protocol', *Age and Ageing*, 38(6), pp. 745-748.

Petajan, J.H., Gappmaier, E., White, A.T., Spencer, M.K., Mino, L. and Hicks, R.W. (1996) 'Impact of aerobic training on fitness and quality of life in multiple sclerosis', *Ann Neurol*, 39, pp. 432 - 441.

Pette, D. and Staron, R. (2001) 'Transitions of muscle fiber phenotypic profiles', *Histochemistry and Cell Biology*, 115(5), pp. 359-372.

Pfeffer, G. and Chinnery, P. (2013) 'Diagnosis and treatment of mitochondrial myopathies', *Annals of Medicine*, 45(1), pp. 4-16.

Pfeffer, G., Majamaa, K., Turnbull, D., Thorburn, D. and Chinnery, P. (2012) 'Treatment for mitochondrial disorders (Review)', *Cochrane Library*

Phillips, M., Flemming, N. and Tsintzas, K. (2009) 'An exploratory study of physical activity and perceived barriers to exercise in ambulant people with neuromuscular disease compared with unaffected controls', *Clinical Rehabilitation*, 23(8), pp. 746-755.

Picard, M., Taivassalo, T., Gouspillou, G. and Hepple, R.T. (2011) 'Mitochondria: isolation, structure and function', *The Journal of Physiology*, 589(18), pp. 4413-4421.

Pilar, C., Juan, B., Teodoro, M., Rafael, V., Luis, J., Francisco, O., Yolanda, C., Hildegard, S. and Joaquín, A. (2005) 'Exercise training in mitochondrial myopathy: A randomized controlled trial', *Muscle & Nerve*, 32(3), pp. 342-350.

Podsiadlo D, S., R. and . (1991) 'The timed "Up & Go": a test of basic functional mobility for frail elderly persons', *J Am Geriatr Soc.* , Feb;.(39(2):), pp. 142-8.

Potter, J.M., Evans, A.L. and Duncan, G. (1995) 'Gait speed and activities of daily living function in geriatric patients', *Archives of Physical Medicine and Rehabilitation*, 76(11), pp. 997-999.

Powell, K.E., Paluch, A.E. and Blair, S.N. (2011) 'Physical Activity for Health: What Kind? How Much? How Intense? On Top of What?', *Annual Review of Public Health*, 32(1), pp. 349-365.

Quaney, B.M., Boyd, L.A., McDowd, J.M., Zahner, L.H., Jianghua, H., Mayo, M.S. and Macko, R.F. (2009) 'Aerobic Exercise Improves Cognition and Motor Function Poststroke', *Neurorehabilitation and Neural Repair*, 23(9), pp. 879-885.

Rabadi, M.H. and Blau, A. (2005) 'Admission Ambulation Velocity Predicts Length of Stay and Discharge Disposition Following Stroke in an Acute Rehabilitation Hospital', *Neurorehabilitation and Neural Repair*, 19(1), pp. 20-26.

Rahman, S. and Hanna, M.G. (2009) 'Diagnosis and therapy in neuromuscular disorders: diagnosis and new treatments in mitochondrial diseases', *Journal of Neurology, Neurosurgery & Psychiatry*, 80(9), pp. 943-953.

Rampello, A., Franceschini, M., Piepoli, M., Antenucci, R., Lenti, G., Olivieri, D. and Chetta, A. (2007) 'Effect of Aerobic Training on Walking Capacity and Maximal Exercise Tolerance in Patients With Multiple Sclerosis: A Randomized Crossover Controlled Study', *Physical Therapy*, 87(5), pp. 545-555.

Reed, R., Den Hartog, R., Yochum, K., Pearlmutter, L., Ruttinger, A. and Mooradian, A. (1993) 'A comparison of hand-held isometric strength measurement with isokinetic muscle strength measurement in the elderly', *J Am Geriatr Soc.* , Jan(41(1)), pp. 53-6.

Reisman, D.S., McLean, H., Keller, J., Danks, K.A. and Bastian, A.J. (2013) 'Repeated Split-Belt Treadmill Training Improves Poststroke Step Length Asymmetry', *Neurorehabilitation and Neural Repair*, 27(5), pp. 460-468.

Remes, A.M., Kärppä, M., Rusanen, H., Majamaa, K., Hassinen, I.E., Moilanen, J.S., Uimonen, S., Sorri, M., Salmela, P.I. and Karvonen, S.L. (2003) 'Epidemiology of the mitochondrial DNA 8344A>G mutation for the myoclonus epilepsy and ragged red fibres (MERRF) syndrome', *Journal of Neurology, Neurosurgery & Psychiatry*, 74(8), pp. 1158-1159.

Rice, W.R. (1989) 'Analyzing Tables of Statistical Tests', *Evolution*, 43(1), pp. 223-225.

Ries, J.D., Echternach, J.L., Nof, L. and Gagnon Blodgett, M. (2009) 'Test-Retest Reliability and Minimal Detectable Change Scores for the Timed “Up & Go” Test, the Six-Minute Walk Test, and Gait Speed in People With Alzheimer Disease', *Physical Therapy*, 89(6), pp. 569-579.

Rochester, L., Galna, B., Lord, S. and Burn, D. (2014a) 'The nature of dual-task interference during gait in incident Parkinson’s disease', *Neuroscience*, 265, pp. 83-94.

Rochester, L., Galna, B., Lord, S., Mhiripiri, D., Eglon, G. and Chinnery, P.F. (2014b) 'Gait impairment precedes clinical symptoms in spinocerebellar ataxia type 6', *Movement Disorders*, 29(2), pp. 252-255.

Rochester, L., Hetherington, V., Jones, D., Nieuwboer, A., Willems, A.-M., Kwakkel, G. and Van Wegen, E. (2004) 'Attending to the task: Interference effects of functional tasks on walking in Parkinson’s disease and the roles of cognition, depression, fatigue, and balance', *Archives of Physical Medicine and Rehabilitation*, 85(10), pp. 1578-1585.

Rochester, L., Yarnall, A.J., Baker, M.R., David, R.V., Lord, S., Galna, B. and Burn, D.J. (2012) 'Cholinergic dysfunction contributes to gait disturbance in early Parkinson's disease', *Brain*, 135(9), pp. 2779-2788.

Ruiz, J.R., Sui, X., Lobelo, F., Morrow, J.R., Jackson, A.W., Sjöström, M. and Blair, S.N. (2008) 'Association between muscular strength and mortality in men: prospective cohort study', *Bmj*, 337.

Sabapathy, N.M., Minahan, C.L., Turner, G.T. and Broadley, S.A. (2011) 'Comparing endurance- and resistance-exercise training in people with multiple sclerosis: a randomized pilot study', *Clinical Rehabilitation*, 25(1), pp. 14-24.

Saltin, B. and Gollnick, P. (1983) *Skeletal muscle adadptability; significance for metabolism and performance*. Oxfoed University Press.

Sapega, A.A. (1990) 'Muscle performance evaluation in orthopaedic practice', *J Bone Joint Surg Am*, 72(10), pp. 1562-1574.

Sardinha, L.B., Lohman, T.G., Teixeira, P.J., Guedes, D.P. and Going, S.B. (1998) 'Comparison of air displacement plethysmography with dual-energy X-ray absorptiometry and 3 field methods for estimating body composition in middle-aged men', *The American Journal of Clinical Nutrition*, 68(4), pp. 786-93.

Schaefer, A.M., McFarland, R., Blakely, E.L., He, L., Whittaker, R.G., Taylor, R.W., Chinnery, P.F. and Turnbull, D.M. (2008) 'Prevalence of mitochondrial DNA disease in adults', *Annals of Neurology*, 63(1), pp. 35-39.

Schaefer, A.M., Phoenix, C., Elson, J.L., McFarland, R., Chinnery, P.F. and Turnbull, D.M. (2006) 'Mitochondrial disease in adults: A scale to monitor progression and treatment', *Neurology*, 66(12), pp. 1932-1934.

Schepens, S., Goldberg, A. and Wallace, M. (2010) 'The short version of the Activities-specific Balance Confidence (ABC) scale: Its validity, reliability, and relationship to balance impairment and falls in older adults', *Arch Gerontol Geriatr. 2010* 51(1), pp. 9–12.

Schmid, A., Duncan, P.W., Studenski, S., Lai, S.M., Richards, L., Perera, S. and Wu, S.S. (2007) 'Improvements in Speed-Based Gait Classifications Are Meaningful', *Stroke*, 38(7), pp. 2096-2100.

Schniepp, R., Wuehr, M., Neuhaeusser, M., Kamenova, M., Dimitriadis, K., Klopstock, T., Strupp, M., Brandt, T. and Jahn, K. (2012) 'Locomotion speed determines gait variability in cerebellar ataxia and vestibular failure', *Movement Disorders*, 27(1), pp. 125-131.

Scholtes, V., Dallmeijer, A., Rameckers, E., Verschuren, O., Tempelaars, E., Hensen, M. and Becher, J. (2008) 'Lower limb strength training in children with cerebral palsy - a randomized controlled trial protocol for functional strength training based on progressive resistance exercise principles', *BMC Pediatrics*, 8(1), p. 41.

Schulz, B.W., Ashton-Miller, J.A. and Alexander, N.B. (2007) 'Maximum Step Length: Relationships to age and knee and hip extensor capacities', *Clinical Biomechanics*, 22(6), pp. 689-696.

Scott, O.M., Hyde, S.A., Goddard, C. and Dubowitz, V. (1982) 'Quantitation of muscle function in children: A prospective study in duchenne muscular dystrophy', *Muscle & Nerve*, 5(4), pp. 291-301.

Serrao, M., Pierelli, F., Ranavolo, A., Draicchio, F., Conte, C., Don, R., Fabio, R., LeRose, M., Padua, L., Sandrini, G. and Casali, C. (2012) 'Gait Pattern in Inherited Cerebellar Ataxias', *The Cerebellum*, 11(1), pp. 194-211.

Shanske, S., Pancrudo, J., Kaufmann, P., Engelstad, K., Jhung, S., Lu, J., Naini, A., DiMauro, S. and De Vivo, D.C. (2004) 'Varying loads of the mitochondrial DNA A3243G mutation in different tissues: Implications for diagnosis', *American Journal of Medical Genetics Part A*, 130A(2), pp. 134-137.

Shephard, R.J., Rankinen, T. and Bouchard, C. (2004) 'Test–retest errors and the apparent heterogeneity of training response', *European Journal of Applied Physiology*, 91(2-3), pp. 199-203.

Sheridan, P.L., Solomont, J., Kowall, N. and Hausdorff, J.M. (2003) 'Influence of Executive Function on Locomotor Function: Divided Attention Increases Gait Variability in Alzheimer's Disease', *Journal of the American Geriatrics Society*, 51(11), pp. 1633-1637.

Shumway-Cook, A., Brauer, S. and Woollacott, M. (2000) 'Predicting the Probability for Falls in Community-Dwelling Older Adults Using the Timed Up & Go Test', *Physical Therapy*, 80(9), pp. 896-903.

Silvestri G, Ciafaloni E, Santorelli FM, Shanske S, Servidei S, Graf WD, Sumi M and S., D. (1993) 'Clinical features associated with the A-->G transition at nucleotide 8344 of mtDNA (''MERRF mutation'')', *Neurology*, 43((6)Jun:.), pp. 1200-6.

Singh, S.J., Morgan, M.D., Scott, S., Walters, D. and Hardman, A.E. (1992) 'Development of a shuttle walking test of disability in patients with chronic airways obstruction', *Thorax*, 47(12), pp. 1019-1024.

Smulders, K., Esselink, R.J., Weiss, A., Kessels, R.C., Geurts, A.H. and Bloem, B. (2012) 'Assessment of dual tasking has no clinical value for fall prediction in Parkinson's disease', *Journal of Neurology*, 259(9), pp. 1840-1847.

Snook, E.M. and Motl, R.W. (2009) 'Effect of Exercise Training on Walking Mobility in Multiple Sclerosis: A Meta-Analysis', *Neurorehabilitation and Neural Repair*, 23(2), pp. 108-116.

Society, A.T. (2002) 'Guidelines for the Six-Minute Walk Test', *Am. J. Respir. Crit. Care Med.*, 166(1), pp. 111-117.

Spencer, R.M.C., Zelaznik, H.N., Diedrichsen, J. and Ivry, R.B. (2003) 'Disrupted Timing of Discontinuous But Not Continuous Movements by Cerebellar Lesions', *Science*, 300(5624), pp. 1437-1439.

St-Onge, M., Mignault, D., Allison, D.B. and Rabasa-Lhoret, R. (2007) 'Evaluation of a portable device to measure daily energy expenditure in free-living adults', *The American Journal of Clinical Nutrition*, 85(3), pp. 742-749.

Steffen, T.M., Hacker, T.A. and Mollinger, L. (2002) 'Age- and Gender-Related Test Performance in Community-Dwelling Elderly People: Six-Minute Walk Test, Berg Balance Scale, Timed Up & Go Test, and Gait Speeds', *Physical Therapy*, 82(2), pp. 128-137.

Stewart, J.M., Xu, X., Ochoa, M. and Hintze, T.H. (1998) 'Exercise reduces epicardial coronary artery wall stiffness: roles of cGMP and cAMP', *Medicine and science in sports and exercise*, 30(2), pp. 220-228.

Stockton, K., Mengersen, K., Paratz, J., Kandiah, D. and Bennell, K. (2011) 'Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis', *Osteoporosis International*, 22(3), pp. 859-871.

Stolze, H., Kuhtz-Buschbeck, J.P., Drücke, H., Jöhnk, K., Illert, M. and Deuschl, G. (2001) 'Comparative analysis of the gait disorder of normal pressure hydrocephalus and Parkinson's disease', *Journal of Neurology, Neurosurgery & Psychiatry*, 70(3), pp. 289-297.

Stringer, W.W., Hansen, J.E. and Wasserman, K. (1997) 'Cardiac output estimated noninvasively from oxygen uptake during exercise', *Journal of Applied Physiology*, 82(3), pp. 908-912.

Stryer, L. (1995.) *Biochemistry* 4th ed. New York : edn. W.H. Freeman, .

Studenski, S., Perera, S., Patel, K. and et al. (2011) 'Gait speed and survival in older adults', *JAMA*, 305(1), pp. 50-58.

Studenski, S., Perera, S., Wallace, D., Chandler, J.M., Duncan, P.W., Rooney, E., Fox, M. and Guralnik, J.M. (2003) 'Physical Performance Measures in the Clinical Setting', *Journal of the American Geriatrics Society*, 51(3), pp. 314-322.

Stump, C.S., Henriksen, E.J., Wei, Y. and Sowers, J.R. (2006) 'The metabolic syndrome: Role of skeletal muscle metabolism', *Annals of Medicine*, 38(6), pp. 389-402.

Svedahl, K. and MacIntosh, B.R. (2003) 'Anaerobic Threshold: The Concept and Methods of Measurement', *Canadian Journal of Applied Physiology*, 28(2), pp. 299-323.

Taivassalo, T., De Stefano, N., Argov, Z., Matthews, P.M., Chen, J., Genge, A., Karpati, G. and Arnold, D.L. (1998) 'Effects of aerobic training in patients with mitochondrial myopathies.', *Neurology.*, Apr;50(4), pp. 1055-60.

Taivassalo, T., De Stefano, N., Chen, J., Karpati, G., Arnold, D.L. and Argov, Z. (1999a) 'Short-term aerobic training response in chronic myopathies', *Muscle & Nerve*, 22(9), pp. 1239-1243.

Taivassalo, T., Dysgaard Jensen, T., Kennaway, N., DiMauro, S., Vissing, J. and Haller, R.G. (2003) 'The spectrum of exercise tolerance in mitochondrial myopathies: a study of 40 patients', *Brain*, 126(2), pp. 413-423.

Taivassalo, T., Fu, K., Johns, T., Arnold, D., Karpati, G. and Shoubridge, E.A. (1999b) 'Gene Shifting: A Novel Therapy for Mitochondrial Myopathy', *Human Molecular Genetics*, 8(6), pp. 1047-1052.

Taivassalo, T., Gardner, J.L., Taylor, R.W., Schaefer, A.M., Newman, J., Barron, M.J., Haller, R.G. and Turnbull, D.M. (2006) 'Endurance training and detraining in mitochondrial myopathies due to single large-scale mtDNA deletions', *Brain*, 129(12), pp. 3391-3401.

Taivassalo, T. and Haller, R.G. (2004) 'Implications of exercise training in mtDNA defects--use it or lose it?', *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1659(2-3), pp. 221-231.

Taivassalo, T., Matthews, P., De Stefano, N., Sripathi, N., Genge, A., Karpati, G. and Arnold, D. (1996) 'Combined aerobic training and dichloroacetate improve exercise capacity and indices of aerobic metabolism in muscle cytochrome oxidase deficiency.', *Neurology*, Aug;47(2), pp. 529-34.

Taivassalo, T., Shoubridge, E.A., Chen, J., Kennaway, N.G., Dimauro, S., Arnold, D.L. and Haller, R.G. (2001) 'Aerobic conditioning in patients with mitochondrial myopathies: Physiological, biochemical, and genetic effects', *Annals of Neurology*, 50(2), pp. 133-141.

Takeuchi, Y., Katsuno, M., Banno, H., Suzuki, K., Kawashima, M., Atsuta, N., Ito, M., Watanabe, H., Tanaka, F. and Sobue, G. (2008) 'Walking capacity evaluated by the 6-minute walk test in spinal and bulbar muscular atrophy', *Muscle & Nerve*, 38(2), pp. 964-971.

Tarnopolsky, M. (2004) 'Exercise testing as a diagnostic entity in mitochondrial myopathies', *Mitochondrion*, 4(5-6), pp. 529-542.

Tarnopolsky, M.A. (2014) 'Exercise as a Therapeutic Strategy for Primary Mitochondrial Cytopathies', *Journal of Child Neurology*.

Taylor, R.W. and Turnbull, D.M. (2005) 'Mitochondrial DNA mutations in human disease', *Nat Rev Genet*, 6(5), pp. 389-402.

Tiedemann, A., Sherrington, C., Close, J.C.T. and Lord, S.R. (2011) 'Exercise and Sports Science Australia Position Statement on exercise and falls prevention in older people', *Journal of Science and Medicine in Sport*, 14(6), pp. 489-495.

Tiffreau, V., Ledoux, I., Eymard, B., Thévenon, A. and Hogrel, J.-Y. (2007) 'Isokinetic muscle testing for weak patients suffering from neuromuscular disorders: A reliability study', *Neuromuscular Disorders*, 17(7), pp. 524-531.

Tiivel, T., Kadaya, L., Kuznetsov, A., Käämbre, T., Peet, N., Sikk, P., Braun, U., Ventura-Clapier, R., Saks, V. and Seppet, E.K. (2000) 'Developmental changes in regulation of mitochondrial respiration by ADP and creatine in rat heart in vivo', *Molecular and Cellular Biochemistry*, 208(1), pp. 119-128.

Tillerson, J.L., Caudle, W.M., Reverón, M.E. and Miller, G.W. (2003) 'Exercise induces behavioral recovery and attenuates neurochemical deficits in rodent models of Parkinson's disease', *Neuroscience*, 119(3), pp. 899-911.

Toro, B., Nester, C.J. and Farren, P.C. (2003) 'The status of gait assessment among physiotherapists in the United Kingdom', *Archives of Physical Medicine and Rehabilitation*, 84(12), pp. 1878-1884.

Trenell, M., Sue, C., Kemp, G., Sachinwalla, T. and Thompson, C. (2006) 'Aerobic exercise and muscle metabolism in patients with mitochondrial myopathy', *Muscle & Nerve*, 33(4), pp. 524-531.

Tudor-Locke, C., Washington, T.L. and Hart, T.L. (2009) 'Expected values for steps/day in special populations', *Preventive Medicine*, 49(1), pp. 3-11.

Tuppen, H.A.L., Blakely, E.L., Turnbull, D.M. and Taylor, R.W. (2010) 'Mitochondrial DNA mutations and human disease', *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1797(2), pp. 113-128.

Turnbull, H.E., Lax, N.Z., Diodato, D., Ansorge, O. and Turnbull, D.M. (2010) 'The mitochondrial brain: From mitochondrial genome to neurodegeneration', *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1802(1), pp. 111-121.

Tyson, S. and Connell, L. (2009) 'The psychometric properties and clinical utility of measures of walking and mobility in neurological conditions: a systematic review', *Clinical Rehabilitation*, 23(11), pp. 1018-1033.

van der Krogt, M.M., Delp, S.L. and Schwartz, M.H. (2012) 'How robust is human gait to muscle weakness?', *Gait & Posture*, 36(1), pp. 113-119.

van Eijkeren, F.J.M., Reijmers, R.S.J., Kleinveld, M.J., Minten, A., Bruggen, J.P.t. and Bloem, B.R. (2008) 'Nordic walking improves mobility in Parkinson's disease', *Movement Disorders*, 23(15), pp. 2239-2243.

van Gelder, B.M., Tijhuis, M.A.R., Kalmijn, S., Giampaoli, S., Nissinen, A. and Kromhout, D. (2004) 'Physical activity in relation to cognitive decline in elderly men: The FINE Study', *Neurology*, 63(12), pp. 2316-2321.

Vandervelde, L., Van den Bergh, P.Y.K., Renders, A., Goemans, N. and Thonnard, J.L. (2009) 'Relationships between motor impairments and activity limitations in patients with neuromuscular disorders', *Journal of Neurology, Neurosurgery & Psychiatry*, 80(3), pp. 326-332.

VanSwearingen, J.M., Perera, S., Brach, J.S., Wert, D. and Studenski, S.A. (2011) 'Impact of Exercise to Improve Gait Efficiency on Activity and Participation in Older Adults With Mobility Limitations: A Randomized Controlled Trial', *Physical Therapy*, 91(12), pp. 1740-1751.

Verghese, J., Holtzer, R., Lipton, R.B. and Wang, C. (2009) 'Quantitative Gait Markers and Incident Fall Risk in Older Adults', *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 64A(8), pp. 896-901.

Verghese, J., Holtzer, R., Lipton, R.B. and Wang, C. (2012) 'Mobility Stress Test Approach to Predicting Frailty, Disability, and Mortality in High-Functioning Older Adults', *Journal of the American Geriatrics Society*, 60(10), pp. 1901-1905.

Verghese, J., Robbins, M., Holtzer, R., Zimmerman, M., Wang, C., Xue, X. and Lipton, R.B. (2008) 'Gait Dysfunction in Mild Cognitive Impairment Syndromes', *Journal of the American Geriatrics Society*, 56(7), pp. 1244-1251.

Verghese, J., Wang, C., Lipton, R.B., Holtzer, R. and Xue, X. (2007) 'Quantitative gait dysfunction and risk of cognitive decline and dementia', *Journal of Neurology, Neurosurgery & Psychiatry*, 78(9), pp. 929-935.

Voet, N., van der Kooi, E., Riphagen, I., Lindeman, E., van Engelen, B. and ACH., G. (2010) ' Strength training and aerobic exercise training for muscle disease. ', *Cochrane Database of Systematic Reviews. Art. No.:* , (1).

Voet NBM, v.d.K.E., Riphagen II, Lindeman E, van Engelen BGM, Geurts ACH. (2013) 'Strength training and aerobic exercise training for muscle disease.', *Cochrane Database of Systematic Reviews* (7).

Voss, M.W., Prakash, R.S., Erickson, K.I., Basak, C., Chaddock, L., Kim, J.S., Alves, H., Heo, S., Szabo, A.N., White, S.M., Wójcicki, T.R., Mailey, E.L., Gothe, N., Olson, E.A., McAuley, E. and Kramer, A.F. (2010) 'Plasticity of Brain Networks in a Randomized Intervention Trial of Exercise Training in Older Adults', *Frontiers in Aging Neuroscience*, 2, p. 32.

Wade, D. (1992) *Measurement in Neurological Rehabilitation.* . Oxford: Oxford University Press.

Wade, D. (2003) 'Outcome measures for clinical rehabilitation trials: impairment, function, quality of life, or value?', *Am J Phys Med Rehabil*, Oct(82), pp. 26-31.

Wallace, D.C. (1992) 'Diseases of the Mitochondrial DNA', *Annual Review of Biochemistry*, 61(1), pp. 1175-1212.

Walsh Riddle, M. and Blumenthal, J.A. (1989) 'Cardiovascular responses during upright and semi-recumbent cycle ergometry testing.', *Med Sci Sports Exerc.*, Oct(21), pp. 581-5.

Ware, J.E., Jr., Kosinski, M. and Keller, S.D. (1996) 'A 12-Item Short-Form Health Survey: Construction of Scales and Preliminary Tests of Reliability and Validity', *Medical Care*, 34(3), pp. 220-233.

Watkins, M.P., Harris, B.A. and Kozlowski, B.A. (1984) 'Isokinetic Testing in Patients with Hemiparesis: A Pilot Study', *Physical Therapy*, 64(2), pp. 184-189.

Wenger, H. and Bell, G. (1986) 'The Interactions of Intensity, Frequency and Duration of Exercise Training in Altering Cardiorespiratory Fitness', *Sports Medicine*, 3(5), pp. 346-356.

Wert, D.M., Brach, J., Perera, S. and VanSwearingen, J.M. (2010) 'Gait Biomechanics, Spatial and Temporal Characteristics, and the Energy Cost of Walking in Older Adults With Impaired Mobility', *Physical Therapy*, 90(7), pp. 977-985.

Weuve, J., Kang, J.H., Manson, J.E., Breteler, M.M.B., Ware, J.H. and Grodstein, F. (2004) 'Physical Activity, Including Walking, and Cognitive Function in Older Women', *JAMA*, 292(12), pp. 1454-1461.

Whitney, S.L., Wrisley, D.M., Marchetti, G.F., Gee, M.A., Redfern, M.S. and Furman, J.M. (2005) 'Clinical Measurement of Sit-to-Stand Performance in People With Balance Disorders: Validity of Data for the Five-Times-Sit-to-Stand Test', *Physical Therapy*, 85(10), pp. 1034-1045.

Whittaker, R.G., Blackwood, J.K., Alston, C.L., Blakely, E.L., Elson, J.L., McFarland, R., Chinnery, P.F., Turnbull, D.M. and Taylor, R.W. (2009) 'Urine Heteroplasmy Is the Best Predictor of Clinical Outcome in the m.3243A>G mtDNA Mutation', *Neurology*, 72(6), pp. 568-569.

Willis, T., Hollingsworth, K., Sveen, M., Morrow, J., Hanna, M.G., Vissing, J. and Straub, V. (2011) *Assessing muscle pathology by MRI in LGMD2I*. Fourth UK Neoromuscular Translational Research Conference. 2011.

Willis, T.A., Hollingsworth, K.G., Coombs, A., Sveen, M.-L., Andersen, S., Stojkovic, T., Eagle, M., Mayhew, A., de Sousa, P.L., Dewar, L., Morrow, J.M., Sinclair, C.D.J., Thornton, J.S., Bushby, K., Lochmüller, H., Hanna, M.G., Hogrel, J.-Y., Carlier, P.G., Vissing, J. and Straub, V. (2013) 'Quantitative Muscle MRI as an Assessment Tool for Monitoring Disease Progression in LGMD2I: A Multicentre Longitudinal Study', *PLoS One*, 8(8), p. e70993.

Wolfe, R.R. (2006) 'The underappreciated role of muscle in health and disease', *The American Journal of Clinical Nutrition*, 84(3), pp. 475-482.

Wong, L.-J.C. (2007) 'Diagnostic challenges of mitochondrial DNA disorders', *Mitochondrion*, 7(1-2), pp. 45-52.

World Health Organization (2001) 'International Classification of Functioning, Disability and Health (ICF)', [Online].

Wright, N.C., Kilmer, D.D., McCrory, M.A., Aitkens, S.G., Holcomb, B.J. and Bernauer, E.M. (1996) 'Aerobic walking in slowly progressive neuromuscular disease: Effect of a 12-week program', *Archives of Physical Medicine and Rehabilitation*, 77(1), pp. 64-69.

Yaffe K, Barnes D, Nevitt M, Lui L and K., C. (2001;) ' A Prospective Study of Physical Activity and Cognitive Decline in Elderly Women: Women Who Walk. ', *Arch Intern Med.*, 161(14), pp. 1703-1708. .

Yogev-Seligmann, G., Hausdorff, J.M. and Giladi, N. (2008) 'The role of executive function and attention in gait', *Movement Disorders*, 23(3), pp. 329-342.

Yogev, G., Giladi, N., Peretz, C., Springer, S., Simon, E.S. and Hausdorff, J.M. (2005) 'Dual tasking, gait rhythmicity, and Parkinson's disease: Which aspects

of gait are attention demanding?', *European Journal of Neuroscience*, 22(5), pp. 1248-1256.

Yoward, L.S., Doherty, P. and Boyes, C. (2008) 'A survey of outcome measurement of balance, walking and gait amongst physiotherapists working in neurology in the UK', *Physiotherapy*, 94(2), pp. 125-132.

Zeviani, M., Moraes, C.T., DiMauro, S., Nakase, H., Bonilla, E., Schon, E.A. and Rowland, L.P. (1988) 'Deletions of mitochondrial DNA in Kearns-Sayre syndrome', *Neurology*, 38(9), pp. 1339-1346.