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Muscle and Bone Health in Adult Males with Muscular Dystrophy

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ABSTRACT

Muscular Dystrophy (MD) results in muscle atrophy, loss of ambulation and reduced physical activity through chronic inflammation leading to muscle damage and derangement of muscle physiology. Reduced physical activity relating to muscle weakness and reduced muscle anatomical cross-sectional area (ASCA) due to atrophy lead to a reduction in tensile and compression strain required to maintain bone mineral density (BMD), resulting in adverse bone health. Studies have demonstrated reductions in BMD and muscle size, primarily in children with Duchenne Muscular Dystrophy (DMD) – a severe variant – however there is no extant data describing muscle size in adults with MD, and data describing BMD in adults with other variants of MD is sparse (e.g. Becker's (BeMD), Limb Girdle (LGMD), and Fascioscapluohumeral dystrophy (FSHD)). The aim of this study was to measure BMD, tibialis anterior (TA) muscle ASCA, grip strength and physical activity in adult male individuals with four distinct variants of MD compared to unaffected adult male controls. TA ASCA was measured via B-mode ultrasound; BMD was measured using speed-of-sound ultrasound of midshaft Tibia (MST) and distal Radius (DR). Grip strength was assessed through isometric dynamometer contraction; and physical activity through use of two questionnaires. 40 adult males with MD and 10 unaffected male controls were measured for demographic information, T- & Z-scores of BMD, TA ASCA, maximum grip strength and physical activity. BMD assessed by Z-score was lower in DMD individuals compared to all other participants in tibia and radius and lower in BeMD and FSHD individuals compared to CTRL ($P<0.05$). There was no difference in T or Z scores between CTRL and LGMD participants. TA ASCA was smaller in all MD individuals compared to controls (BeMD -39.7%; DMD -59.7%; FSHD -58.9%; LGMD -62.4%) ($P<0.05$). Physical activity was lower in DMD individuals compared with all other MD individuals (assessed by the Bone-specific Physical Activity Questionnaire) ($P<0.05$) and all MD groups demonstrated significant reductions in grip strength compared to control (BeMD -51.2%; FSHD -46.3%; LGMD -49.9%) ($P<0.05$). In conclusion, decreased TA ASCA was observed in all MD individuals and reduced T and Z scores observed in three of four groups compared to controls. MD individuals demonstrated reduced physical activity and grip strength compared to controls. Due to the link between BMD and physical activity it is recommended that efforts be made to encourage weight bearing activity and exercise in individuals with MD and that future research investigate the specific benefits of physical exercise in MD individuals to establish best practice for healthcare professionals.

Acknowledgements

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List of Abbreviations

ASCA – Anatomical Cross-sectional Area
BMD – Bone Mineral Density
BeMD – Becker Muscular Dystrophy
BPAQ - Bone-specific Physical Activity Questionnaire
CT - Computed Tomography
CTRL - Control
DMD – Duchenne Muscular Dystrophy
DR – Distal Radius
DXA - Dual-Energy X-ray Analysis
FSHD – Facioscapulohumeral Muscular Dystrophy
IL-6 - Interleukin-6
LGMD – Limb Girdle Muscular Dystrophy
MD – Muscular Dystrophy
MDC – Muscular Dystrophy Campaign
MST – Midshaft Tibia
NMC – Neuromuscular Centre
OP – Osteoporosis
PASIPD - Physical Activity Scale for Persons with Physical Disabilities
pQCT - Peripheral Quantitative Computed Tomography
SoS – Speed of Sound
TA – Tibialis Anterior
VEGF - Vascular Endothelial Growth Factor

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CHAPTER 1- INTRODUCTION AND LITERATURE REVIEW

Introduction

The Muscular Dystrophy Campaign (MDC) estimates that there are over 70,000 individuals in the UK affected by Muscular Dystrophy (MD) and related neuromuscular conditions (www.muscular-dystrophy.org). These conditions vary in severity but often lead to significant disability; loss of ambulation (Sussman, 2002), function and independence (Yamaguchi & Suzuki, 2013); and respiratory as well as cardiac complications (Ishikawa et al, 2011) in those affected with some severe variants which are life limiting. These individuals also pose a challenging issue in their medical and non-pharmacological management. Research is currently ongoing into genetic and pharmacological management however with an estimated 8,000 individuals affected by neuromuscular conditions in the North-West of England alone (Bell, 2010), there is a need for quantitative investigation into the non-pharmacological management of these individuals to maximise quality of life and independence. The subsequent review will address the extant literature for functional, skeletal and muscular impairments in a range of muscular dystrophies; these dystrophies represent the most severe form - Duchenne MD (representing a clinical condition resulting in loss of life by the third decade), to milder forms of the condition such as Facioscapulohumeral Muscular Dystrophy (FSHD), where individuals present later in life and can maintain ambulation throughout their lifespan.

Aetiology

There is well-established molecular and genetic data describing the biochemical defects in human muscle in individuals with MD. Hoffman et al (1987) described the mechanism of weakness and functional loss in Duchenne Muscular Dystrophy (DMD) – the most severe and disabling form – with preliminary work into exon deletion carried out by Monaco et al (1985). Similarly, biological data for Becker Muscular Dystrophy (BeMD) undertaken during the same decade indicated the two diseases share a common genetic origin – the defective coding of the muscular cytoskeleton membrane protein Dystrophin. The resultant absence or mutation of Dystrophin in human muscle leads to reduced cohesion of muscle fibres during whole muscle contraction and a subsequent 'shearing' effect within individual fibrils – the so-called 'membrane hypothesis' (Hutter, 1992). An absence of Dystrophin has more recently been demonstrated to cause aberrant regulation of membrane Nitric Oxide synthesis. Nitric Oxide is normally generated through membrane shear force and causes intramuscular vasodilation – improving blood supply and reducing ischaemic damage to contracting muscle tissue (Brenman et al, 1995). Over years of repeated contraction this manifests as a low-level inflammatory response, with biochemical markers of muscle degeneration (such as Plasma DNA Fragmentation) significantly elevated and markers of muscle regeneration (such as Vascular Endothelial Growth Factor – VEGF) significantly reduced *in vitro* compared to unaffected, age- and sex-matched controls (Abdel-Salam et al, 2009). Muscle tissue is subsequently destroyed; compromising function and force output (Villalta et al, 2014).

Pathophysiology of functional impairments

Muscle atrophy and weakness in DMD individuals result in a clinical presentation of proximal muscle weakness resulting in reducing ambulation through childhood and wheelchair dependency by mid-teens, upper limb and distal muscle weakness occur as the condition progresses with age. A three-year study of 6-minute walk tests and North Star Ambulatory Assessments (a DMD specific 26-point motor function scale) of 96 paediatric DMD individuals (5-16 years, mean: 8.82), compiled by a collaboration of European hospitals, revealed significantly reduced scores on both measures at every point of measurement (12, 24, 36 months) (Pane et al, 2014), demonstrating an accelerating decline in ambulatory function. Additionally, statistics compiled by the United States MDStar Net analysed in 2007 demonstrate a wheelchair dependence of DMD individuals concurrent with the ambulation figures described above of 29, 82 and 90% for 5-9, 10-14 and 15-24 yr olds, respectively (CDC, 2007).

Within DMD non-invasive ventilation support is usually required by the second decade – typically associated with a loss of ambulation and subsequent reduction in lung volume and respiratory muscle strength due to positioning and disuse (Bushby et al, 2010). Life expectancy, despite marked improvements owing to medical advances in the last two decades, is usually the third decade (Eagle et al 2002). Despite observations on clinical appearance there are no long-term studies of functional ambulatory performance in participants other than DMD individuals (Pane et al, 2014), despite well-established data indicating muscle weakness as a predictor of ambulatory function in other clinical populations.

Although respiratory and skeletal muscle impairments are reported in children with DMD and associated with an absence of dystrophin, other dystrophic conditions present. A mutated version of healthy dystrophin is a cardinal feature of Becker Muscular Dystrophy (BeMD) – with individuals experiencing reduced muscle strength, loss of ambulation and cardiac involvement due to similar pathological origins, however with a reduced severity in clinical presentation.

Cytoskeletal muscle protein defects are also responsible for proximal muscle weakness in Limb Girdle Muscular Dystrophy (LGMD). There are currently two gross types of LGMD with further subdivisions (LGMD Type 1A-H; LGMD Type 2A-Q) with each classification relating to a specific myoprotein genetic defect. For example, mutated Dysferlin – a protein involved in the regulation of muscle membrane repair – in LGMD Type 2B results in compromised sarcolemma regeneration in human muscle due to impaired calcium-mediated membrane repair and delayed myoblast fusion in response to muscle damage (Bushy and Laval, 2008). Subsequent accumulation of microtrauma and injury leads to reduced muscle tissue cohesion, (evidenced by fibre derangement and abnormality observed on biopsy) and a clinical presentation of weakness.

The pathogenesis of FSHD is less well understood. Research indicates that a defect on chromosome 4 – specifically a contraction of the D4Z4 subunit – was identified in all FSHD individuals (Lemmers et al, 2010). It is likely that this defect results in a mis- or overexpression of DUX4, a gene that when bound to specific human defensins inhibits muscle cell differentiation from stem cells, reduces innate immune response to virus invasion and encourages apoptotic cell death thereby leading to muscle atrophy and weakness.

Atrophy and strength in muscular dystrophy

Despite ongoing genetic, biomolecular and pharmacological research into these conditions there is a paucity of published evidence describing whole muscle structure and function in individuals affected by MD. Research undertaken in mice affected by a form of dystrophy (mdx) describes comparative muscle weakness when compared with unaffected controls as assessed by electrically-elicited isometric muscle contractions (Lynch et al, 2001). Lynch et al also describes an enlargement of certain limb muscles (particularly those involved in locomotion) compared to control mice. This 'pseudohypertrophy' (an apparent increase in tissue size without an increase in cell number), caused by infiltration of fibrous and adipose tissue into the muscle, secondary to an inflammatory response to microscopic muscle damage is described in mdx mice and is demonstrated in human DMD individuals in Fig 1.0 (Wang et al, 2011).

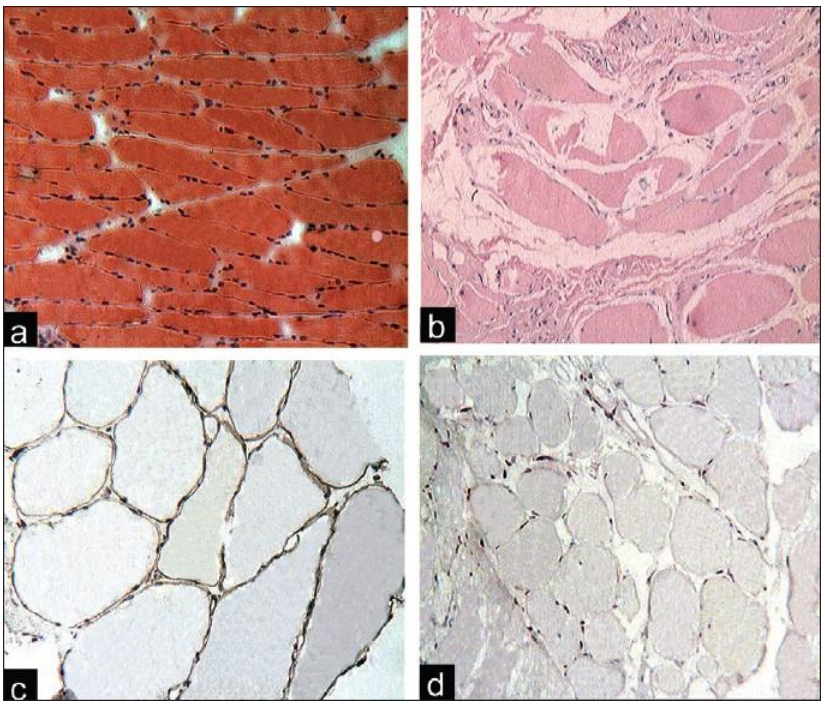

Fig 1.0 – A) Haemotoxylin and Eosin (HE) staining of control sample B) HE staining of 5-year-old DMD child C) Snap frozen quadriceps tissue of control sample D) Snap frozen quadriceps tissue of 5-year-old DMD child. B) and D) - Evident fibre disorganisation, variable fibre size, intramuscular adipose/fibrous tissue infiltration and evidence aggregated muscle fibres attempting regeneration (Wang et al, 2011).

In humans, a comprehensive descriptive study undertaken by Jones et al (1983) analysed the composition of calf and quadriceps musculature as assessed by Computed Tomography (CT) and in biopsy samples, in 12 boys (7-18 years) affected by DMD. The study identifies at length the constitution of dystrophic muscle tissue compared to 5 healthy (though non-age matched) controls. Similar to MDX mice (Dellorusso et al 2001), Jones et al conclude that muscle enlargement in DMD is the result of accumulations of intramuscular fat and connective tissue (identified as increased intramuscular shadowing on X-ray) in the presence of relatively normal amounts of muscle tissue (Fig 1.1). This pseudohypertrophy, is further confirmed by circumferential calf measurements in DMD compared to a non-dystrophic group of age-matched boys (Ichiyama 1991). However it is impossible to distinguish between the muscular and non-muscular content using circumferential measures.

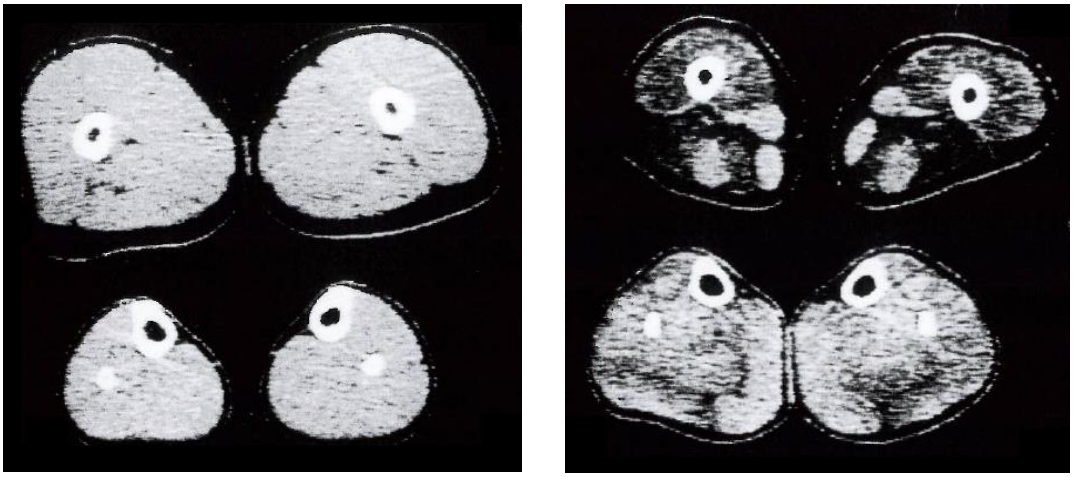

Fig 1.1 Quadriceps (top) and Calf CT scans of control (left) and DMD individuals. Dark areas of CT indicate areas of less dense adipose infiltrates in muscle tissue. Gross Cross-sectional Area (ASCA) of calves is increased in DMD in presence of reduced muscle mass (Jones et al, 1983).

Preceding the observation of dystrophic skeletal muscle tissue in mice carried out by Lynch (2001), De Bruin et al (1997) described diaphragm muscle thickness (as assessed by ultrasonography) compared to inspiratory strength (mouth pressure) in 10 human DMD participants (Aged 7-12 years, mean age 10.3years). Diaphragmatic thickness was significantly higher in DMD participants than Control (0.26mm, 14.9%) during diaphragm relaxation indicating a potential pseudohypertrophy similar to limb muscles. However, during inspiration the diaphragm of non-affected control groups became visibly larger, likely indicative of a stronger muscle contraction. There were markedly reduced inspiratory strength values in the participants with DMD. Although this study is limited by the sample size and single-pathology design, it nonetheless represents the only evidence to date describing a comparison between muscle size and strength in humans affected by muscular dystrophy.

All current published data of muscle structure in humans with MD has focussed on DMD children or adolescents as participants (Table 1.0). To date, there is has been no investigation

of muscle area or architecture in FSHD, BeMD or LGMD, or DMD participants at later stages of life. It is possible that this discrepancy arises from a predominance of paediatric medical services available to individuals with DMD. DMD individuals present with symptoms earlier, with more marked impact upon function and health, and as such may attend specialist clinic environments (where participant recruitment is more likely) more often than individuals with other forms of MD. Furthermore, the complexity of managing individuals with DMD compared to other, less severe, forms of MD, results in availability of DMD sample populations for study compared to conditions where individuals are more disparate or access health services less regularly.

Table 1.0 Summary of muscle size measurements in dystrophic populations (Both human & animal models)

Author	Dystrophy	Population	Methods	Results
Lynch et al 2001	Mdx Mice	6-, 17-, 24-, 28-month old mdx and control mice	EDL, soleus and gastrocnemius muscles (*in vitro*) measured as total muscle mass, cross-sectional area and force output	Muscle mass of mdx mice was larger throughout life (17-22%) relative to control Muscle power was lower in mdx mice (~20%) at every stage of life
Dellorusso et al 2001	Mdx Mice	2-19 month old mdx and control mice	Stretch of maximally activated TA muscle (40% strain relative to muscle fibre length) followed by isometric contraction	Force deficits in injured muscle in mdx mice 4-7 times more significant than control mice
Jones et al 1983	DMD	12 DMD boys (7-18 years) and 5 control	Computed CT and needle biopsy mid-thigh and calf	Gross Volume: DMD > Control (calf) DMD < Control (Quads) Fat-free mass DMD < Control (calf & quads)
Ichiyama 1991 (abstract)	DMD	7 DMD boys (3-5 years) & 165 controls (3-6 years)	Circumference tape measurement of thigh, knee calf and ankle	DMD > controls (calf circumference) DMD > controls (calf/knee ratio)
De Bruin et al 1997	DMD	10 DMD boys (7-12 years) 12 control boys (6-12 years)	B-mode U/S measurement of diaphragm thickness FEV1, FVC and PEF measured on spiro-bank	Resting thickness of diaphragm increased in DMD (by mean 0.26mm) Reduced forced expiratory pressure and volume in DMD and reduced muscle contraction

Physical activity and muscular dystrophy

Immobilisation or significant reductions in physical activity (such as those associated with physical disability and dependence upon powered mobility aids) are known to reduce muscle mass and strength (Creditor, 1993). A study of step activity in DMD boys through computerised activity monitors identified significant reductions in overall physical activity; both medium and high step-rate activity; and reduced maximum heart rates in 16 ambulatory DMD individuals compared to sex-matched, age-matched, able-bodied controls (McDonald et al, 2005). McDonald et al also demonstrated a significant correlation between overall step activity and knee extensor muscle strength in the DMD participants but not the able-bodied controls, indicating a possible causal link between muscle weakness and immobility. Bed rest studies indicate a loss of thigh muscle cross-sectional area of 14% following 6-weeks

enforced immobilisation when assessed by MRI (Berg et al, 1997), with immobilised muscle tissue characterised by substantial reductions in muscle protein synthesis (Wall et al, 2013), impaired oxygen absorption by muscle tissue and changes to connective tissue architecture (Williams & Goldspink, 1984). It is likely therefore that higher levels of sedentary behaviour, and/or immobilisation of lower limb muscle groups associated with frequent, long-term use of powered mobility aids in individuals affected by muscular dystrophy will contribute to differences in muscle mass between predominantly seated individuals and those remaining ambulant (allowing for marked variations between individuals of the same age and condition). To date there is no extant data from adult humans comparing muscle mass between dystrophic conditions, where the sarcopenic effect (i.e. muscle loss compared to healthy age-matched counterparts) may be expected to be worse than that seen in children and adolescents, owing to the duration of low habitual physical activity.

The role of the inflammatory response and muscle atrophy

Chronic, low-level inflammation generated from repeated, abnormal repair and structural damage in MD is known to have deleterious effects on muscle health. Elevated levels of the regulatory cytokine Interleukin-6 (IL-6) has been demonstrated in disease states such as Chronic Obstructive Pulmonary Disease (COPD) and following muscle damage from excessive exercise (Bruunsgaard, 2005), with IL-6 believed to play a central role in muscle metabolism, satellite cell proliferation and suppression of macrophage invasions (Haddad et al, 2005). Increased IL-6 levels are demonstrated alongside chronic intramuscular inflammation in mdx mice (Kostek et al, 2012) and it is proposed that this represents a biomarker of catabolic muscle metabolism.

In vitro study of biochemical markers of muscle regeneration and degeneration in a DMD population reported significant increases in biomarkers of muscle degeneration (such as Fas; FasL – associated with Tumour Necrotising Factor) and a simultaneous increase in some biomarkers of muscle regeneration in the presence of a decrease in others, as compared to normal blood biomarker levels in age- & sex-matched, unaffected controls. This indicates an environment of accelerated muscle degeneration and insufficient muscle regeneration in DMD individuals leading to overall tissue damage and disorganisation (Abdel-Salam et al, 2009).

Furthermore, frequent physical activity in geriatric populations has demonstrated a regulatory effect on levels of IL-6 (Jankord & Jemiolo, 2004), indicating an anti-inflammatory effect of physical activity on muscle tissue. A combination of chronic, low-level inflammation evidenced by elevation of intramuscular IL-6, deranged balance of muscle degeneration and regeneration markers and a lack of the protective effects of regular physical activity in individuals with MD provide a further mechanism to suggest that muscle atrophy should exist in adults with the condition. However, as previously mentioned, there is no published data from adult males with any form of muscular dystrophy compared to age-matched controls.

Skeletal health in muscular dystrophy

Inexorably linked to this descriptive evidence of muscular alterations in MD is its association with Bone Mineral Density (BMD) and the regulatory effect of significant and substantial tensile and compression forces on BMD maintenance. As described by Frost's 'mechanostat' theory and subsequent Utah Paradigm a minimum threshold of microstrain force must be achieved before bone tissue enters a homeostatic or anabolic metabolism (Frost, 2003). Forces below a minimum threshold do not promote regulatory osteocyte or constructive osteoblast activity. Frost further described how the intensity of the force experienced by the bone tissue correlated to the regulatory effect on BMD i.e. the more significant the force (within limitations of a stress-strain relationship) the more prominent the metabolic response in preserving BMD. Rittweger, in a festschrift review in 2008, identifies the critical link between BMD preservation and tensile strain through muscle force generation (particularly during adolescence where bone mass is most significantly accrued). Despite the supposed clarity of this linear model, Rittweger identified a further component of BMD regulation; Eventual adult joint size - governed by forces experienced in bone during childhood – directly limits peak force transmission in bone during adulthood. This therefore provides a caveat explaining the unpredictability in linear models describing the effect of interventions (such as bone loading exercise) and their impact in generating improvements in BMD as adult joint size appears to impose limitations on BMD gains through appropriate bone loading exercise.

Previous studies have demonstrated how immobility forms part of a complex of factors in BMD regulation (Zerwekh et al, 1998) and prolonged immobility secondary to skeletal muscle weakness will have a negative impact upon BMD. Additionally, recent medical advances in condition management have led to the routine prescription of long-term corticosteroid therapies in DMD adolescents. Individuals with DMD using frequent corticosteroidal anti-inflammatory therapy experience fewer skeletal deformities and prolonged ambulation relative to those untreated, however they also experience a significant increase in incidence of vertebral fractures; with an estimated incidence in one study of between 17-32% of corticosteroid treated individuals compared to no fractures in untreated individuals over a three year period (King et al, 2007). Long bone fracture rates have been reported to be similar between corticosteroid and non-corticosteroid users in DMD children (mean age 13.1 years). However in some of the literature (Houde et al, 2008) elevated levels of lower limb fractures in MD populations (9% DMD – 0% controls) has also been described (Vestergaard et al, 2001). It is worth noting that the populations in these studies differed in mean age significantly (13.1 years – Houde et al, 23.9 years – Vestergaard et al). Older DMD individuals are likely to experience more marked levels of physical disability; more significant impairments to ambulation and mobility and therefore may have more significant impairments of bone health compared to younger individuals who may remain ambulant or have lost ambulation more recently.

Fracture incidence was recorded by Larson & Henderson (2000) as part of demographic data collected during their study of BMD in DMD individuals. The authors identified that 44% (18 individuals) of the 41 adolescent participants scanned sustained a fracture, with 66% of these occurring in the lower limbs. BMD as assessed by Z-score (a relative score of BMD compared to the reference mean of an age-matched demographic) was reduced in DMD individuals most significantly in the proximal femur (mean z-score -1.6) - (despite ambulation) and additionally in the lumbar spine but only following loss of ambulation (mean z-score -1.7).

Confirming anticipation of compromised bone health in DMD individuals, Bianchi et al (2003) investigated cortical steroid impact on BMD in their study of 32 DMD boys (22 – steroid, aged 4-17years, mean age 9.9; 10 – non-steroid, aged 4-15 years, mean age 10) by Dual-Energy X-ray Absorptiometry (DXA). They identified reduced BMD z-scores at lumbar spine vertebral bodies in both groups; with further significant reductions demonstrated in the corticosteroid group compared to the non-corticosteroid group (mean t-score; steroid -4.0, non-steroid -3.5). Furthermore, Bianchi identified through blood analysis, marked reduction in hydroxyvitamin D levels and hypocalciuria – markers of calcium metabolism – indicating deranged bone turnover at the site investigated. Further specific assessment of 10 ambulatory individuals with DMD (mean age 8 years) was undertaken by Aparicio et al (2002) whereby substantial reductions in femoral and vertebral Z-scores (-4.32 and -3.47 respectively) were identified.

Whilst BMD is likely to be significantly reduced in DMD individuals due to factors such as enforced immobilisation and the degree of bone loading, medical factors such as corticosteroid use can further impact upon bone health. Soderpalm et al (2008), in addition to confirming Bianchi et al's (2003) biochemical data of bone biomarker derangement, completed DXA scans of whole-body (with/without head excluded), spine, hip and heel – identifying reduced Z-scores at all sites in 24 DMD boys (2-19 years), the majority of whom were undergoing prednisolone therapy. Reductions in all sites were confirmed with age after a 4-year follow-up.

Corticosteroid therapy has been demonstrated to contribute to significant reductions in BMD in the earlier work of Hawker et al (2005). This observation of impaired bone health was not affected by dependency on dose in subsequent studies by Crabtree et al (2010) and Houston et al (2014) who used BMD assessed by DXA as a primary outcome measure for dosage trials of corticosteroid therapy – all studies identified reductions in BMD at specific sites in Table 2.0. It is worth noting that Crabtree et al in their study adjusted recorded BMD Z-scores for lean body mass and height measurements. This post-hoc analysis was not performed by other authors and in doing so recorded BMD Z-scores did not achieve statistical significance compared to control data.

The only study currently to investigate BMD in adults with MD was undertaken by Philippe et al (2011). A total of 15 DMD individuals (mean age 27); 18 LGMD individuals (mean age 40); and 12 FSHD individuals (mean age 45) were scanned at the femoral neck and spine with results describing reduced T-scores (a relative score of BMD as compared to the mean score of a healthy, thirty-year-old reference adult) in all groups as assessed by DXA (Figure 1.2). Particular reductions were observed in those individuals who were non-ambulant compared to those who remained independently mobile in both anatomical sites. DMD individuals presented with the lowest relative BMD – the severity of their condition perhaps leading to earlier dependence upon wheelchair mobility aids and reducing overall lifetime loading of lower limb bones, whereas there was some preservation of BMD in the MD in which ambulation was possible (Table 2.0). Philippe et al represents the broadest investigation of BMD in individuals affected by a variety of MD. However their use of T-scores as an outcome measure is less age-appropriate than a Z-score and their omission of a distal lower limb scan site prevents completion of the data (it is estimated that up to 35% of DMD individuals experience a tibial fracture and 44% experience foot/ankle fractures (Vestergaard et al 2001).

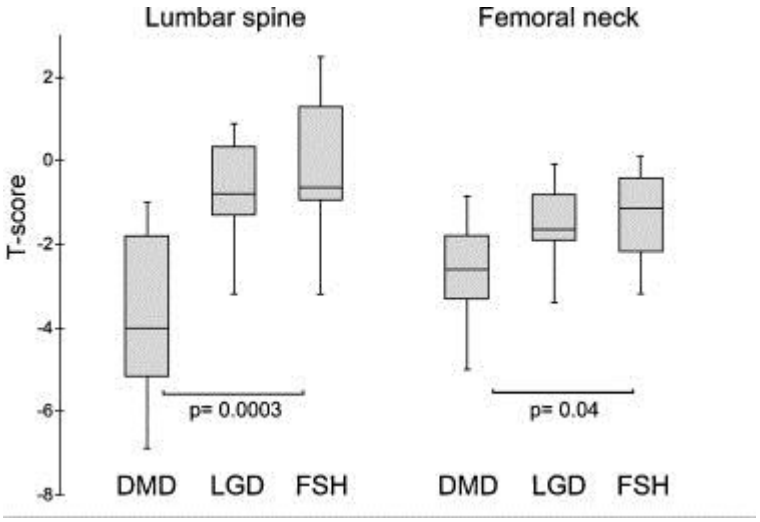

Fig 1.2 Relative T-scores of adult individuals affected by three types of dystrophy at femoral neck and lumbar spine highlighting particular reductions in individuals affected by DMD (Philippe et al 2011)

While literature investigating BMD in MD has been conducted more recently and by a larger number of studies than those describing muscle composition there is a near unanimous focus on DMD participants (particularly adolescent populations) and no study has yet investigated the correlation between muscle mass and BMD in any form of MD.

Table 2.0 Summary of Bone Density measures from individuals with MD.

Author	Dystrophy	Population	Methods	Results
Bianchi et al 2003	DMD	N = 32 Mean age = 10 years	DXA Lumbar spine vertebrae Biomarker blood test	Steroid z-score mean = - 4.0 Non-steroid z-score mean = - 3.5 Reduced markers of bone anabolism
Houston et al, 2014	DMD retrospective study	N = 39 Mean age at study start = 3.5 Mean age at study end = 18.6	DXA Lumbar Spine DXA femur	Steroid z-score mean = - 1.12 Non-steroid z-score mean = - 2.04 Steroid z-score mean = - 3.30 Non-steroid z-score mean = - 3.30
Hawker et al, 2005	DMD	N = 42 Mean age = 11 years	DXA Lumbar spine vertebrae	Z-score mean = -1.94
Crabtree et al, 2010	DMD	N = 25 5-12 years	DXA subcranial bone and Lumbar spine	Z-score mean Lumbar spine = - 1.4 Subcranial = - 3.0
Soderpalm et al, 2008	DMD	N = 24 Mean age = 12 years	DXA – whole body (+/- cranium inclusion), lumbar spine, hip & heel Biomarker blood test	Mean Z-score = - 2.5 Reduced markers of bone anabolism
Larson & Henderson, 2000	DMD	N = 41 Ambulant and non-ambulant	DXA Lumbar spine vertebrae DXA femur	Z-score amb = - 0.8 Non-amb = - 1.7 Z-score amb = - 1.6 Non-amb = - 3.9
Aparicio et al, 2002	DMD	N = 10 Mean age = 8 years	DXA Lumbar spine vertebrae DXA femur	Z-score mean = - 4.32 Z-score mean = - 3.47
Philippe et al, 2011	DMD, LGMD, FSHD	DMD = 15 LGMD = 18 FSHD = 12 Adults	DXA Lumbar spine vertebrae (T-score mean) Total amb = - 0.25 Total non-amb = - 3.16 DXA femur (T-score mean) Total amb = - 1.17 Total non-amb = - 2.81	DMD t-score mean = - 4.0 LGMD t-score = - 0.8 FSHD t-score = - 0.7 DMD t-score mean = - 2.5 LGMD t-score = - 1.7 FSHD z-score = - 1.1

Physical activity is known to contribute significantly in all-cause mortality and general health (Iannuzzi-Sucich et al 2002). More specifically, intense physical activity provides stimuli for muscle accumulation and maintenance. The effects of bone loading through physical activity have also been demonstrated to have a profound effect on bone geometry and density thus having a positive effect on its quality and quantity (Kelley et al, 2013). Combination effects of ongoing corticosteroid therapy and reduced physical activity have also been demonstrated to reduce bone tensile strength and geometric architecture of bone in mdx mice (Novotny et al, 2012). Muscle weakness and detraining contribute to reductions in physical activity in all demographics, with previous studies identifying a correlation between lower limb strength and functional activities (e.g. stair climb) in elderly populations (Bean et al, 2007). It is reasonable to expect this to be more pronounced in individuals affected by muscle wasting conditions - particularly in severely affected individuals reliant upon powered wheelchairs for mobility.

As evidenced, a complex number of factors including physical activity and bone loading influence BMD and muscle size, particularly in children (Table 1 and 2). Characteristic of MD is a high intra- and inter-condition variability in clinical presentation, muscle weakness and disease progression with MD representing a spectrum of functional deficit - with some individuals requiring permanent support of powered mobility aids from early adolescence, some individuals maintaining high levels of regular physical activity (vocational, leisure, etc.) into late adulthood. Individuals who are less severely affected by functional impairment related to muscle tissue damage are likely to maintain higher levels of physical activity and maintain independent ambulation for a higher proportion of lifespan. Given the importance of stress and loading in physiological feedback systems of muscle and bone regulation, early loss of ambulation is likely to accelerate muscle atrophy and loss of BMD (Fig 1.3), whereas prolonged physical activity is likely to delay deleterious effects on muscle and bone. It is therefore reasonable to assume; individuals with more severe levels of muscle impairment are more likely to demonstrate decline in physiological markers of muscle size and BMD earlier than those with less severe disease presentations.

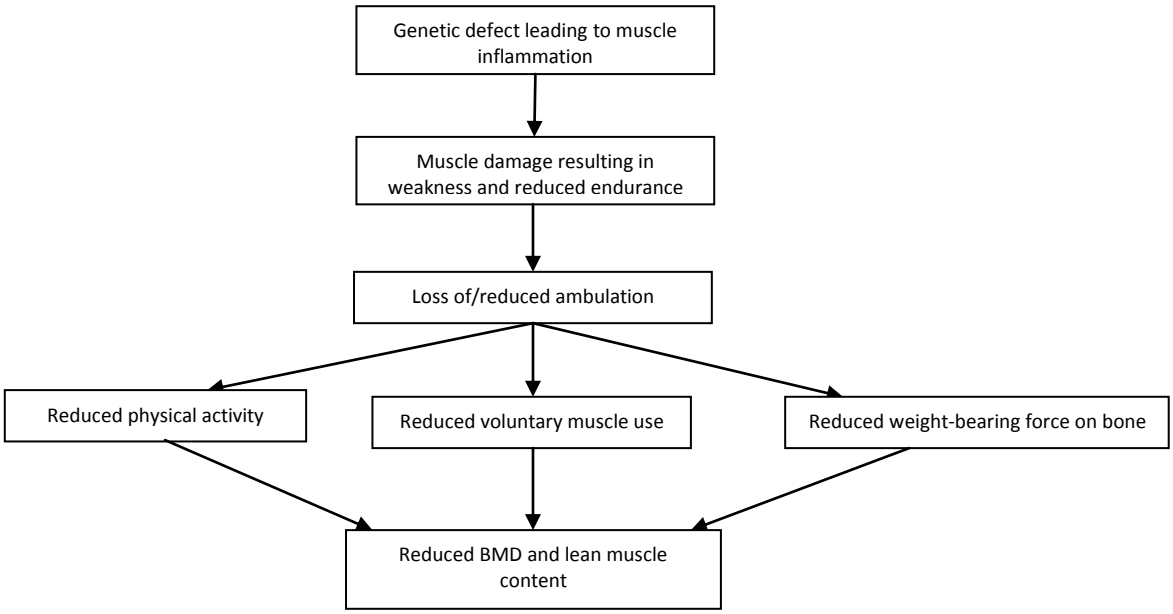

Fig 1.3 Flowchart describing process effects on BMD and muscle as a result of muscle condition presence

The focus of previous published studies in muscle and bone has largely been small, adolescent DMD populations; this represents a minor (though severely affected) proportion of the population of individuals affected by MD. Previous studies have also largely focused on either muscle structure or bone density, with no study thus far describing the two variables in one group of participants.

Aim

The aim of this study is to describe a data set of muscle cross-sectional area (tibialis anterior (TA)) and bone mineral density in upper (distal radius (DR)) and lower (midshaft tibia (MST)) limbs in adult males affected by a variety of dystrophies.

CHAPTER 2- METHODS

Participants

A total of 39 males with four distinct variants of MD - 10 individuals with DMD (Aged 25.4 ± 5.4 years), 10 individuals with BeMD (Aged 38.2 ± 11.7 years), 9 individuals with LGMD (Aged 37.1 ± 13.8 years), 10 individuals with FSHD (Aged 46.3 ± 10.3 years) - were recruited prospectively from a cohort of adults (>18 years old) regularly attending the Neuromuscular Centre (NMC), Cheshire, UK – a national centre of excellence for adults with MD. A group of 10 adult males without any form of neuromuscular impairment were recruited as a control group (Aged 23.0 ± 4.9 years) (CTRL) (Table 3.0).

All individuals with MD had previously received a laboratory confirmed diagnosis of MD through genetic testing at specialist NHS clinics.

All MD individuals recruited for the project were medically fit enough to attend a physiotherapy appointment (MD individuals attended the NMC on a regular (1-, 2-, 4-weekly) basis as part of a program of physiotherapy intervention aimed at maintaining function and independence). All CTRL participants were untrained, recreationally active and self-reported as undertaking no more than 1 hour of intense physical activity per week (Activities approximately >6 Metabolic Equivalents such as competitive sport, aerobics etc). CTRL participants were staff and students at Manchester Metropolitan University; staff members of the NMC physiotherapy or administrative team; carers or personal assistants to individuals attending the NMC.

Ethics

Ethical approval was obtained through the Department of Exercise and Sport Science, Manchester Metropolitan University and all participants signed informed consent prior to taking part in the study. All procedures complied with the latest revision of the Declaration of Helsinki (WMA 2013).

Condition	Population (n)	Ambulant (n)	Age (years)	Mass (kg)	Stature (cm)
CTRL	10	10	23 ± 5.0 (Range 19-37)	84.5 ± 10.5	182.4 ± 9.5
DMD	10	0	25 ± 5.8 (Range 20-38)	67.0 ± 17.5	164.7 ± 12.9
BeMD	10	8	38 ± 11.9 (Range 22-59)	87.3 ± 15.7	179.6 ± 8.0
FSHD	10	9	46 ± 10.6 (Range 33-58)	91.5 ± 20.6	183.9 ± 7.9
LGMD	9	7	37 ± 13.8 (Range 18-63)	90.1 ± 20.5	177.3 ± 10.2

Table 3.0: Participant demographic data, population size and ambulatory status for adult males unaffected by MD (CTRL); adult males affected by Duchenne MD (DMD); adult males affected by Becker MD (BeMD); adult males affected by facioscapulohumeral MD (FSHD); and adult males affected by Limb Girdle MD (LGMD). Data are presented as Mean ± Standard Deviation.

Protocol

Participants were tested on a single occasion with bone and muscle measurements performed in a random order during the same session in an outpatient clinic environment. All muscle, bone, and grip measurements were taken from the participant's dominant upper and lower limb as identified by subjective verbal questioning.

Ultrasound setting such as depth, brightness, gain etc were optimised to permit ease of identification of connective tissue septa during site identification and scanning. Surface markings were made on the skin with indelible pen over the region of interest.

Anthropometric measures were collected as follows:

Stature: 34 Ambulatory individuals able to stand erect were measured using a stadiometer (Harpenden stadiometer, Holtain Crymych, UK). 15 individuals dependent upon a wheelchair or able to stand but unable to achieve an erect posture were measured via arm span measurements with use of a tape measure (individuals with marked contractures were measured point-to-point; middle finger, elbow crease, corocoid process, midline sternum). Arm span measurements have been demonstrated to be a valid method of measuring height in adults (Brown et al, 2000).

Mass: All individuals were weighed using a digital seated scale system (6875, Detecto, Webb City, MO, USA) sensitive to the nearest 100 grams. In MD participants, the weight of slings, splints, shoes etc was subtracted from gross weight following separate measurement. CTRL participants were weighed standing, using a digital scale (Seca model 873, Seca, Germany), clothed, but unshod. The degree of agreement between the two scales was assessed by use of a 5kg metal weight. The degree of agreement was within 2% of weight measured.

Steroid Therapy

As previously demonstrated, long-term steroid therapy is known to adversely affect BMD. None of the individuals of the DMD group were currently on long-term steroid therapy, with 2 individuals having discontinued long-term steroid therapy at least 2 years prior to enrolment on the study. No individuals of any other group were using or had previously been prescribed long-term steroid therapy for a period of more than 1 year.

Muscle ASCA

ASCA of the Tibialis Anterior (TA) muscle was assessed using a (MyLab25, Esaote Biomedica, Genoa, Italy) brightness mode (B-mode), real-time ultrasound scanning device at 7.5 MHz frequency (linear array) with aqueous gel as contact medium (Cardiacare Ltd Ultrasound Gel, Cardiacare Limited, Brentwood, UK). During assessment of the lower limb individuals were in a relaxed, seated position with the knee fully extended and the calcaneus supported to ensure limb stability due to the fibrous nature of tissue associated with MD – particularly evident in dystrophinopathies – full knee extension to a straight leg was often unachievable due to joint and soft tissue contractures. Muscle deformation as a function of stretch in this position should not cause differences in muscle Anatomical Cross-Sectional Area (ASCA) between those able to achieve full knee extension and those with restricted joint range due to the positioning of the TA. Confounding muscle deformation as a result of stretch

during a ASCA assessment may occur during observation of a passively insufficient, two-joint muscle such as the Gastrocnemius Medius in knee extension; however this position does not affect the TA due to its sole role in ankle dorsiflexion. The TA was chosen as it was accessible in the non-ambulatory MD participants and has been extensively used in studies observing human muscle ASCA, volume and body composition (Abe et al, 1994).

TA insertion to tibial periosteum was established proximally and distally by sonographic assessment of its osseotendinous junction and a tape measurement from surface markers allowed the site to be calculated based upon TA length. The transducer head was applied in a transverse plane to the limb and a single, real-time image was sufficient to capture the TA muscle boundary. Care was taken to maintain a consistent application of pressure on the ultrasound transducer to prevent deformation of soft tissue through mechanical means. Real-time ultrasound recording was performed at 25 fps (Adobe Premier pro Version 6). Images taken through transverse scanning were digitally reconstructed (ImageJ 1.45, National Institutes of Health, USA) and ASCA established through computerised measurement. ASCA measurements were taken at 20% muscle length distal to the proximal origin where the muscle thickness has been demonstrated to be maximal (Martinson & Stokes, 1991).

B-mode ultrasound has been demonstrated to be valid in obtaining accurate data on muscle ASCA. It has previously compared favourably in terms of accuracy in delineating muscle/bone and muscle/fat divisions with gold-standard assessments such as MRI (Miyatani et al, 2004). Deviations from data obtained by MRI have been estimated to range from -0.15% to 5.17% (Esformes et al, 2002) indicating a valid and reproducible outcome measure, with a reported interclass correlation of 0.998 (reliability) and 0.999 (validity) compared with MRI (Reeves et al, 2004). Furthermore, ultrasound machines do not carry the burden of cost and impracticality compared to MRI and are realistic and viable alternatives in a field setting such as the NMC physiotherapy department.

Grip Strength

Muscle strength has previously been demonstrated to indicate bone health (Snow-Harter et al, 1994). Specifically, grip strength has been demonstrated to be a valid, independent indicator of upper limb bone health and density in healthy populations – higher grip strength correlates positively with higher BMD and overall bone health. This correlation has been estimated to account for up to 16.8% of the variation of BMD within a healthy population (Kritz-Silverstein & Barrett-Conner, 1994). At present, grip strength is considered as part of a complex of factors predicting BMD and is not clinically used as a predictor of BMD; therefore specific data concerning the predictive accuracy of grip strength for BMD is not available. Individuals who were capable of producing a measurable contraction of the hand completed a grip strength assessment by means of an analogue dynamometer. Dynamometer measurements were made subsequent to muscle ultrasound scans to prevent significant fluid

shifts influencing muscle volume assessment (Berg et al, 1993). Individuals were required to produce three, 5-second duration, 1-minute interval, maximal-effort grip contractions with their self-reported dominant hand whilst in a seated position. Maximal recorded reading of grip strength was reported.

11 individuals (22% of the total cohort) with advanced disease progression or severity (10 DMD; 1 BeMD) could not generate significant muscle force to effect a measurement on the equipment.

Dynamometers have been used to measure strength for a number of decades and are an accepted means of obtaining a value for muscle strength more accurately than therapist-performed manual muscle testing. Hand-held dynamometers have been demonstrated to have a re-test accuracy of up to 99% (Janssen and Le-Ngoc, 2009).

Bone Mineral Density

BMD assessment of the distal radius and mid-shaft tibia were recorded using a (Sunlight Omnisense 8000 portable, BeamMed Ltd, Petah Tikva, Israel) Speed of Sound (SoS) bone density ultrasound scanner, with aqueous gel as contact medium. Prior to any data collection the machine was calibrated using a Lucite reference block to correct for ambient temperature fluctuations and ensure reliability. During assessment of the limb individuals were seated in a relaxed position with their upper limb resting upon the armrest of the chair/wheelchair, separated from the surface by a foam spacer, with elbow flexed to 90°, fingers fully extended and the wrist in a neutral position. During lower limb observation the individual was seated in a relaxed position with hip and knee aligned, full knee extension (with respect to limitations imposed by soft tissue and joint contractures) and their calcaneus supported to ensure limb stability during testing. Data collection of BMD values in the upper and lower limb was based on sites recommended by the manufacturer for use with their equipment; distal radius and midshaft tibia.

DR measurements were obtained by measuring the distal arm length from the olecranon process of the elbow to the tip of the third distal phalanx. Measurement was performed on the dorsal aspect of the limb to avoid complications with individuals with contractures at elbow and/or wrist. All individuals were assessed on their self-reported dominant hand to exclude confounding influences of asymmetry. The investigation site was determined at 25% of total limb length proximal to the tip of the third phalanx at the lateral aspect of the radius relative to standard anatomical position following manufacturer's instructions. The transducer was placed in a coronal plane and aligned caudad-cephalad relative to the limb in anatomical position. A total of three data collections were performed by a slow, repeated oscillation of the transducer in an arc over the surface of the radius to ensure ultrasonic transduction through the entire bone.

This observation site has a physiological, in addition to, clinical significance. At the point of assessment the radius is composed of primarily cancellous bone with a trabecular arrangement indicating a substantial role in stress transmission through the upper limb (Hsu et al, 1993). Proximal to this site the bone transitions to a cortical structure with intermedullary marrow. Distal to this site and the bone transitions to an epiphyseal structure with greater concentrations of sclerosed bone (once adulthood has been reached). Transmission of SoS ultrasound at these sites would be compromised by changes in the refractive value of the bone tissue and a true indication of BMD would be more difficult to accurately obtain. Furthermore, the DR is a common area of fractures related to falls. The 'Colles' fracture of the distal radius is one of the most common upper limb fractures with an estimated incidence of 0.29% in male populations of every age (Mallmin and Lljunghall, 1992). Recently, DR sites have been used in addition to the established sites of lumbar spine and femoral neck in BMD scanning as part of detection of osteoporosis. Forearm fractures are reliable determinants of future fracture risk in other sites, with a cumulative incidence of whole-body fractures of 55% 10 years following a forearm fracture and 80% 20 years post-fracture (Cuddihy et al, 1999).

MST measurements were based upon a tape measurement of lower leg length from tibial plateau (identified through palpation of knee joint line) to base of calcaneus. All individuals were assessed on their dominant lower limb as identified by subjective verbal questioning. Individuals with MD often report asymmetrical symptoms in lower limbs, particularly those associated with muscle weakness; however there is often variable weakness between individual muscle groups on the same leg (e.g. weak glutei may lead to an ipsilateral calf musculature hypertrophy as a compensatory mechanism). As such it would be difficult to accurately assess a dominance of lower limbs through muscle strength testing alone, particularly as these tests are often not sensitive enough to detect subtle differences in muscle strength. The investigation site was determined at 50% of the total limb length proximal to the calcaneus on the anterior aspect of the tibia (tibial spine). The transducer was placed in a sagittal plane and aligned caudad-cephalad relative to the limb. Three data collections were performed with a slow, repeated oscillation of the transducer in an arc over the surface of the tibia to ensure ultrasonic transduction through the entire bone.

The site of the MST is an important area of trabecular concentration (similar in composition to the DR). Given associated reductions in BMD in individuals affected by MD, a pattern of reduction will be more prevalent distally in individuals who are non-weightbearing. In a cohort of 378 ambulant and non-ambulant DMD children (Aged 1-25years, mean age 12 years), 40% of 102 fractures sustained during the period of investigation occurred in the lower limb – with 15% of all fractures involving the tibia, implicating the distal lower limb as an important clinical site for BMD investigation (McDonald et al, 2002)

The ultrasound data outputs are expressed as - T-scores (a relative score of BMD as compared to the mean score of a healthy, thirty-year-old reference adult) and a Z-score (a relative score of BMD compared to the reference mean of an age- & gender-matched demographic) – relative indications of BMD. T-scores are routinely used as a screening and diagnostic tool in osteoporosis (OP); with a T-score of <-2.5 SD considered as manifest osteoporosis (Johnell et al, 2005). While T-scores remain prevalent in the diagnosis of OP, the Z-score represents deviation from a norm of demographically-matched individuals and may be of more significance in individuals undergoing long-term intervention (such as steroids) or those with long-term conditions. Z-scores as a statistical device are frequently seen in large-scale studies of chronic conditions such as Chronic Liver Disease (Sokol and Stall, 1990) and childhood obesity (Cole et al, 2000).
Assessment of BMD is clinically performed more commonly by either Peripheral Quantitative Computed Tomography (pQCT) or Dual-energy X-Ray Assessment (DXA) being the gold standard for diagnostic accuracy. However, both devices use ionising radiation and within the cohort of the DMD patients it is difficult to maintain posture during the duration of the scan, alongside issues of gantry size within the pQCT.

SoS quantitative ultrasound, while lacking credentials for use in definitive clinical diagnoses of adverse bone health, has been shown to be an effective screening tool in the identification of OP, particularly in conjunction with known risk factors (Minnock et al, 2008). Ultrasound as a means of assessing BMD is as sensitive as DXA to OP risk factors (Frost et al, 2001) and compares favourably with pQCT measures of BMD and bone composition (Lee et al, 1997). It is also of a non-invasive ionising radiation thus being an ideal portable screening tool for bone tissue analysis. Furthermore, due to soft tissue contracture and pseudohypertrophy of lower limb musculature, reliable pQCT measurements from certain clinical populations (e.g. dystrophinopathies) are impossible due to complications regarding limb position and size as previously highlighted.

Physical Activity

Physical activity is a key determinant of life-long BMD. Due to reductions in ambulation and functional ability, individuals with mobility-limiting disabilities often present with significant reductions in physical activity. As such, standard subjective measurements of physical activity are unlikely to be sensitive enough to capture individuals with MD – particularly those with the most significant physical impairment.

Washburn et al (2002) developed a disability specific physical activity questionnaire (PASIPD – Physical Activity Score for Individuals with Physical Disabilities) to account for reduction in physical activity associated with various physical disabilities. This encompasses a lower activity threshold and has subsequently been validated as a reliable outcome measure for physical activity in disabled persons (van der Ploeg et al, 2007).

The Bone Specific Activity Questionnaire (BPAQ) was developed by Weeks & Beck (2008) to account for variations in mechanical bone loading activity and their effect on BMD. It has subsequently been used in multiple studies describing interventions in bone loading (e.g. Beck & Norling, 2010) and as part of data abstraction in meta-analyses of bone changes post-exercise intervention (e.g. Kelley et al, 2012).

MD individuals were required to complete both physical activity questionnaires with support of the researcher.

Statistics

IBM SPSS Statistics 21 was used to analyse the data. Parametricity was established with use of a Shapiro-Wilk test of normality and a Kolgomorov-Smirnov test of equal variance – TibAnt ASCA and PASIPD score data satisfied the criteria of parametricity. Age, BPAQ score, Grip strength, Mass, Span, T-score Radius, T-score Tibia, Z-score Radius and Z-score Tibia all failed to satisfy criteria of normal distribution.

All data (including that generated by the PASIPD and BPAQ) was ordinal in nature. Intergroup means of demographic, muscle ASCA, bone, physical activity, and grip data were compared with use of a one-way ANOVA with post-hoc pairwise comparisons, using LSD adjustments. GreenHouse Geisser corrections were applied to the above data failing to satisfy normal distribution. Intergroup percentage deviations and figures were produced using functions in Microsoft Excel 2013. Data are presented as Mean± Standard Deviation (STDEV) unless otherwise indicated. Statistical significance was set with α=0.05.

CHAPTER 3- RESULTS

DMD had a lower age compared with all groups except CTRL (BeMD -50.5%; FSHD -82.4%; LGMD -46.3%) (P<0.05, Table 4.0).
All groups (except DMD participants) demonstrated higher age compared with CTRL (BeMD +65.4%; FSHD +100.0%; LGMD +60.7%). There were no differences demonstrated between any other groups in age.

DMD had lower mass compared to all groups (BeMD -23.3%; FSHD -26.8%; LGMD -25.6%; CTRL -20.7%) (P<0.05 Table 4.0). There was no difference between FSHD, LGMD, BeMD or CTRL in mass.

DMD demonstrated lower stature compared with all other groups (BeMD -8.27%; FSHD -10.4%; LGMD -7.11%; CTRL -9.67%) (P<0.05 Table 4.0). There was no difference identified in stature between any other groups.

DMD demonstrated lower radial T-scores compared with all groups except BeMD and FSHD, and lower Z-scores compared with all groups except BeMD and FSHD. All groups demonstrated reduced radial T- and Z-scores compared with CTRL except LGMD. All groups demonstrated reduced radial T- and Z-scores compared with LGMD except CTRL. (P<0.05 Table 4.0, Figure 1.4). There were no further differences between groups.

DMD demonstrated lower tibial T- and Z-scores compared with all groups. There were no differences in tibial T- and Z-scores between any other groups. (P<0.05 Table 1.1, Figure 1.5).

All groups demonstrated lower TA ASCA compared with CTRL (BeMD -39.7%; DMD -59.7%; FSHD -58.9%; LGMD -62.4%). BeMD demonstrated lower TA ASCA compared with CTRL and higher TA ASCA compared with all other groups. There were no further differences between other groups in TA ASCA (P<0.05 Table 1.1, Figure 1.6).

DMD demonstrated lower grip strength compared with all groups. All groups demonstrated lower grip strength compared to CTRL (BeMD -51.2%; FSHD -46.3%; LGMD -49.9%). There was no difference in grip strength between LGMD, FSHD and BeMD. (P<0.05 Table 4.0, Figure 1.7)

DMD demonstrated significantly lower mean BPAQ (P<0.05 Table 4.0, Figure 1.8) and PASIPD (P<0.05 Table 4.0, Figure 1.9) score compared with BeMD, FSHD and LGMD. There were no differences in physical activity scores between with BeMD, FSHD, LGMD.

	CTRL	BeMD	DMD	FSHD	LGMD
Age (years)	23 ± 5.0	38 ± 11.9*†	25 ± 5.7	46 ± 10.6*†	37 ± 13.9*†
Stature (cm)	182.4 ± 9.5	179.6 ± 8.0†	164.7 ± 12.9*	183.9 ± 7.9†	177.3 ± 10.2†
Mass (kg)	84.5 ± 10.5	87.3 ± 15.7†	67.0 ± 17.5*	91.5 ± 20.6†	90.1 ± 20.5†
T-Score Radius	0.7 ± 0.7	-0.68 ± 1.0*	-1.18 ± 1.2*	-0.4 ± 0.8*	0.04 ± 1.0†
Z-score Radius	0.96 ± 0.7	-0.53 ± 1.1*	-0.88 ± 1.2*	-0.2 ± 0.8*	0.22 ± 0.9†
T-score Tibia	-0.09 ± 0.5	-0.4 ± 0.7†	-1.32 ± 1.0*	0.2 ± 1.0†	0.2 ± 0.8†
Z-score Tibia	-0.06 ± 0.6	-0.35 ± 0.8†	-1.29 ± 1.0*	0.3 ± 1.0†	0.29 ± 0.8†
Tib Ant ASCA (cm^2)	9.57 ± 2.6‡	5.77 ± 1.6*	3.86 ± 1.2*‡	3.9 ± 1.5*‡	3.6 ± 0.7*‡
Grip (kg)	44 ± 7.20	21.5 ± 19.2*†	0.0 ± 0.0*$	23.6 ± 15.5*†	22.1 ± 12.1*†
PASIPD score	N/A	18.4 ± 21.1†	1.10 ± 0.6	12.2 ± 11.8	11.7 ± 7.8
BPAQ score	N/A	19.4 ± 15.2†	2.17 ± 2.3	22.2 ± 14.1†	24.9 ± 13.7†

Table 4.0: Participant demographics, bone health scores, TA ASCA, and grip strength for Adult males without muscular dystrophy (CTRL); adult males with Becker muscle dystrophy (BeMD); adult males with Duchenne Muscular Dystrophy (DMD); adult males with Facioscapulohumeral Muscular Dystrophy (FSHD); and adult males with Limb Girdle Muscular Dystrophy (LGMD). Data are presented as Mean ± SD. * Denotes significant difference from CTRL, † denotes significant difference from DMD, ‡ denotes significant difference from Becker's MD, $ denotes inability for participants in this group to perform the required data collection test ($P<0.05$) (PASIPD = Physical Activity Scale for Persons with Physical Disabilities; BPAQ = Bone-specific Physical Activity Questionnaire)

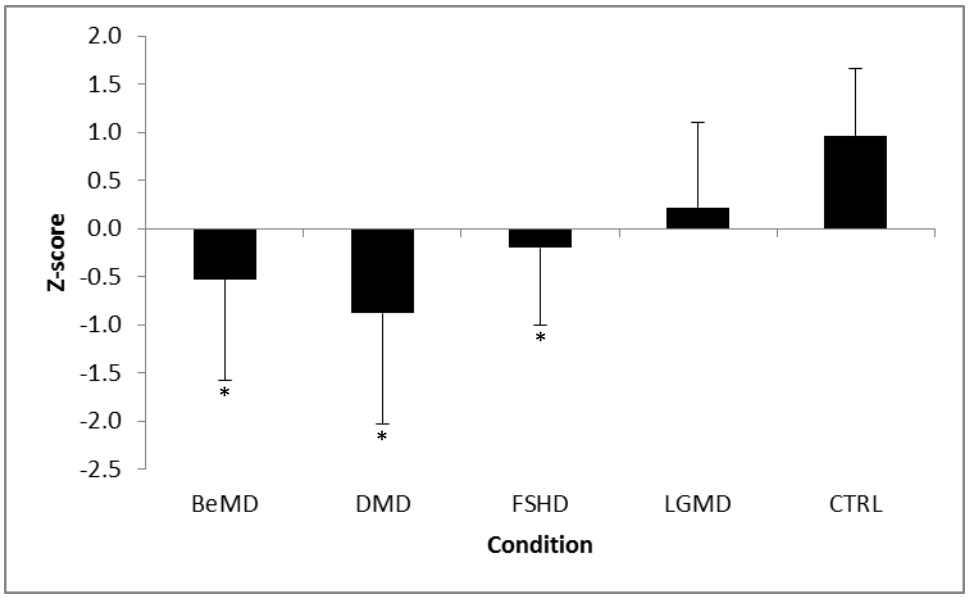

Fig 1.4 Radial Z-scores of adult males affected by Becker Muscular Dystrophy (BeMD), adult males affected by Duchenne Muscular Dystrophy (DMD), adult males affected by Facioscapulohumeral Muscular Dystrophy (FSHD), adult males affected by Limb Girdle Muscular Dystrophy (LGMD) and unaffected adult male controls (CTRL). Data are presented as Mean ± standard deviation error bars. * denotes significant difference from CTRL.

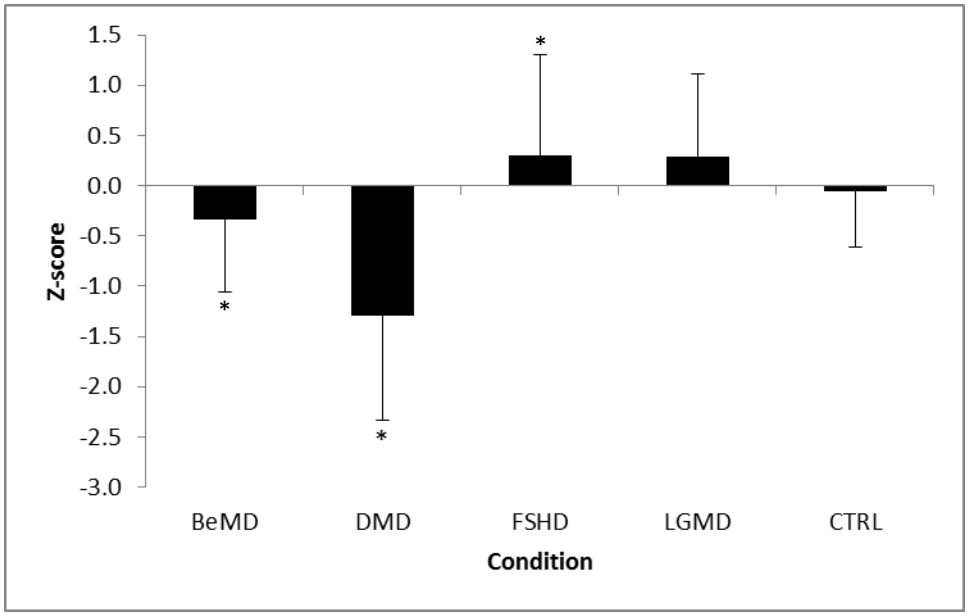

Fig 1.5 Tibial Z-scores of adult males affected by Becker Muscular Dystrophy (BeMD), adult males affected by Duchenne Muscular Dystrophy (DMD), adult males affected by Facioscapulohumeral Muscular Dystrophy (FSHD), adult males affected by Limb Girdle Muscular Dystrophy (LGMD) and unaffected adult male controls (CTRL). Data are represented as Mean ± standard deviation error bars. * denotes significant difference from CTRL.

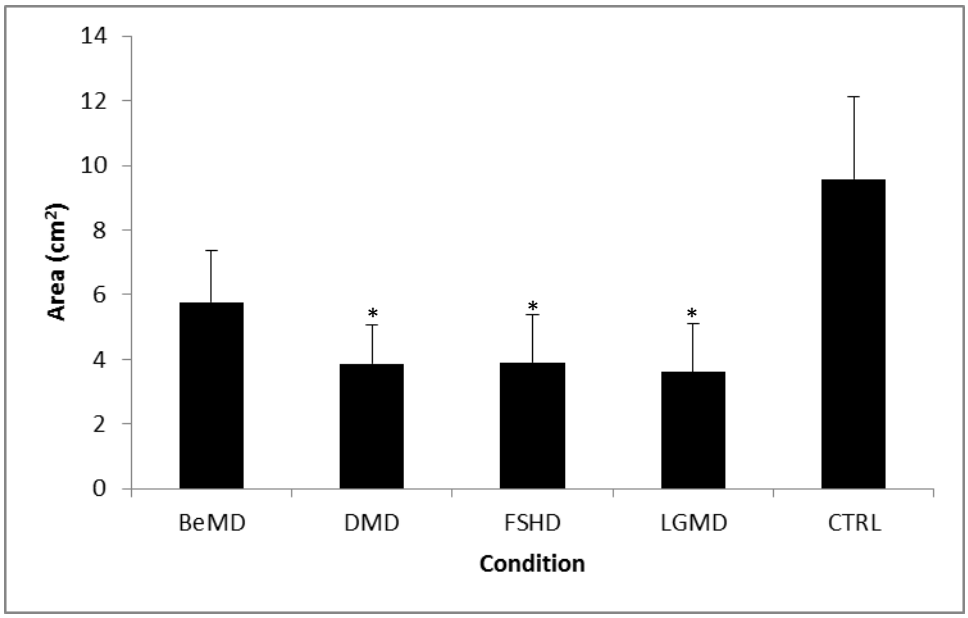

Fig 1.6 TA ASCA of adult males affected by Becker Muscular Dystrophy (BeMD), adult males affected by Duchenne Muscular Dystrophy (DMD), adult males affected by Facioscapulohumeral Muscular Dystrophy (FSHD), adult males affected by Limb Girdle Muscular Dystrophy (LGMD) and unaffected adult male controls (CTRL) presented in cm^2. Data are presented as Mean ± standard deviation error bars. * denotes significant difference from CTRL.

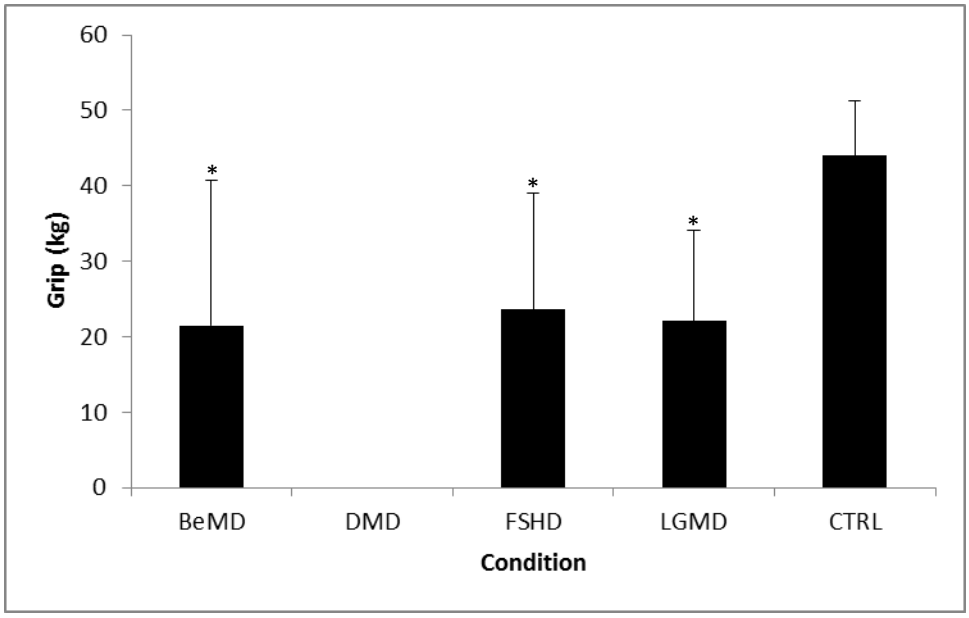

Fig 1.7 Grip strength of adult males affected by Becker Muscular Dystrophy (BeMD), adult males affected by Duchenne Muscular Dystrophy (DMD), adult males affected by Facioscapulohumeral Muscular Dystrophy (FSHD), adult males affected by Limb Girdle Muscular Dystrophy (LGMD) and unaffected adult male controls (CTRL) presented in kg. Data are presented as Mean ± standard deviation error bars. * denotes significant difference from CTRL.

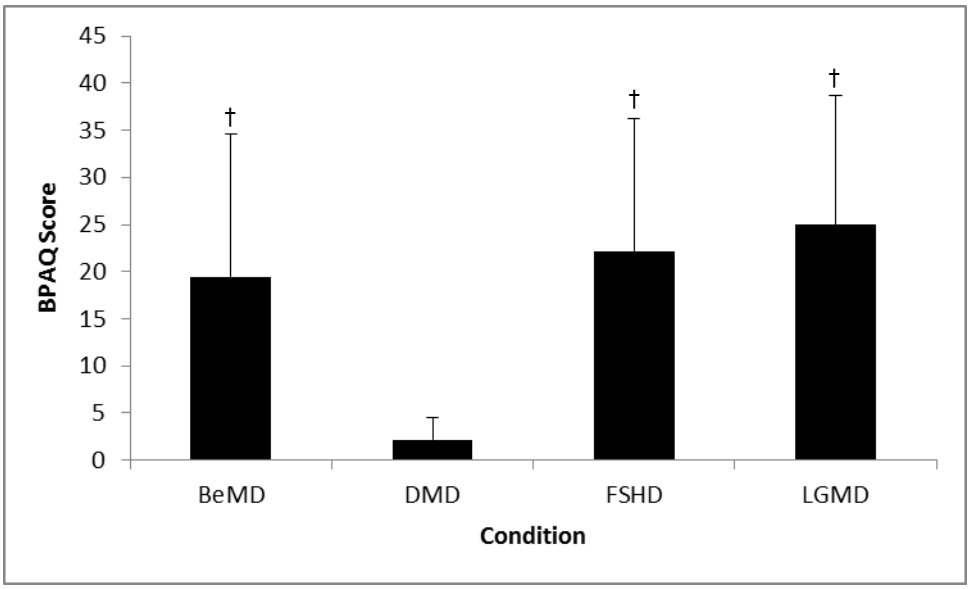

Fig 1.8 BPAQ scores of adult males affected by Becker Muscular Dystrophy (BeMD), adult males affected by Duchenne Muscular Dystrophy (DMD), adult males affected by Facioscapulohumeral Muscular Dystrophy (FSHD), and adult males affected by Limb Girdle Muscular Dystrophy (LGMD). Data are presented as Mean ± standard deviation error bars. † denotes significant difference from DMD.

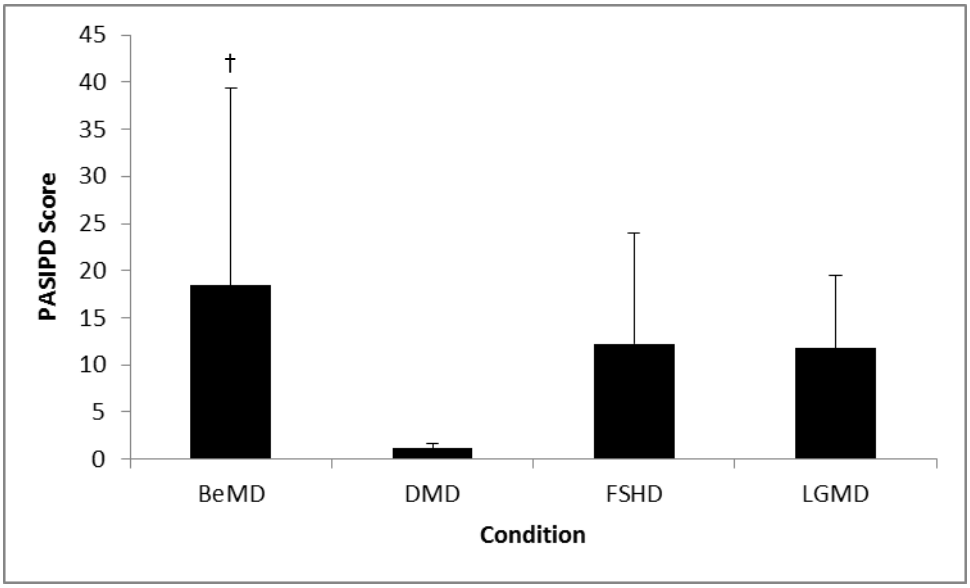

Fig 1.9 PASIPD scores of adult males affected by Becker Muscular Dystrophy (BeMD), adult males affected by Duchenne Muscular Dystrophy (DMD), adult males affected by Facioscapulohumeral Muscular Dystrophy (FSHD), and adult males affected by Limb Girdle Muscular Dystrophy (LGMD). Data are presented as Mean ± standard deviation error bars. † denotes significant difference from DMD.

CHAPTER 4- DISCUSSION

The main finding of the present thesis is that bone health at the assessed sites was demonstrated to be lower in adult males with DMD compared to non-affected adult males and adult males with other variant forms of MD. Furthermore, TA ASCA was observed to be smaller in adult males with MD compared to those of non-affected adult males.
Adult males affected by MD demonstrated lower grip strength compared to adult males unaffected by MD, with DMD participants unable to generate sufficient force to produce a reliable measure of grip strength. Furthermore, adult males affected by DMD demonstrated lower PASIPD scores compared with individuals with BeMD and lower BPAQ scores compared with all other individuals affected by MD.

Subsequent to the observations of previous studies investigating BMD in MD individuals, adverse bone health in this population was anticipated. As demonstrated, studies involving populations of children and adolescents with MD have identified lower Z-scores in whole-body (Soderpalm et al, 2008); Lumbar spine (Bianchi et al, 2003; Hawker et al, 2005); and femoral BMD (Aparicio et al, 2002; Larson & Henderson, 2000). These studies have selected exclusively DMD populations with mean ages often in early childhood. Whilst this is justifiable due to the severity of this condition, the associated early loss of ambulation and the frequent use of long-term corticosteroid therapy to arrest muscle atrophy, it represents a narrow spectrum of MD individuals in both age and variety of impairment during advancement of age. Due to advances in cardiac medication, non-invasive ventilation and enhanced access to regular adult physiotherapy services, individuals with DMD are living longer than at any other point in history (Passamano et al, 2012) and as such, observation of adult individuals is important. This is especially true considering deleterious effects of immobility on bone health are anticipated to continue as an individual's functional impairments become more profound with symptom progression. With peak BMD achieved in early adulthood (Gilsanz et al, 1988), deterioration in bone density occurring after this point is likely to be more pronounced when combined with the effects of prolonged immobility and dependence upon powered mobility aids.

Furthermore, while representing a continuum of less acute physical impairment, individuals with other variants of MD experience muscle atrophy, and loss of ambulation & function as symptoms of their condition. Successively, negative effects on bone health may also be expected in these individuals, as has been demonstrated in reductions in tibial and radial Z-scores of BeMD and FSHD participants during this study.

To date, Phillippe et al (2011), represents the only study to investigate BMD in adults with different forms of MD (inclusive of DMD). However, the use of a T-score – despite its clinical relevance - prevents a direct comparison with an age-matched reference group. Additionally, as discussed earlier, Z-scores are frequently used during observation of cohorts of individuals with long-term condition. With the use of Z-score as an outcome measure of BMD, an age-

matched control group within the study is not critical to the relevance or significance of the data observed (Z-score being the comparison of BMD relative to an age-matched reference group). The data observed in this study is consistent with the reductions in BMD observed in adults with muscular dystrophy (Phillippe et al, 2011), albeit with differing selection of observation sites (lumbar spine and midshaft femur – Phillippe et al, 2011), with specific reductions in adult DMD individuals compared to other individuals with FSHD and LGMD.

Phillippe et al, in their study, also do not include BeMD individuals as part of the participant cohort. The BeMD group in this study demonstrated lower T- and Z-scores for radius and tibia compared to other participants with MD and, while this was not statistically significant, it may prove to be clinically relevant particularly in a group of less ambulant individuals. Therefore, this study has progressed knowledge in this area due to its use of adult participants; its inclusion of participants with a variety of pathologies; and its use of a variety of outcome measures for BMD which may be more relevant for samples comprising individuals with long-term conditions.

The reduction in BMD in individuals affected by MD observed in this study is consistent with current theory of bone tissue maintenance and the influence of loss of bone loading on catabolic bone metabolism. As described by Frost's 'mechanostat' theory (and subsequent Utah Paradigm) (Frost, 2003) sufficient mechanical stress acquired through bone loading is required to regulate osteoclast activity and prevent loss of bone density. The lower BMD observed in DMD individuals compared with unaffected controls and other ambulatory individuals with MD is consistent with the severity of the condition (earlier loss of ambulation; all participants wheelchair-dependent; reduced levels of physical activity). Reduced BMD was also observed in FSHD and BeMD individuals compared with unaffected controls, although this was only observed in the radius and not the tibia. This may be due to the continued bone loading of lower limbs in walking etc. (the majority of participants in LGMD, BeMD and FSHD cohorts were ambulant).

TA ASCA was observed to be smaller in all participant groups affected by MD, with DMD individuals demonstrating the most significant reduction compared to unaffected controls. There were significant reductions in TA ASCA in DMD individuals compared with those participants affected by other variants of MD. The TA has been used as an experimental site in previous studies investigating muscle size in individuals with long-term conditions (Kent-Braun et al, 1997 – Multiple Sclerosis; Johansen et al, 2003 – Kidney Dialysis; Castro et al, 1999 – Acute Spinal Cord Injury). TA is selected for its functional impact on ambulation (ankle dorsiflexion in gait) and its ease of analysis with clinical imaging equipment due to its superficial position and clear delineating osseous boundaries (Abe et al, 1994). The data reported in this study is similar to reductions in ASCA identified in individuals affected by disease states known to have associated reductions in physical activity and muscle strength

as reported by previous authors (Johansen et al, 2003; Kent-Braun et al, 1997). This presentation of reduced TA ASCA in individuals known to have mobility-limiting muscle conditions is consistent with current comprehension of muscle regulation. Models of disuse have been demonstrated in the sarcopenic elderly (Morley et al, 2011) and specifically functional impairment and physical disability have been identified as significant causes of reduction in muscle size and strength preservation (Janssen et al, 2002).

Reduced ASCA in MD individuals compared with CTRL participants is anticipated due to the inflammatory damage associated with genetic protein defects within muscle tissue affected by MD (Kostek et al, 2012). Adipose and fibrous infiltrations replace contractile elements of muscle tissue (Wang et al, 2011) leading to gross loss of muscle power output and accelerated muscle fatigue on sustained contraction as well as promoting catabolic muscle metabolism in the presence of chronic elevation of inflammatory markers (IL6) (Haddad et al, 2005). Reduced gross muscle strength proliferates an environment of muscle disuse, ultimately leading to clinical presentations of muscle weakness (e.g. TA weakness in individuals with FSHD presenting as a clinical sign of foot drop (Tawil, 2008)). Consistent with reductions in muscle power which have been previously reported, the present data observes lower handgrip strength from adult males with MD, compared to unaffected male controls.
Reduced ASCA and grip strength in DMD individuals compared with other variants of MD is consistent with an increased severity of clinical presentation; including early loss of ambulation and dependence upon powered mobility aids in DMD individuals. This provides an environment of muscle disuse much earlier than other MD participants in this study (of whom the majority remained ambulant at the time of observation).

In this study, BeMD participants demonstrated significantly larger TA ASCA than other individuals affected by MD. This is unlikely to be explained by differences in physical activity as the highest proportion of non-ambulant individuals (excluding DMD) was observed in the BeMD cohort. Pseudohypertrophy of crural musculature has been documented previously in BeMD individuals (Bradley et al, 2004). However, due to the imaging used in this study it is not possible to discount non-contractile elements of a muscle complex from the overall ASCA and as such the effects of these fibrous infiltrates on gross muscle ASCA cannot be estimated.

Grip strength was significantly reduced in all MD participants compared to CTRL (BeMD -51.2%; FSHD -46.3%; LGMD -49.9%), with those affected by DMD further significantly reduced compared with other MD participants. Due to the nature of the dynamometer equipment used, a minimum threshold of force was required to overcome inertia, the resistance of the machine, and generate a measurable outcome. While some DMD individuals participating in the trial maintain hand function sufficient to write, operate a computer/wheelchair joystick etc., they lacked the strength to overcome this threshold and as

such were unable to generate a reading of grip strength. A highly sensitive dynamometer would mitigate this issue however, due to clinical presentation and the results presented in this study, it is extremely unlikely that DMD individuals would achieve a generation of force comparable to other participants.

Physical activity was significantly reduced in individuals with DMD compared to other MD participants when assessed using the BPAQ (developed by Weeks & Beck, 2008) and lower compared to BeMD individuals using the PASIPD score (developed by Washburn et al, 2002). There are key differences between the BPAQ and the PASIPD score which may affect the outcomes of these results. Firstly, the BPAQ takes into account historical, as well as current, reports of physical activity – this is not the case with the PASIPD. As MD presents as a progressive condition, historical reports of physical activity during childhood tended to be higher than current reports of activity within the last 12 months. As such the BPAQ captured data relevant specifically to bone health that the PASIPD did not and this may explain the higher intergroup mean difference.

It is also worthy of consideration that individuals taking part in this study were undergoing regular physiotherapy. Regular, on-going physiotherapy is currently unavailable to individuals with MD through NHS services in the UK - where a patchwork of services prevails. This ranges from no intervention; to annual contact with a specialist therapist at a tertiary centre; to sporadic contact with a generic or neurological therapy service offering short-term manual therapy, exercise and advice; to on-going therapy (as part of home exercise programs administered by carers or relatives, or through private/voluntary sector services such as NMC). It may therefore be assumed that the participants in this study are not a sample applicable to the wider population of MD individuals. However, as previously discussed, the most significant period in life of accumulation of BMD and muscle mass is in childhood (pre-15 years) (Rittweger, 2008) – the participants in this study are therefore unlikely to demonstrate gains in BMD or muscle mass through specific therapy, particularly if they experienced reduced levels of activity as children as a result of non-participation of sports etc. due to their condition. Furthermore, the physiotherapy provided by the NMC is of a broad-spectrum; addressing multiple therapeutic problems such as pain, quality of life etc. and not specifically focused on strengthening exercise. Additionally, the input provided to individuals attending NMC often comprises low-intensity, low-load stretching, exercise and soft tissue therapy aimed at maintaining strength and function, which are likely to be of insignificant strain and frequency to stimulate positive changes in muscle size and BMD. Regardless, it is likely that due to the effects of the physiotherapy provided on a regular basis, the participants in this study may present less severely than similarly affected individuals who have not historically received regular therapy and supervised or accessible exercise. They may therefore represent a sample of individuals with a less profound presentation than individuals who do not regularly access physiotherapy and, as such, populations of MD individuals who

do not access regular physiotherapy are likely to be more severely affected by their condition in terms of muscle mass and BMD.

Due to the variety of the conditions reported in this study, there were substantial ranges of BPAQ and PASIPD scores within all groups except DMD, even in a cohort of individuals receiving therapeutic input. It is therefore reasonable to suggest that larger sample sizes of individuals not clinically assessed as needing therapeutic input may yield more significant results when utilising the PASIPD. However, it should be noted that the population described in the present study represents the first adult male data in regards to muscle and bone from the dystrophic conditions, and represents a random selection of individuals (albeit a convenience sample) with different conditions.

Conclusion

Adult, male DMD individuals demonstrate significantly lower T- and Z-scores of bone health, TA ASCA and grip strength compared with unaffected male controls as well as other adult individuals with differing variants of MD. Adult male individuals affected by BeMD, FSHD, and LGMD demonstrate significantly lower TA ASCA compared to unaffected male controls. All MD individuals demonstrated reduced grip strength compared to controls and DMD individuals reported significantly lower physical activity scores than other MD participants.

Study Limitations

Participants in the study were not age-matched. This is due to the participant sample available (clients attending the NMC in Winsford) representing a cross-section of individuals with MD. Furthermore, control participants were recruited from a student cohort, whereby mean age is low. This issue is partly ameliorated by the use of a Z-score outcome measure, which provides an age-matched reference value for BMD.

Clinical Implications

The present data is meaningful in the following ways. It suggests the negative impact of prolonged immobility on BMD (specifically bone unloading) and muscle. Given the effects of muscle atrophy on ambulation and balance, dependence upon powered mobility aids and reduced physical activity is common in individuals with MD. As prolonged periods of immobility or reduced physical activity are known to negatively impact upon bone and muscle tissue regulation, it is reasonable to envisage a multi-factorial, self-propagating feedback whereby reduced activity and limb loading (brought upon by gradual, unremitting muscle atrophy) produces further reductions in activity and limb loading until loss of ambulation, functional transfer etc. occurs. Implications on individual independence are significant, as reductions in function often necessitate external support from technology or persons. There are further connotations on fracture risk and incidence – with reductions in BMD leading to an

increased lifelong prevalence of fractures and associated medical treatment (including further risk and expense).

Presently, there are no national guidelines advising healthcare professionals on the most adequate practice for the preservation of BMD and muscle mass in MD individuals, however, given the current theory of bone loading on preservation of BMD, efforts should be made to encourage and promote weight bearing in individuals with MD, particularly during childhood where the most significant accumulations of lifelong bone and muscle are made. This should be achieved through specific loading activities where suitable stress can be achieved as to satisfy the threshold for effective BMD regulation (such as ambulation). Furthermore, as BMD in particular is known to be regulated by a complex of factors, there may be implications other than loading exercise - such as diet (factors such as vitamin D and calcium uptake) – to consider in the clinical setting.

Recommendations for future work

It is the recommendation of this study that further study into this area is specifically required to establish the most efficacious method in preserving BMD and muscle size and strength in individuals with MD. Furthermore, it is of critical importance to assist healthcare professionals in providing evidence-based advice and therapy to prolong independence and function in those affected by MD.

References

Abe, T., Kondo, M., Yasuo, K., Fukunaga, T. (1994) Prediction equations for body composition of Japanese adults by B-mode ultrasound, American Journal of Human Biology, 6(2):161-170

Abdel-Salam, E., Abdel-Maguid, I., Korraa, S. (2009) Markers of degeneration and regeneration in Duchenne muscular dystrophy, Acta Myologia, 28(3), 94-100

Aparicio, LF., Jurkovic, M., DeLullo, J. (2002) Decreased bone density in ambulatory patients with Duchenne muscular dystrophy, Journal of Paediatric Orthopaedics, 22(2):179-81

Barber, L., Barrett, R., Lichtwark, G. (2009) Validation of a freehand 3D ultrasound system for morphological measures of the medial gastrocnemius muscles, Journal of Biomechanics, 42(9):1313-9

Bean, JF., Kiely, DK., LaRose, S., Alian, J., Frontera, WR. (2007) Is stair climb power a clinically relevant measure of leg power impairments in at-risk older adults?, Archives of Physical Medicine and Rehabilitation, 88(5):604-9

Beck, BR., Norling, TL. (2010) The effects of 8 mos of twice-weekly low- or higher intensity whole body vibration on risk factors for postmenopausal hip fracture, American Journal of Physical Medicine and Rehabilitation, 89(12):997-1009

Bell, S. (2010) Our future services for people living with Neuromuscular Disorders in the North West, NHS White Paper

Berg, H., Larsson, L., Tesch, P. (1997) Lower limb skeletal muscle function after 6 wk of bed rest, Journal of Applied Physiology, 82(1), 182-88

Berg, WA., Caskey, CI., Hamper, UM., Chang, BW., Sheth, S., Zerhouni, EA., Kuhlman, JE. (1993) Diagnosing breast implant rupture with MR imaging, US, and mammography, Radiographics, 13(6), DOI: 10.1148/radiographics.13.6.8290727

Bianchi, ML., Mazzanti, A., Galbiati, E., Saraifoger, S., Dubini, A., Cornelio, F., Morandi, L. (2003) Bone mineral density and bone metabolism in Duchenne muscular dystrophy, Osteoporosis International, 14(9):761-7

Bradley, WG., Jones, MZ., Mussini, JM., Fawcett, PRW. (1978) Becker-type muscular dystrophy, Muscle and Nerve, 1(2), 111-132

Brenman, JE., Chao, DS., Xia, H., Aldape, K., Bredt, DS. (1995) Nitric Oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy, Cell, 82(5), 743-52

Association WM (2013) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects, Journal of the American Medical Association 310(20):2191-4

Brown, JK., Whittemore, KT., Knapp, TR. (2000) Is arm span an accurate measure of height in young and middle-age adults, Clinical Nursing Research, 9(1):84-94

Bruunsgaard, H. (2005) Physical activity and modulation of systemic low-level inflammation, Journal of Leukocyte Biology, 78(4), 819-835

Castro, MJ., Apple Jr, DF., Hillegrass, EA., Dudley, GA (1999) Influence of complete spinal cord injury on skeletal muscle cross-sectional area within the first 6 months of injury, European Journal of Applied Physiology and Occupational Physiology, 80(4), 373-8

Centre for Disease Control and Prevention (CDC) (2007) Prevalence of Duchenne/Becker muscular dystrophy among males 5-24 years – four states, 2007, Morbidity and mortality weekly, 58(40), 1119-1122

Muscular Dystrophy Campaign UK, *Types of Conditions*, Available from: <http://www.muscular-dystrophy.org/about_muscular_dystrophy/conditions> [10 October 2014]

Cole, TJ., Bellizzi, MC., Flegal, KM., Dietz, WH. (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey, British Medical Journal, 320: DOI: 10.1136/bmj.320.7244.1240

Crabtree, NJ., Roper, H., McMurchie, H., Shaw, NJ. (2010) Regional changes in bone area and bone mineral content in boys with Duchenne muscular dystrophy receiving corticosteroid treatment, Journal of Paediatrics, 156(3):450-5

Creditor, M. (1993) Hazards of hospitalisation of the elderly, Annals of internal medicine, 118(3), 219-223

Crytzer, TM., Dicianno, BE., Fairman, AD. (2013) Effectiveness of an upper extremity exercise device and text message reminders to exercise in adults with spinda bifida: a pilot study, Assistive Technology, 25(4):181-193

Cuddihy, MT., Gabriel, SE., Crowson, CS., O'Fallon, WM. Melton, LJ 3rd (1999) Forearm fractures as predictors of subsequent osteoporotic fractures, Osteoporosis International, 9(6):469-75

De Bruin, PF., Ueki, J., Bush, A., Khan, Y., Watson, A., Pride, NB. (1997) Diaphragm thickness and inspiratory strength in patients with Duchenne muscular dystrophy, Thorax, 52(5), 472-475
Dellorusso, C., Crawford, RW., Chamberlain, JS., Brooks, SV. (2001) Tibialis anterior muscles in mdx mice are highly susceptible to contraction-induced injury, Journal of Muscle Research and Cell Motility, 22(5), 467-475
Eagle, M., Baudouin, SV., Chandler, C., Giddings, DR., Bullock, R., Bushby, K. (2002) Survival in Duchenne muscular dystrophy: improvements in life expectancy since 1967 and the impact of home nocturnal ventilation, Neuromuscular Disorders, 12(10), 926-9
Esformes, JI., Narici, MV., Maganaris, CN. (2002) Measurement of human muscle volume using ultrasonography, European Journal of Applied Physiology, 87(1):90-2
Frost, ML., Blake, GM., Fogelman, L. (2001) Quantitative ultrasound and bone mineral density are equally strongly associated with risk factors for osteoporosis, Journal of Bone and Mineral Research, 16(2):406-416
Frost, HM. (2003) Bone "mass" and the "mechanostat": a proposal, The Anatomical Record, 219(1), 1-9
Gilsanz, V., Gibbens, DT., Carlson, M., Boechat, MI., Cann, C., Schulz, EE. (1988) Peak trabecular vertebral density: A comparison of adolescent and adult females, Calcified Tissue International, 43(4), 260-2
Haddad, F., Zaldivar, F., Cooper, DM., Adams, GR. (2005) IL-6-induced skeletal muscular atrophy, Journal of Applied Physiology, 98(3), 911-917
Hawker, GA., Ridout, R., Harris, VA., Chase, CC., Fielding, LJ., Biggar, WD. (2005) Alendronate in the treatment of low bone mass in steroid-treated boys with Duchenne muscular dystrophy, Archives of Physical Medicine and Rehabilitation, 86(2):284-288
Hoffman, EP., Brown, RH Jr., Kunkel, LM. (1987) Dystrophin: the protein product of the Duchenne muscular dystrophy locus, Cell, 51 (6), 919-28
Houde, S., Filiatrault, M., Fournier, A., Dube, J., D'Arcy, S., Berube, D., Brousseau, Y., Lapierre, G., Vanasse, MD. (2008) Deflazacort use in Duchenne muscular dystrophy: An 8-year follow-up, Pediatric Neurology, 38(3), 200-206
Houston, C., Mathews, K., Shibli-Rahhal, A. (2014) Bone density and alendronate effects in Duchenne muscular dystrophy, Muscle and Nerve, 49(4):506-11
Hutter, OF. (1992) The membrane hypothesis of Duchenne muscular dystrophy: quest for functional evidence, Journal of Inherited Metabolic Disease, 15(4), 565-77
Hsu, E., Patwardhan, A., Meade, K., Light, T., Martin, W. (1993) Cross-sectional geometrical properties and bone mineral contents of the human radius and ulna, Journal of Biomechanics, 26(11), 1307-09, 1311-1318
Iannuzzi-Sucich, M., Prestwood, KM., Kenny, AM. (2002) Prevalence of sarcopenia and predictors of skeletal muscle mass in healthy, older men and women, Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 57(12):772-7
Ichiyama, NT. (1991) Quantitative evaluation of pseudohypertrophy in Duchenne muscular dystrophy, No To Hattatsu, 23(6), 567-70 [Abstract only]
Jankord, R., Jemiolo, B. (2004) Influence of physical activity on serum IL-6 and IL-10 levels in healthy old men, Medicine and Science in Sports and Exercise, 36(6), 960-4
Janssen, I., Heymsfield, SB., Ross, R. (2002) Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability, Journal of American Geriatrics Society, 50(5), 889-896
Janssen, JC., Le-Ngoc, L. (2009) Intratester reliability and validity of concentric measurements using a new hand-held dynamometer, Archives of Physical Medicine and Rehabilitation, 90(9):1541-7
Johnell, O., Kanis, JA., Oden, A., Johansson, H., De Laet, C., Delmas, P., Eisman, JA., Fujiwara, S., Kroger, H., Mellstrom, D., Meunier, PJ., Melton III, LJ., O'Neill, T., Pols, H., Reeve, J., Silman, A., Tenenhouse, A. (2005) Predictive value of BMD for hip and other fractures, Journal of Bone and Mineral Research, 7:1185-94
Johansen, KL., Shubert, T., Doyle, J., Soher, B., Sakkas, GK., Kent-Braun, JA. (2003) Muscle atrophy in patients receiving haemodialysis: effects on muscle strength, muscle quality, and physical function, Kidney International, 63, 291-7
Jones, DA., Round, JM., Edwards, RHT., Grindwood, SR., Tofts, PS. (1983) Size and composition of the calf and quadriceps muscles in Duchenne muscular dystrophy: A tomographic and histochemical study, Journal of Neurological Sciences, 60(2), 307-322

Kelley, GA., Kelley, KS., Kohrt, WM. (2012) Effects of ground and joint reaction force exercise on lumbar spine and femoral neck bone mineral density in postmenopausal women: a meta-analysis of randomised controlled trials, BMC Musculoskeletal Disorders, 13(177): DOI: 10.1186/1471-2474-13-177

Kelley, GA., Kelley, KS., Kohrt, WM. (2013) Exercise and bone mineral density in premenopausal women: a meta-analysis of randomised controlled trials, International Journal of Endocrinology, DOI: 10.1155/2013/741639

Kent-Braun, JA., Ng, AV., Castro, M., Weiner, MW., Gelianis, D., Dudley, GA., Miller, RG. (1997) Strength, skeletal muscle composition, and enzyme activity in multiple sclerosis, Journal of Applied Physiology, 83(6), 1998-2004

King, WM., Ruttencutter, R., Nagaraja, HN., Matkovic, V., Landoll, J., Hoyle, C., Mendell, JR., Kissel, JT. (2007) Orthopaedic outcomes of long-term daily corticosteroid treatment in Duchenne muscular dystrophy, Neurology, 68(19), 1607-13

Kostek, MC., Nagaraju, K., Pistilli, E., Sali, A., Lai, SH., Gordon, B., Chen, YW. (2012) IL-6 signaling blockade increases inflammation but does not affect muscle function in mdx mice, BMC Musculoskeletal Disorders, 106(13), doi:10.1186/1471-2474-13-106

Kritz-Silverstein, D., Barrett-Connor, E. (1994) Grip strength and bone mineral density in older women, Journal of Bone and Mineral Research, 9(1), 45-51

Larson, CM., Henderson, RC. (2000) Bone mineral density and fractures in boys with Duchenne muscular dystrophy, Journal of Paediatric Orthopaedics, 20(1) 71-4

Laval, SH., Bushby, KM. (2008) Limb girdle muscular dystrophies – from genetics to molecular pathology, Neuropathology and Applied Neurobiology, 30(2), 91-105

Lee, SC., Coan, BS., Bouxsein, ML. (1997) Tibial ultrasound velocity measured in situ predicts the material properties of tibial cortical bone, Bone, 21(1):119-125

Lemmers, RJLF., van der Vliet, PJ., Klooster, R., Sacconi, S., Camano, P., Dauwerse, JG., Snider, L., Straasheijm, KR., van Ommen, GJ., Padberg, GW., Miller, DG., Tapscott, SJ., Tawil, R., Frants, RR., van der Maarel, SM. (2010) A unifying genetic model for Facioscapulohumeral muscular dystrophy, Science, 329(5999), 1650-53

Lindboe, CF., Platou, CS. (1984) Effect of immobilisation of short duration on the muscle fibre size, Clinical Physiology, 4(2), 183-8

Lynch, GS., Hinkle, RT., Chamberlain, JS., Brooks, SV., Faulkner, JA. (2001) Force and power output of fast and slow skeletal muscles from mdx mice 6-28 months old, Journal of Physiology, 535(2), 591-600

Mallmin, H., Ljunghall, S. (1992) Incidence of Colles' fracture in Uppsala. A prospective study of quarter-million population, Acta Orthopaedica Scandinavica, 63(2):213-5

McDonald, DGM., Kinali, M., Gallagher, AC., Mercuri, E., Muntoni, F., Roper, H., Jardine, P., Hilton Jones, D., Pike, MG. (2002) Fracture prevalence in Duchenne muscular dystrophy, Developmental Medicine and Child Neurology, 44, 695-698

McDonald, CM., Widman, LM., Walsh, DD., Walsh, SA., Abresch, RT. (2005) Use of step activity monitoring for continuous physical activity assessment in boys with Duchenne muscular dystrophy, Archives of Physical Medicine and Rehabilitation, 86(4), 802-8

Minnock, E., Cook, R., Collins, D., Tucker, J., Zioupos, P. (2008) Using risk factors and quantitative ultrasound to identify postmenopausal Caucasian women at risk of osteoporosis, Journal of Clinical Densitometry, 11(4): 485-493

Monaco, AP., Bertelson, CJ., Middlesworth, W., Colletti, CA., Aldridge, J., Fischbeck, KH., Bartlett, R., Pericak-Vance, MA., Roses, AD., Kunkel, LM. (1985) Detection of deletions spanning the Duchenne muscular dystrophy locus using a tightly linked DNA segment, Nature, 316(6031), 842-5

Morley, JE., Abbatecola, AM., Argiles, JM., Baracos, V., Bauer, J., Bhasin, S., Cederholm, T., Stewart-Coats, AJ., Cummings, SR., Evans, WJ., Fearon, K., Ferrucci, L., Fielding, RA., Guralnik, JM., Harris, TB., Inui, A., Kalantar-Zadeh, K., Kirwan, BA., Mantovani, G., Muscaritoli, M., Newman, AB., Rossi-Fanelli, F., Rosano, GMC., Roubenoff, R., Schambelan, M., Sokol, GH., Storer, TW., Vellas, B., van Haehling, S., Yeh, SS., Anker, SD. (2011) Sacropenia with limited mobility: an international consensus, Journal of post-acute and long-term care medicine, 12(6), 403-9

Miyatani, M., Kanehisa, H., Ito, M., Kawakami, Y., Fukunaga, T. (2004) The accuracy of volume estimates using ultrasound muscle thickness measurements in different muscle groups, European Journal of Applied Physiology, 2(3):264-272

Novotny, SA., Warren, GL., Lin, AS., Guldberg, RE., Baltgalvis, KA., Lowe, DA. (2012) Prednisolone treatment and restricted physical activity further compromise bone of mdx mice, Journal of Musculoskeletal and Neuronal Interactions, 12(1):16-23

Pane, M., Mazzone, ES., Sivo, S., Sormani, MP., Messina, S., D'Amico, A., Carlesi, A., Vita, G., Fanelli, L., Berardinelli, A., Torrente, Y., Lanzillotta, V., Viggiano, E., D'Ambrosio, P., Cavallaro, F., Frosini, S., Barp, A., Bonfiglio, S., Scalise, R., De Sanctis, R., Rolle, E., Graziano, A., Magri, F., Palermo, C., Rossi, F., Donati, MA., Sacchini, M., Arnoldi, MT., Baranello, G., Mongini, T., Pini, A., Battini, R., Pegoraro, E., Previtali, S., Bruno, C., Politano, L., Comi, GP., Bertini, E., Mercuri, E. (2014) Long term natural history data in ambulant boys with Duchenne muscular dystrophy: 36-month changes, 9(10), Published online 2014 Oct 1. doi: 10.1371/journal.pone.0108205

Passamano, L., Taglia, A., Palladino, A., Viggiano, E., D'Ambrioso, P., Scutifero, M., Cecio, MR., Torre, V., De Luca, F., Picillo, E., Paciello, O., Piluso, G., Nigro, G., Politano, L (2012) Improvement of survival in Duchenne muscular dystrophy: retrospective analysis of 835 patients, Acta Myologica, 31(2), 121-5

Philippe, V., Pruna, L., Abdel Fattah, M., Pascal, V., Kaminsky, P. (2011) Decreased bone mineral density in adult patients with muscular dystrophy, Joint Bone Spine, 78(6):651-2

Reeves, ND., Maganaris, CN., Narici, MV. (2004) Ultrasonographic assessment of human skeletal muscle size, European Journal of Applied Physiology, 91(1):116-8

Rittweger, J. (2008) Ten years muscle-bone hypothesis: what have we learned so far? –almost a festschrift-, Journal of Musculoskeletal and Neuronal Interactions, 8(2), 174-8

Snow-harter, CM. (1994) Bone health and prevention of osteoporosis in active and athletic women, Clinics in Sports Medicine, 13(2):389-404

Soderpalm, AC., Magnusson, P., Ahlander, AC., Karlsson, J., Kroksmark, AK., Tulinius, M., Swolin-Eide, D. (2008) Bone markers and bone mineral density in Duchenne muscular dystrophy, J Musculoskelet Neuronal Interact, 8(1):24

Martinson, H., Stokes, MJ. (1991) Measurement of anterior tibial muscle size using real-time ultrasound imaging, European Journal of Applied Physiology and Occupational Physiology, 63(3-4), 250-254

Soderpalm, AC., Magnusson, P., Ahlander, AC., Karlsson, J., Kroksmark, AK., Tulinius, M., Swolin-Eide, D. (2012) Bone mass development in patients with Duchenne and Becker muscular dystrophies: a 4-year clinical follow-up, Acta Pediatrica, 101(4):424-432

Sokol, RJ., Stall, C. (1990) Anthropometric evaluation of children with Chronic Liver Disease, American Journal of Clinical Nutrition, 52(2), 203-8

Sussman, M. (2002) Duchenne muscular dystrophy, Journal of American Academy of Orthopaedic Surgery, Mar-Apr; 10(2), 138-51

Ishikawa, Y., Miura, T., Ishikawa, Y., Aoyagi, T., Ogata, H., Hamada, S., Minami, R. (2011) Duchenne muscular dystrophy: Survival by cardio-respiratory interventions, Neuromuscular Disorders, 21(1), 47-51

Tawil, R. (2008) Facioscapulohumeral muscular dystrophy, Neurotherapeutics, 5(4), 601-6

Van der Ploeg, HP., Streppel, KR., van der Beek, AJ., van der Woude, LH., Vollenbroek-Hutten, M., van Mechlen, W. (2007) The physical activity scale for individuals with physical disabilities: test-retest reliability and comparison with accelerometer, Journal of Physical Activity and Health, 4(1):96-100

Vestergaard, P., Glerup, H., Steffensen, Rejnmark, L., Rahbek, J., Mosekilde, L. (2001) Fracture risk in patients with muscular dystrophy and spinal muscular atrophy, Journal of Rehabilitative Medicine, 33, 150-55

Villalta, SA., Rosenthal, W., Martinez, L., Kaur, A., Sparwasser, T., Tidball, J., Margeta, M., Spencer, M., Bluestone, J. (2014) Regulatory T cells suppress muscle inflammation and injury in muscular dystrophy, Science Translational Medicine, 6(258), 142

Washburn, RA., Zhu, W., McAuley, E., Frogley, M., Figoni, SF. (2002) The physical activity scale for individuals with physical disabilities: development and evaluation, Archives of Physical Medicine and Rehabilitation, 83(2):193-200

Weeks, BK., Beck, BR. (2008) The BPAQ: a bone-specific physical activity assessment instrument, Osteoporosis International, 19(11):1567-77

Wall, B., Snijders, T., Senden, J., Ottenbros, C., Gijsen, A., Verdijk, L., van Loon, L. (2013) Disuse impairs the muscle protein synthetic response to protein ingestion in healthy men, J Clin Endocrinol Metab, 98(12), 4872-81

Williams, P., Goldspink, G. (1984) Connective tissue changes in immobilised tissue, J Anat, 138(Pt 2), 343-350

Yamaguchi, M., Suzuki, M. (2013) Independent living with Duchenne muscular dystrophy and home mechanical ventilation in areas of Japan with insufficient national welfare services, International Journal of Qualitative Studies on Health and Well-being, 8, doi: 10.3402/qhw.v8i0.20914

Zerwekh, JE., Ruml, LA., Gottschalk, F., Pak, CYC. (1998) The effects of twelve weeks of bed rest on bone histology, biochemical markers of bone turnover, and calcium homeostasis in eleven normal subjects, Journal of Bone and Mineral Research, 13(10), 1594-1601

Figures

Wang, Q., Yang, X., Yan, Y., Song, N., Lin, C., Jin, C. (2011) Duchenne or Becker muscular dystrophy: a clinical, genetic and immunohistological study in China, Neurology India, 59(6), 797-802

Jones, DA., Round, JM., Edwards, RHT., Grindwood, SR., Tofts, PS. (1983) Size and composition of the calf and quadriceps muscles in Duchenne muscular dystrophy: A tomographic and histochemical study, Journal of Neurological Sciences, 60(2), 307-322

Philippe, V., Pruna, L., Abdel Fattah, M., Pascal, V., Kaminsky, P. (2011) Decreased bone mineral density in adult patients with muscular dystrophy, Joint Bone Spine, 78(6):651-2