Advancing the evidence base for nutritional management of boys with Duchenne muscular dystrophy

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Submitted May 2012

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Declaration

This manuscript does not contain any material that has been accepted for the award of another degree or diploma in any other university or institution. To the best of my knowledge, this thesis does not contain material published or written by another person, except where due reference is made in the text.

Zoe Davidson

Abstract

Duchenne muscular dystrophy (DMD) is the most common and the most severe of the neuromuscular disorders. Affecting 1 in 3,500 boys, the disease causes progressive and relentless muscle weakness, limiting life to the second or third decade. The mainstay of medical management involves the use of glucocorticosteroids which has significantly altered the natural history of the disease. The nutritional issues associated with DMD are complex, yet poorly understood. To date, these issues have not been an important priority of care; but as life expectancy slowly inches forward, such concerns warrant immediate attention. This thesis aims to progress the understanding and management of nutritional issues for boys with DMD by advancing the evidence base. Five distinct lines of investigation were undertaken under this broader theme. Firstly, a comprehensive literature review highlighted many gaps in the literature specifically in the areas of energy requirements, bone health, micronutrient requirements (vitamin D), growth monitoring, and use of novel amino acids and nutritional supplements. Secondly, a retrospective case series documented the genetic, physical and clinical profile of boys with DMD living in Australia, and for the first time a relationship between genotype and function was elucidated; boys with a deletion in the dystrophin gene are more likely to stop walking before age 10 years. Whilst the relationship between growth and walking ability was unclear, a higher body mass index (BMI) may be protective against a decline in lung function. This analysis also provides evidence for commencing steroid treatment earlier, as a longer duration of steroid treatment was associated with walking past age 10 years, and increased lung function. Next, vitamin D status in boys with DMD was explored. Young, ambulatory boys with DMD who attend regular school were found to have low serum 25-hydroxyvitamin D when compared to their healthy siblings, a finding that was not explained by differences in sun exposure. Also, a series of meta-analyses demonstrated that steroid therapy is associated with suboptimal serum vitamin D concentrations, and may

potentially explain the observed reduction in 25-hydroxyvitamin D. Based on data obtained by meta-analysis, recommendations for vitamin D supplementation should, at a minimum, be 1800 IU (45 µg)/d for boys with DMD receiving steroid therapy. Chapter four presents data and literature to support the use of selected outcome measures for dietary trials in DMD, specifically: StepWatch™ activity monitoring, the six minute walk test, and a three day food diary. These functional outcome measures were found to be sensitive to differences in ambulatory capacity in boys with DMD when compared to control participants. Furthermore, self-reported energy intake using food diaries was similar to total energy expenditure as determined by doubly labelled water in boys with DMD. Finally, a pilot randomised control trial is described in which a novel nutritional supplement incorporating whey protein, creatine monohydrate, glutamine and β-hydroxy-β-methylbutyrate is investigated. Results indicate that a 50 week nutriceutical intervention is feasible, and may improve functional outcome measures when used in conjunction with steroid treatment.

This thesis comprises of several smaller studies that are linked in a framework of empirical research. Firstly, the evidence base for nutritional management of boys with DMD, and their nutritional status across two major paediatric clinic populations nationally is examined. The thesis then further explores confounding factors of nutritional status and determines methodology to measure these before piloting a novel nutriceutical intervention informed by the best available evidence. This body of work, with its purely nutritional focus, is the first of its kind, and will assist dietitians to provide evidence based care to this courageous group of children.

Acknowledgements

Many colleagues from both Queensland and Victoria and a spirit of generous collaboration have contributed to this body of work. Please indulge me as I thank and acknowledge some of the key contributors to my candidature.

Professor Helen Truby has been my constant and supportive mentor over the last four years. She has unstintingly provided me with encouragement, personal support and professional guidance throughout my candidature. As a leader in the field, Helen has taught me so much about conducting research, including the value of personal resilience. In particular, I will be forever grateful to her for the support she provided me when I transferred my candidature from Queensland to Victoria. I acknowledge and thank A/Professor Karen Walker for her practical and generous support in helping me develop my writing skills and for her valuable intellectual input throughout the drafting process. I also acknowledge Dr Robyn Littlewood, who encouraged me to consider enrolling in a PhD. I thank her for the seed of opportunity planted in 2007 and for her ongoing support across both the clinical and research domains.

There have also been many 'enablers' involved in this project without whose willing collaboration this body of work could not have proceeded: to the Melbourne team at The Royal Children's Hospital, A/Professor Andrew Kornberg, and A/Professor Monique Ryan, thank you for being so supportive of allied health research; to the neuromuscular clinic coordinator, Daniella Villano and physiotherapists Katy de Valle, Dr Kate Carroll, and Rachel Kennedy, thank you for helping me with study assessments and for teaching me so much about clinical trials. Thank you also to Dr Eppie Yiu, Dr Tyson Ware and Dr Katherine Howell for your support in completing the medical assessments in the nutriceuticals study. I also gratefully acknowledge the assistance and support I have received from the Brisbane

group at Royal Children's Hospital, Mater Children's Hospital, and MontroseAccess: in particular to Dr Anita Cairns, Dr Kate Sinclair, Helen Posselt, Sue Nicklin and Robyn Peters, thank you for giving us a start in this area and for continuing to support this research in the ongoing study. Likewise, I am indebted to my many colleagues at the Nutrition and Dietetics Departments at Monash University and Royal Children's Hospital, Brisbane for their ongoing encouragement. I particularly acknowledge and thank Professor Peter Davies, Dr Ian Hughes and Marea Fox from the Children's Nutrition Research Centre in Brisbane, where I commenced my candidature in 2008.

And to the numerous individuals who have helped the work progress: to Deanna Pattieson, who was in the (un)fortunate position as both the head "shake maker" and my housemate over the last 12 months, I thank you for your careful consideration and effort in making a quality product and for your personal support in my final year; to Sarah Elliott, for analysis of the doubly labelled water; to Nicola Abel, for completing the FoodWorks entry for the nutriceutical study; and to Aimee Segman who facilitated the recruitment of control subjects for exploration of functional outcome measures, I thank you all for your assistance with this work.

Thank you to my amazing family for believing in PLZ, and helping me through the hard times; and to some fantastic friends, Kim Baxter and Sarah Elliott for sharing the journey with me. My Luke, although we have been miles apart, your support has been unwavering even in my most irrational moments. Thank you.

And finally and so importantly, my deepest appreciation goes out to the boys and their families who live with Duchenne every day. I thank you for your participation in these studies. Your courage is truly inspiring.

Academic achievements during candidature

Publications

Elliott SA, Davidson ZE, Davies PSW, Truby H. Predicting resting energy expenditure in boys with Duchenne muscular dystrophy. Eur J Paediatr Neurol. 2012;(Epub ahead of print). (Impact Factor = 1.994, ERA B)

Davidson ZE, Walker KZ, Truby H. Do Glucocorticosteroids Alter Vitamin D Status? A Systematic Review with Meta-Analyses of Observational Studies. J Clin Endocrinol Metab. 2012;97(3):738-44. (Impact Factor = 6.202, ERA A*)

Davidson ZE, Truby H. A review of nutrition in Duchenne muscular dystrophy. J Hum Nutr Diet. 2009;22(5):383-93. (Impact Factor = 1.789, ERA C, citations = 8)

Published Abstracts

Davidson ZE, Kornberg AJ, Ryan MM, Kimlin M, Truby H. Conference Abstracts Nutrition Society of Australia and Nutrition Society of New Zealand 2011: I: The relationship between serum vitamin D and sun exposure in boys with Duchenne muscular dystrophy treated with corticosteroids compared with their healthy siblings. Australas Med J. 2011;4(12):789-813. (**Oral Presentation**)

Davidson ZE, Walker KZ, Truby H. Conference Abstracts Nutrition Society of Australia and Nutrition Society of New Zealand 2011: IV: Serum 25 hydroxyvitamin D in patients treated with glucocorticosteroids: a systematic review with meta-analyses. Australas Med J. 2011;4(12):739-88. (**Poster**)

Davidson ZE, Elliott SA, Sinclair K, Wallace G, Posselt H, Truby H. Oral Program: Vitamin D insufficiency in boys with Duchenne muscular dystrophy. Nutr Diet. 2009;66:A1-A24. (**Oral Presentation, 'Best of the Best' student presentations**)

Davidson ZE, Elliott SA, Davies PSW, and Truby H, Conference Abstracts Nutrition Society of Australia and Nutrition Society of New Zealand 2009: III: Energy requirements in boys with Duchenne muscular dystrophy. Australas Med J. 2010;1(1):74-96. (**Oral Presentation**)

Elliott SA, Davidson ZE, Davies PSW, and Truby H, Conference Abstracts Nutrition Society of Australia and Nutrition Society of New Zealand 2009: III: Body composition in boys with Duchenne muscular dystrophy. Australas Med J. 2010;1(1):74-96.

Invited Presentations

Davidson ZE. Nutrition and supplements in neuromuscular disorders. Parent Project Muscular Dystrophy Conference. Denver. June 2010. (**Oral and Poster Presentation**)

Davidson ZE. Nutrition for neuromuscular disorders. Duchenne Foundation Conference. Sydney. February 2010. (**Oral Presentation**)

Other Presentations

Davidson ZE, Sinclair K, Kornberg AJ, Ryan MM, Kimlin M, Davies PSW, Truby H. Let the sunshine in? Exploring Vitamin D in DMD. Duchenne Foundation Conference. Sydney. February 2010. (**Poster Presentation**)

Awards

Best Clinical Research Poster. Awarded at Duchenne Foundation Conference. Sydney. 2010.
Received travel grant to attend and speak at Parent Project Muscular Dystrophy Conference, 2010 (Denver, USA).

Funding

$43,000 - John T Reid Charitable Trusts, Project Grant, 2010.
$9,000 - Murdoch Children's Research Institute, Critical Care & Neurosciences Theme, 2010.
$5,000 - Muscular Dystrophy Australia, Project Grant, 2010.

List of Abbreviations

BMD	Becker muscular dystrophy
BMI	body mass index
BMR	basal metabolic expenditure
CDC	Centers for Disease Control and Prevention
CrM	creatine monohydrate
DLW	doubly labelled water
DMD	Duchenne muscular dystrophy
DMD-G	Dystrophin gene
DXA	dual energy X-ray absorptiometry
FEV1%	forced expiratory volume as a percentage of predicted
FVC%	forced vital capacity as a percentage of predicted
GCS	glucocorticosteroids
GIO	glucocorticoid-induced osteoporosis
HMB	β-hydroxy-β-methylbutyrate)
LOA	loss of ambulation
REE	resting energy expenditure
SMD	standardised mean difference
TEE	total energy expenditure
25(OH)D	25-hydroxyvitamin D
6MWT	six minute walk test

1 A review of nutrition in Duchenne muscular dystrophy

This chapter relates to the published manuscript, presented in Appendix 1:

Davidson ZE, Truby H. A review of nutrition in Duchenne muscular dystrophy. J Hum Nutr Diet. 2009;22(5):383-93.

1.1 Introduction

The neuromuscular dystrophies comprise a diverse group of genetic disorders characterised by progressive muscle weakness and reduced muscle tone. Although over 30 genetic types have been identified, the most well documented and also the most severe, is the recessive X linked Duchenne muscular dystrophy (DMD). Affecting only boys, Duchenne is relentless in its progression, limiting life to the second or third decade. The body composition changes seen as the disease progresses are unique, not well understood and complicate nutritional management. Boys with DMD can move between spectrums of over to under nutrition within their shortened lifespan. In the past, nutritional issues have not been an important focus for treatment; but as life expectancy slowly inches forward with improvements in medical management, such concerns warrant immediate attention. There is no current consensus on nutritional management so dietitians remain unguided in trying to deal with the complex nutritional issues arising from DMD. This chapter aims to synthesise the current literature on nutritional issues and management in DMD.

1.2 Methods

An initial literature search was conducted using MEDLINE (via OVID, from January 1950 to March 2008) and the Cochrane Library using the term "Duchenne muscular dystrophy". This search was then updated in January 2012. A total of 2,640 articles were recovered, although only articles in English were examined for this review (N = 2,384). Studies were included (n=418) if outcome data considered: body composition (n = 14), energy expenditure (n = 16), nutrition (n = 16), corticosteroid therapy (n = 60), or gene therapy (n = 312). Reference lists were examined for additional papers not identified by the search strategy. Nutritional textbooks and chapters on the management of neuromuscular conditions were also examined for content and references.

Key themes were identified from the literature relevant to nutritional management and dietetic practice. Practice points were agreed between this author and the primary supervisor (Helen Truby, (HT)) as the most helpful to practitioners. Papers relevant to these key themes were assigned a level of evidence and grade of recommendation according to the Oxford Centre for Evidence-based Medicine criteria (1), selected because they include 'expert opinion' as a fifth level of evidence, which is appropriate for this review of the DMD literature. The practice points and supporting literature are summarised in Table 1-3 with the grade of recommendation (from A, strongest through to D lowest quality of evidence). A summary of the search and literature appraisal process is provided in Figure 1-1. Due to the many methodologies and outcome measures reported in reviewed studies, a systematic review with a meta-analysis was not possible. Where systematic or Cochrane reviews were available, these were reviewed in preference to individual papers because of the higher level of evidence provided.

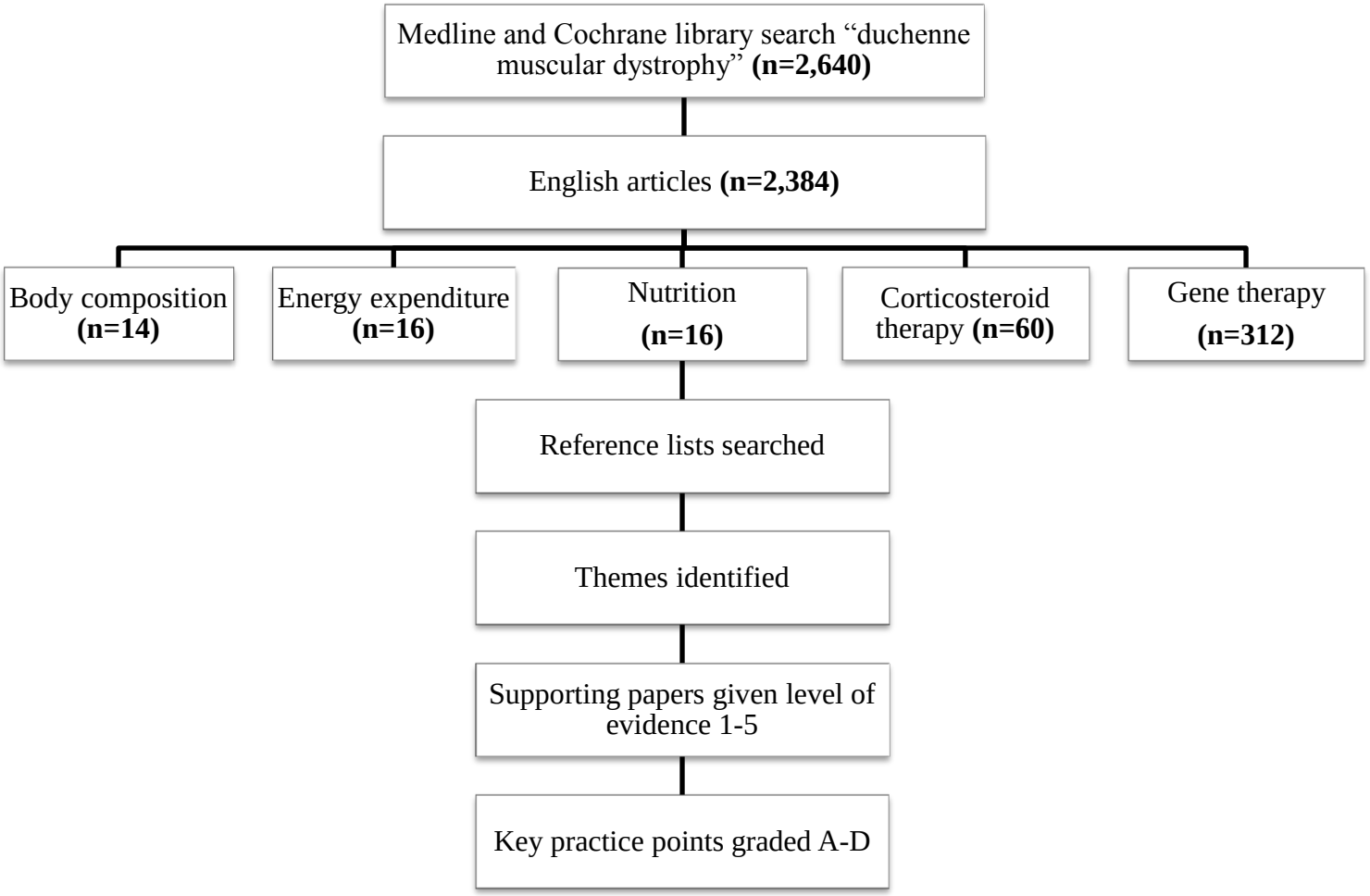

Figure 1-1 Summary of search and literature appraisal process

1.3 Duchenne muscular dystrophy

1.3.1 Prevalence and aetiology

DMD affects between 1 in 3,500-6,000 live male births (2, 3). It is caused by a large variety of mutations in the dystrophin gene located on chromosome Xp21 (4). In smooth, cardiac and skeletal muscle, dystrophin binds actin to the membrane-bound dystroglycan complex (Figure 1-2) (5). Lack of dystrophin expression leads to progressive muscle weakness and an aggressive inflammatory response associated with chronic degeneration and necrosis of muscle fibres, replacement of muscle by fat cells and progressive endomysial fibrosis. These pathological changes provoke the inexorable deteriorating clinical course of DMD (5).

1.3.2 Signs, symptoms and progression

Boys with DMD present in early childhood with failure to meet developmental milestones, and soon exhibit delayed walking, increased falls, decreased activity, difficulty climbing stairs or difficult getting up off the floor (6). Unique identifiers of DMD include a positive "Gower's sign", whereby the boy uses his hands to walk himself up his legs; and pseudo-hypertrophy of the calf muscles which is indicative of fibrous infiltration of the muscle. Toe walking may also be present. Affected boys may also be identified by the presence of elevated serum transaminases (performed for other reasons) resulting from the breakdown of muscle; although this may delay diagnosis as hepatic injury or gastrointestinal causes will often be the first line of investigation (7). Diagnosis can be confirmed by genetic analysis with or without elevated serum creatine kinase or a muscle biopsy demonstrating an absence of dystrophin (3). A family history may or may not be present, as approximately 30% of cases result from spontaneous mutations (8). The mean age of diagnosis is approximately five years of age and is often delayed by extensive investigations prior to a definitive diagnosis (9, 10). A milder phenotype, Becker muscular dystrophy (BMD), may be identified in older boys presenting with similar symptoms.

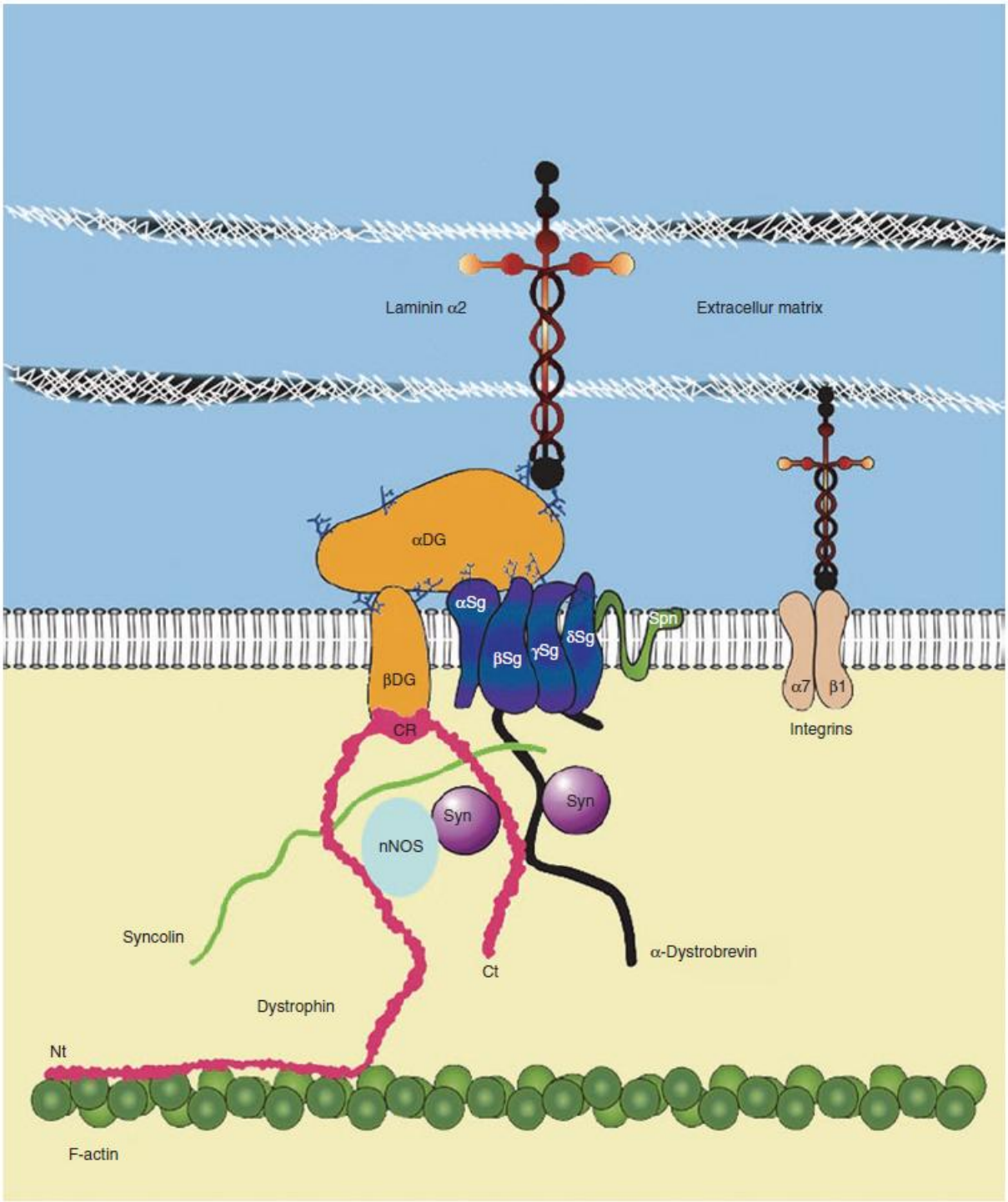

Figure 1-2 Pictorial representation of the dystroglycan complex

Dystrophin is represented by the pink rod. Other components of the complex include: C-terminal region (Ct), cysteine-rich region (CR), and N-terminal (Nt) region, DG, dystroglycan; nNOS, neuronal nitric oxide synthase; Sg, sarcoglycan; Syn, syntrophin (11).

Characteristic progressive muscle wasting becomes noticeable in early childhood and by their early teens, boys with DMD will have become wheelchair bound. Strength and function of distal muscles in the upper extremities will be best preserved (12). As boys with DMD age, they are faced with numerous medical complications: lung and cardiac function will decline at a rate of 5% and 1% per year, respectively, while 75-90% of non-ambulant boys will develop scoliosis (13, 14). Cardiac disease often presents as cardiomyopathy and/or arrhythmias (15).

Without intervention, premature death before the age of 20 years will result from failure of the respiratory or cardiac musculature (3). With dramatic improvements in recent years in the understanding and coordination of clinical care, boys diagnosed today with DMD now have the possibility of living into their fourth decade and fulfilling their educational and vocational potential (3, 16, 17).

A range of other complicating disease features affecting nutritional management can occur such as learning disabilities and gastrointestinal symptoms. These are summarised in Table 1-1.

Table 1-1 Complicating disease features that may affect nutritional management in DMD

Potential issue		Study	n	Overall findings
Gastrointestinal	**Delayed gastric emptying**	Barohn et al. (1988) (18)	11 males with DMD aged 13-27 years	The mean time for gastric emptying in DMD patients was significantly lower when compared to controls.
		Borrelli et al. (2005) (19)	10 males with DMD and 10 males with BMD aged 3-7 years.	Gastric emptying time was significantly delayed in DMD and BMD patients vs controls.
	Bowel problems	Backhouse and Harding (2006) (20)	48 males with DMD aged 4-28 years	40% of males diagnosed with DMD experience bowel and bladder problems, including constipation, daytime urgency, or pain. Bowel problems were not statistically associated with immobility but with dietary factors.
	Acute gastric dilation	Barohn et al. (1988) (18)	11 males with DMD aged 13-27 years	Of the patients, two had previous episodes of acute gastric dilation. As this can be potentially fatal, recognising this aspect of DMD may lead to earlier treatment and prevention. Other symptoms reported included postprandial belching and abdominal bloating.
		Bensen et al. (1996) (21)	Case report: 19 year old male with DMD	Gastrointestinal symptoms are more common in patients with DMD than previously appreciated and are associated with both morbidity and mortality. Although the incidence of gastric dilation in this patient population is uncertain, clinical gastrointestinal symptoms appear to increase with age. For patients with DMD who prolong their lives with mechanical ventilation, the incidence of gastric dilation may increase over time.
	Intestinal pseudo-obstruction	Leon et al. (1986) (22)	Case report: male with DMD deceased at 16 years	DMD may be associated with significant intestinal smooth muscle atrophy and fibrosis. Recognition of this complication is imperative in order to avoid unnecessary procedures and surgery.
	Other symptoms	Jaffe et al. (1990) (23)	55 males with DMD aged 4.3-20.3 years	DMD patients experienced six symptoms of upper gastrointestinal dysfunction more frequently than age matched controls: nasal quality voice, dysphagia, choking while eating, heartburn, and vomiting after meals. Only heartburn was found to be significantly more frequent in non-ambulatory boys compared to ambulatory.
		Pane et al. (2006) (24)	118 DMD aged 13.8-35.8 years	Of the 118 participants, 47% reported gastrointestinal symptoms: mostly heartburn; gastro oesophageal reflux requiring treatment 4%; constipation 36% (more frequent in patients over 18 yrs); and meteorism 23%.
	Physiology	Barohn et al. (1988) (18)	Post-mortem examination of 13 year old male with DMD	Extensive loss of smooth muscle could be seen in the stomach, particularly in the outer longitudinal muscle layer. The lost tissue had been replaced by connective tissue. The inner, circular muscle layer of the stomach was better preserved although a mild degree of fibrosis was found. Similar changes were observed in the distal oesophagus and duodenum, with milder degrees of fibrosis throughout the small and large intestines. In every area the outer, longitudinal muscle was more affected than the circular muscle layer.

Potential issue		Study	n	Overall findings
Gastrointestinal	**Physiology**	Jaffe et al. (1990) (23)	Post-mortem examination two males with DMD aged 15 and 20 years	Cross-sectioned smooth muscle in oesophagus, stomach, small and large bowel revealed a moderate increase in the amount of intramuscular connective tissue.
	Dental characteristics	Ghafari et al. (1988) (25)	43 males with DMD mean age 10.5 years	In the DMD group, 58% had a posterior cross bite; 21% had an anterior open bite; 47% were observed mouth breathing; and 42% were determined to have a 'large tongue'. DMD patients exhibited a significantly delayed dental emergence (1.06 years). The study emphasizes the influence of muscular environment on dental development in general.
Bladder problems		Backhouse and Harding (2006) (20)	48 males with DMD aged 4-28 years	40% of males diagnosed with DMD experience bowel and bladder problems including daytime urgency, voiding postponement, daytime urinary hesitancy, and urinary frequency. High levels of urinary problems were reported in the transition group. Voiding postponement was significantly related to reduced drinking (fluid intake).
Learning difficulties		Berardinelli et al. (2001) (26)	9 males with DMD aged 6-12 years	Mean IQ of the study group was below average intelligence.
		Fabbro et al. (2007) (27)	8 males with DMD with mean age 16 ± 4.7 years.	Results showed mild mental retardation associated with a specific deficit in Verbal rather than Performance IQ. At the linguistic assessment, 7 of 8 participants showed moderate to severe difficulties with oral language processing.
		Hinton et al. (2000) (28)	80 males with DMD aged 6 – 16 years.	Boys with DMD have a specific cognitive profile, regardless of their general level of cognitive function. Specifically, boys with DMD performed more poorly on tests requiring attention to complex verbal information than they did on other verbal or memory measures. The possibility that the missing dystrophin brain products may contribute to selective cognitive processing is considered.
		Cyrulnik et al. (2008) (29)	20 males with DMD aged 3-6 years. 20 familial controls	Children with DMD exhibit impairments on multiple measures of cognition, including measures of receptive language, expressive language, visuo-spatial skills, fine-motor skills, attention, and memory skills. Across all domains examined, the young children with DMD performed more poorly than their familial controls.
		Donders and Taneja (2009) (30)	22 males with DMD aged 6-16 years. 18 unaffected siblings	Boys with DMD were weaker in verbal and non-verbal measures of delayed recall and response generation compared to their siblings. Parent reported more difficulties with social interaction, initiation and adaption for their sons with DMD compared to their other children.
		Hendriksen and Vles (2008) (31)	351 males with DMD mean age 11.9 ± 5.2 years	Using a parent reported questionnaire, the prevalence of attention-deficit hyperactivity disorders, autism spectrum disorders, and obsessive–compulsive disorders was 11.7%, 3.1%, and 4.8%, respectively.
		Mochizuki et al. (2008) (32)	194 males with DMD aged 10-44 years	Mental retardation, defined as intelligence quotient score < 70, was demonstrated in 38% of the population. Males with DMD with mental retardation started walking later, required ventilator and tube nutrition support earlier, and died earlier than those without mental retardation.

1.3.3 Clinical management

1.3.3.1 Recent advances

In the period between the original literature review (May, 2009) and its update (January, 2012), there have been several key publications reporting on consensus statements for many aspects of clinical care for individuals with DMD. The most important of these are the practice guidelines for the diagnosis and management of DMD published as part 1: *Diagnosis, and pharmacological and psychosocial management*, and part 2: *Implementation of multidisciplinary care* (3, 15). Other consensus statements published in the last three years cover the molecular diagnosis of Duchenne and Becker's muscular dystrophies, respiratory management, endocrine aspects, and bone protection (33-36). Whilst it is evident from these publications that more research is still needed across many areas, these publications are significant and should greatly improve the coordination of clinical care. Notably, despite the documented side effects, the use of glucocorticosteroids (GCS) for the treatment of DMD is supported in all consensus statements.

1.3.3.2 Glucocorticosteroids

GCS are now accepted as best practice for the medical management for DMD usually given either as prednisone or deflazacort (3, 37, 38). GCS have clear benefits in the maintenance of muscle strength and functional ability (39). Their precise mode of action is unclear but is thought to be related to improving muscle fibre regeneration, increasing the size and strength of muscle fibres, decreasing cytokine activity, and suppressing the inflammatory process (40). The optimal time to commence GCS treatment is unclear (38). The recent practice guidelines suggest GCS should be commenced when a boy reaches a plateau; that is, when there is no longer any progression of motor skills but prior to any decline (3). This will usually occur between the ages of 4-8 years. The standard initial therapeutic dose is 0.75mg/kg/day for

prednisone and 0.9mg/kg/day for deflazacort (38) with preference for a daily regimen over alternative regimens (alternate day, high dose weekend, or 10 day on/10 day off cycling) (3). During the course of the disease, boys treated with GCS have improved functional capacity, less scoliosis and better pulmonary function than those not taking GCS (41-44). Side effects of treatment such as excessive weight gain, cushingoid features, osteoporosis, impaired growth, hirtsuism, glucose intolerance, cataracts and behavioural changes, can be of sufficient concern to cause their withdrawal (35, 42, 45). GCS therapy can also delay puberty, which in itself has repercussions for bone health and psychosocial wellbeing (35, 46). This gamut of endocrinological issues is now the focus of much discussion (35).

Data on the longer term effects of GCS are beginning to emerge, although it would seem overall that the body of evidence is still catching up with the now regular use of GCS in clinical practice (39, 42, 47). Data from the United States demonstrates that GCS use for DMD has increased from 20% in 1991 to 44% in 2005 (48). GCS therapy is also changing the natural history of DMD. A recent review by Moxley et al. demonstrates that long term GCS therapy in boys with DMD prolongs ambulation by 2-5 years, reduces the need for spinal surgery, improves cardiopulmonary function, delays the need for ventilation, and increases survival and quality of life (49). Further research is required to remap the natural history of DMD in GCS treated boys. The evaluation of the literature on DMD needs to be appraised critically in light of the advent of GCS therapy. In many publications it is unclear if patients received GCS therapy or were steroid-naïve.

1.3.3.3 Multidisciplinary team management

The goals of multidisciplinary treatment are supportive and work in conjunction with GCS treatment. The multidisciplinary team involves many specialities: cardiology, respiratory, orthopaedics, gastroenterology, physiotherapy, occupational therapy, psychology, social

work, and nutrition and dietetics (3). With the exception of physiotherapy, boys may only require these specialities sporadically. Daily physiotherapy, on the other hand, aims to maintain mobility, flexibility and independence in boys with DMD and may reduce complications such as the development of contractures and scoliosis (50).

1.3.3.4 Gene therapy for the future

Identification of the dystrophin gene and the development of the *mdx* mouse (a mouse model of DMD) in the 1980's have contributed to our understanding of the disease process. The DMD gene is the largest gene, spanning ~2.5Mb of genomic sequence, and is composed of 79 exons (5). Developing gene therapy has been hampered by the complexity, size, and sheer number of mutations of the dystrophin gene; over 4,700 mutations are known (11).

Despite these complexities, many promising advances into treatments for DMD have emerged since 2008. Therapeutics can be divided into two categories: pharmaceutical interventions that aim to restore dystrophin expression, or gene therapy whereby a functional copy of the DMD gene is introduced into the muscle fibres. Pharmaceutical therapies appear to be the most promising treatment option to date. Ataluren (PTC124®) is an orally administered drug that promotes ribosomal read-through of nonsense mutations. Phase 2b clinical trials were well tolerated and produced an improvement in the six minute walk test (6MWT) of 29.7m; however, the trial was halted as statistical significance was not reached (which was a 30 metre change in the 6MWT) (51). PTC Therapeutics however, has recently announced an open label study of ataluren for all previous study participants, regardless of ambulatory status (52). This treatment will have limited reach (10-15% of DMD patients), as nonsense mutations are one of the least common genotypes (11). Conversely, pharmaceuticals targeted at exon skipping could theoretically be applied to 80% of DMD patients (53).

Exon skipping uses antisense molecules that can skip targeted exons. This can restore the open reading frame for dystrophin and allow for the expression of an internally deleted (truncated) dystrophin with some residual functionality (11). While not fully functional, the presence of this truncated dystrophin may ameliorate the most severe symptoms, resulting in the milder phenotype (BMD). Two different types of antisense molecules can skip exon 51 and both have been investigated in Phase 2 clinical trials: phosphorodiamidate morpholino oligomer (AVI-4658) and 2′-O-methylphosphorothioate (PRO051/GSK2402968) (54, 55). Both trials showed positive results including dystrophin restoration and improvements in functional and strength outcomes. Phase 3 trials in PRO051/GSK2402968 are now underway (56).

Whilst pharmaceutical interventions are progressing, few genetic therapies have yet been trialled in DMD. A recent clinical trial in six boys with DMD did not demonstrate gene expression, and some participants displayed an immune response to the therapy (57). Until a cure is found, therapeutic and supportive interventions must assist in preventing or delaying complications and preserving quality of life; and nutrition is integral to those outcomes.

1.4 Best practice guidelines - nutritional management

The original literature review by the candidate on nutritional management in DMD was published in 2009 (Appendix 1). Subsequently, the best practice guidelines for the diagnosis and management of DMD were released with the inclusion of a section on nutritional management (15). Although the two publications are completely separate entities and the candidate was not involved in the guideline process, the recommendations of the practice guidelines are in line with the candidate's published literature review. The risk of patients becoming both over- and underweight is highlighted as a primary concern, in addition to potential deficiencies in energy, protein, vitamins, minerals and fluid as the disease progresses. Regular monitoring of height and weight, and anticipatory guidance for the prevention of over- or under-nutrition are recommended, as these are key triggers for referral to a dietetic professional (Figure 1-3). Beyond this, recommendations for dietetic goals are somewhat broad; the dietitian should aim to provide a well-balanced, nutritionally complete diet. Specific statements regarding appropriate weight classification, micronutrients, and nutriceuticals are discussed below. This literature review supports the practice guidelines by providing the "how and why" for nutritional management of boys with DMD.

Panel 2: Improving underweight and overweight status

Monitor regularly for:

- Weight*
- Linear height in ambulatory patients (measured every 6 months)
- Arm span/segmental length in non-ambulatory patients†

Refer for a nutritional/dietetic assessment:

- At diagnosis
- At initiation of glucocorticoids
- If the patient is underweight (<10th age percentile)‡
- If the patient is at risk of becoming overweight (85–95th age percentile)‡
- If the patient is overweight (>95th age percentile)‡
- If there has been unintentional weight loss or gain
- If there has been poor weight gain
- If major surgery is planned
- If the patient is chronically constipated
- If dysphagia is present

National guidelines and recommendations for diets for underweight and overweight individuals can be found in Kleinman,[118] and are often available from charities/associations for cardiac disorders and diabetes. *In non-ambulatory patients, wheelchairweight should be obtained first, then patient and wheelchairweight, or caregiverweight should be obtained first, then the weight of the patient held by the caregiver. †If the patient has scoliosis, the arm span should be measured if possible. ‡Overweight/underweight status should be judged on the basis of local body-mass index percentiles (weight for age is a possible alternative if height is unavailable). Body composition is altered in Duchenne muscular dystrophy (DMD) owing to the relatively low lean body mass seen in DMD with a relatively higher fat body mass.[119]

Figure 1-3 Current recommendations regarding dietetic assessment

As per recent practice guidelines for DMD (15)

1.5 Nutritional requirements

1.5.1 Energy requirements

It is unclear whether decreased energy expenditure – either from reduced voluntary activity, or a primary defect in energy metabolism – is a contributing factor to obesity in DMD; or if indeed there is no difference in energy expenditure between boys with DMD and their healthy counterparts. As described in Table 1-2, few studies are available in this area and they yield contradictory findings. The eight identified studies all have limitations: small sample sizes, a large age range and a failure to determine total energy expenditure. Only four studies have examined energy requirements in younger children and it is unclear in most studies whether the participants were receiving GCS therapy. In younger boys, absolute resting energy expenditure (REE) appears to be similar to control cohorts. Energy requirements become complicated as boys with DMD age due to changes in their respiratory function. In particular, REE can be affected when gas exchange is impaired, secondary to a decrease in respiratory muscle strength (58). The literature is inconclusive as to energy requirements in advanced stages of DMD. Okada et al. measured basal metabolic rate (BMR) in 310 individuals with DMD aged 11-29 years (59). DMD subjects had an increased BMR when compared with controls, and BMR increased with age. Gonzalez-Bermejo et al. measured REE in a similar patient group of 20 males aged 20-33 years on mechanical ventilation (60). REE was significantly lower in DMD participants compared to controls. The difference in REE findings could be the result of the presence of ventilation support in the latter study. Similarly, the most recent publication exploring energy expenditure suggests a decreased REE compared to controls across several age groups (10-11, 12-14, 15-17, 18-29, and ≥ 30 years) (61). Interestingly, this group found that REE was directly correlated with vital capacity (r=0.51).

There is insufficient evidence to develop predictive energy equations for DMD; however, our recent publication demonstrates that the Schofield weight equation is the most accurate and

precise for predicting REE in boys with DMD (Appendix 2)(62). Dietitians need to make an individualised ‘best guess’ to establish energy requirements using the following algorithm: total energy expenditure = REE (measured if possible or from Schofield weight equation) x physical activity level (63). Individual longitudinal monitoring of change in body weight will provide a crude indication on the accuracy of energy prescription (64).

1.5.2 Micronutrient requirements

There has been little investigation into the micronutrient status and requirements of boys with DMD. Current practice guidelines recommend that boys with DMD receive a daily multivitamin supplement with vitamin D and minerals; although given the lack of evidence on nutrient requirements in these patients, this recommendation is somewhat arbitrary (15). In the advent of GCS therapy, the micronutrients that require immediate attention are vitamin D and calcium.

Canalis et al. describes how pharmacological doses of oral GCS can suppress bone formation and increase bone resorption, thereby leading to osteoporosis (65). The use of GCS therapy combined with decreased mobility suggests that boys with DMD are at an increased risk of fractures and poor bone health (66). Low bone mineral density and increased fracture risk in males with DMD have been reported by several groups (66-69). The effect of a fracture on mobility can be devastating, as 30-50% of boys sustaining a fracture in their lower limbs will never resume walking (69, 70).

Table 1-2 Summary of literature on energy studies in DMD

Study	Year	n (age range)	Results
Okada et al. (59)	1992	310 (11-29 years)	• DMD subjects had an increased BMR (kcal/day) compared to controls. • BMR (kcal/day) was found to increase with age.
Hankard et al. (71)	1996	13 (8-13 years) 5 obese; 8 non-obese	• REE (kcal/hr) was decreased only in non-obese DMD subjects when compared to controls. • There was no difference in REE (kcal/hr/kg BW) between controls and obese and non-obese DMD subjects. • REE (kcal/hr/kg FFM) was higher in only the obese DMD group.
Berardinelli et al. (26)	2001	9 (6-12 years)	• REE (kcal/kg BW) in DMD subjects was similar to controls. • REE (kcal/kg LBM) was increased in DMD subjects when compared to controls.
Satomura et al. (72)	2001	8 (16-24 years)	• REE (kcal/m^2) was similar in DMD subjects compared to controls. • REE (kcal/m^2/FFM) was increased in DMD subjects compared to controls.
Zanardi et al. (73)	2003	9 (6-12 years) 3 obese; 6 non-obese	• REE (kJ/kg BW) of DMD subjects was similar to controls of the same age. • REE/(kJ/kg FFM) was higher in DMD subjects than in controls.
Gonzalez-Bermejo et al. (60)	2005	20 (20-33 years)*	• Measured REE (kJ/day) was significantly lower in DMD subjects compared to controls. • This difference disappeared when REE (kJ/day) was corrected for FFM. • Measured REE (kJ/day) of DMD subjects was 22% lower than the theoretical REE, calculated using standard energy equations (Harris Benedict).
Hogan (74)	2008	4 (11-22 years) REE only in 3 subjects	• On observation only, REE (kJ/day) was reduced compared to control data from literature.
Shimizu-Fujiwara et al. (61)	2011	77 (10-37 years)	• REE (kcal/day) was reduced across all age groups (10-11, 12-14, 15-17, 18-29, and ≥ 30 years) compared to control data from the literature.

*All subjects received respiratory support (nocturnal or continuous), BMR basal metabolic rate; REE resting energy expenditure; BW body weight; FFM fat free mass; LBM lean body mass.

Serum 25-hydroxyvitamin D (25(OH)D) was examined in 24 boys with DMD aged 2.3-19.7 years, most of who were being treated with prednisolone, and in 24 age- matched controls (66). Significantly lower levels of 25(OH)D were found in the DMD group (21 ± 6 ng/ml) than in controls (30 ± 8 ng/ml). Although the levels of vitamin D found in the DMD boys constituted borderline 'insufficiency' (< 20ng/ml) (75), food records from 15 subjects indicated that dietary calcium and vitamin D intake was 114% and 94% of the recommended daily intake, implying either that requirements are increased or there is another mechanism predisposing boys with DMD to vitamin D insufficiency. Bianchi et al. reports similar findings (76). Both authors report uncertainty as to why 25(OH)D levels are reduced in boys with DMD. Nutritional interventions designed to correct 25(OH)D insufficiency and optimise calcium intake could improve bone health, especially in boys using GCS.

Recently, Bianchi et al. reported on results from an intervention in which boys with DMD treated with GCS were provided with vitamin D supplementation (0.8µg/kg/day) together with dietary counselling to optimise calcium intake (77). Participants were observed for a one year period prior to commencing the dietary changes. Following the two year intervention, serum 25(OH)D levels normalised (20-55ng/ml), and an increase in bone mineral density was observed in 65% of boys.

Despite these findings, the appropriate dose of vitamin D supplementation for boys with DMD is unclear. Unpublished findings described in a workshop report detail the variability of responses to vitamin D supplementation in boys with DMD (36). In boys receiving 1200-1300 IU vitamin D daily, 50% showed no response to therapy, whilst the mean increase in 25(OH)D in the remaining children was 13 ng/ml. Biggar et al. recommend daily calcium (750mg) and Vitamin D (1000 IU) supplementation in conjunction with GCS therapy for those participating in a longitudinal trial of deflazacort therapy (39). Vitamin D metabolism and appropriate supplementation doses are areas which would benefit from future research.

1.6 Nutritional assessment

1.6.1 Height

Short stature in boys with DMD has been documented but its precise aetiology is unclear. Eiholzer et al. conducted a retrospective longitudinal analysis of growth in 25 boys with DMD (78). Whilst mean birth length is normal or slightly elevated, growth velocity slows and crosses percentile channels at 1.5 SD below the mean during the first year of life. Growth is maintained at a normal velocity from age 3-4 years. The findings of Eiholzer et al. are supported by Nagel et al. and Willig et al. who determined mean z-scores for height for their populations as -1.0 and -0.936, respectively (79, 80). McDonald et al. demonstrated that reduced stature remains a feature into adulthood (81). Interactions of GCS and growth require long term follow up. Biggar et al. demonstrated that boys treated with deflazacort were significantly shorter than boys not treated at age 10 years, 15 years and 18 years (39). Whilst short stature may offer some benefits in terms of functional ability, it may be detrimental to the psychological health and quality of life for males with DMD (35). Currently, there is a lack of evidence to support the use of growth hormone treatment.

Although standing height is the preferable measurement, accurate measurements of height become difficult to obtain approaching adolescence because of boys' inability to stand combined with the presence of severe scoliosis and/or contractures. Stewart et al. suggest measurement of upper arm length, tibial length or knee height as surrogates for total height in these circumstances (82).

1.6.2 Weight

In steroid-naïve boys, weight is disproportionately distributed throughout the stages of DMD. McDonald et al. reported that 44% of boys aged 9-13 years were above the 90th percentile in weight, whilst, after age 17 years, 65% weighed less than the 10th percentile (81). Similar

findings were presented by Willig et al. (80). Of the 252 boys studied, the prevalence of obesity, defined as weight above the 90th percentile, reached 54% by age 13 years. Under-nutrition (weight below the 10th percentile) then occurred after age 14 years, involving 54% of the boys by age 18 years. Recent data suggests an increasing prevalence of obesity; 73% of patients were obese by 13 years of age and at maximal follow up (mean age 18.3 years), 47% of patients were obese and 34% were underweight (83). All of these studies were conducted in steroid-naïve cohorts. Again, the interaction between weight and GCS treatment is significant; this is discussed in further detail in section 1.8.

1.6.3 Body composition

Body composition in DMD is characterised by a decreased lean body mass and an increased fat mass (84). As much of the fat mass is intramuscular (84), standard anthropometric measures (ie. skinfold thickness) are not as accurate in assessing adiposity as other body composition techniques (84-87). Body composition measures most often used with the DMD population include dual energy X-ray absorptiometry (DXA)(66, 68, 87-89), bioelectrical impedance (60, 71, 86, 87, 90), total body potassium (91, 92), magnetic resonance imaging (MRI)(26, 73, 84, 85, 93, 94), and urinary creatinine excretion (71, 91, 92, 95). The variety of different methodologies and outcome measures has precluded any meta-analysis. Qualitatively, these studies demonstrate that DXA and MRI are accurate and non-invasive measures that would be appropriate for a paediatric population. DXA may be more readily available for clinical evaluations, and has been shown to correlate with strength measures (89).

Classifying boys with DMD as overweight or underweight based solely on height and weight or body mass index (BMI) without concurrent accurate estimates of body composition is problematic. Pessolano et al. cautioned against using BMI for evaluation of nutritional status

in boys with DMD (96). The researchers compared the number of boys classified as overweight using BMI (n=5) to the number of boys classified as overweight using the percentage of expected weight for zero muscle mass (n=30). However, it is necessary to account for reduced stature in boys with DMD when assessing weight. As no other disease specific index exists to classify weight for height, BMI remains a useful clinical tool if these limitations are acknowledged.

1.6.4 Growth charts

A specific DMD weight chart was created using the principle that both creatinine excretion and total body potassium, when related to height, decline at an average rate of 4% per year (97). At 6 and 17 years, boys with DMD have 60% and 20% of predicted muscle mass, respectively. This muscle mass was added to theoretical weight centiles drawn for zero muscle mass to produce an ideal body weight for boys with DMD. When comparing this chart to the Centers for Disease Control and Prevention (CDC) 2000 weight-for-age charts, the percentiles sit lower to take into account the loss of muscle mass.

Willig et al. examined the validity of this ideal weight chart (80). The researchers collected 2,387 individual weights from 252 DMD subjects aged 5-19 years. The researchers confirmed the accuracy of the weight chart up until the age of 12 years for the 10th percentile, and up to the age of 7 years for the 90th percentile. After these ages, the observed curves deviate greatly from the ideal chart. Perhaps the accuracy of the chart is better represented by McDonald et al., documenting the natural history of DMD, who compared weight measures of 162 boys with DMD to the ideal weight chart (81). They found that before age 17 years, a disproportionate percentage of participants (64 – 73%) fell above the 90th percentile for ‘ideal’ weight. Conversely, after age 17 years, 23% of participants fell below the 10th percentile while 53% of participants were above the 90th percentile.

These papers highlight a matter of common contention when using growth charts: should clinicians use charts that illustrate how children should grow (ideal growth charts) or charts that illustrate how children actually grow (standard growth charts)? The best assessment of growth will be made with clinical judgement based on an individual's serial anthropometric measures, hence standard growth charts such as the 'UK 90' or the 'CDC 2000' are appropriate for use when one is aware of the comparison population (64). The recent practice guidelines also recommend the use of national percentile charts (15). Here, the working group has defined good nutritional status as a weight-for-age or BMI-for-age falling between the 10th and 85th percentiles.

1.7 Nutritional concerns

1.7.1 Early onset obesity

Excess weight gain occurs in the early stages of the disease, with a dramatic inflexion in the observed 90th weight percentile occurring from an age of seven years (80). Effects of increased weight can be extremely debilitating. Obesity contributes to the progression of the disease by exerting extra force on already weak muscle groups, essentially decreasing mobility. In an examination of the body composition of 13 boys aged 9-13 years with DMD, Hankard et al. split the group into obese DMD (n = 5) and non-obese DMD (n = 8), in accordance with the Griffiths and Edwards weight chart (71, 97). Their analysis found no difference in muscle mass between obese and non- obese participants, suggesting that obesity should be considered only as a factor increasing the level of disability. Obesity also has implications for increased respiratory involvement, poorer psychosocial development, and can make the activities of daily living and care at home more difficult.

Excess weight gain is exacerbated in boys treated with GCS as their increased appetite contributes to over-consumption of energy (98). Weight gain is the most frequently reported side effect for DMD children on GCS and is a major concern for parents/caregivers (47). A systematic review conducted by Moxley et al., determined that weight gain occurred in boys given prednisone in the dose range of 0.3-1.5mg/kg/day (38). In patients treated for 6 months, 20%-24% of those receiving a placebo increased their weight by ≥ 10% compared with 75-80% of patients receiving 0.75 mg/kg/day prednisone. Likewise, 43% of patients receiving a placebo gained ≥ 20% of their baseline weight after 18 months compared with 75% of patients receiving 0.75 mg/kg/day prednisone. Despite their weight gain, patients showed clinical improvement. Interestingly, the source of the weight gained, ie: a gain in muscle versus a gain in fat, differed between those patients receiving prednisone compared with those receiving a placebo. At the completion of the 18-month prednisone trial a 36% increase in muscle mass in the 0.75 mg/kg/day prednisone group compared

with placebo-treated patients was demonstrated using urinary creatinine excretion. These findings challenge health professionals to re-consider the definition of "excessive weight gain" and to consider the need for measurement of body composition as part of the nutrition assessment.

There is debate about whether the type of GCS contributes to the severity of induced side effects. It has been suggested that those treated with deflazacort do not gain as much weight as those receiving prednisone. Bonifati et al. followed 18 boys over 1 year in a double blind, randomised controlled trial of deflazacort vs prednisone (99). After 9 months, the average weight increase with respect to baseline value was 5% in the deflazacort group compared with 18% in the prednisone group. This change remained significant after 12 months. Balaban et al. report similar results when examining the long term effects of GCS treatment in DMD (42).

1.7.2 Malnutrition

As boys with DMD age, the likelihood that they will become underweight is increased. Significant muscle wasting will result in weight loss. This is accentuated by eating difficulties experienced at meal times usually leading to a reduced food intake. Iannaccone et al. in a retrospective chart review investigated malnutrition following surgical procedures in nine boys with DMD aged 14-17 years (100). Weight loss after surgery was found to be associated with loss of the ability to self-feed. Some possible explanations suggested were: a decrease in the range of motion of the neck, or decreased head control; lack of adequate care-giving; or low cognitive function. Intervention to increase energy intake resulted in weight gain. Pane et al. reported on feeding problems experienced by 118 individuals with DMD aged 13.8-35.8 years (24). Of those in the sample, 20% reported chewing difficulties leading to an increase in meal duration, 10% had difficulties opening their mouth, and 18% experienced episodic

choking. In a similar survey, Willig et al. collected responses from 41 individuals with DMD (mean age 18.7 ± 5.7 years), as well as several other neuromuscular diseases (101). The most prominent problem encountered was getting food to the mouth. Moreover, 53% and 33% of respondents also reported difficulties with chewing, and choking, respectively.

1.8 Nutritional management and monitoring

1.8.1 Obesity

Standard dietetic practice for weight management is to manipulate the energy balance equation by decreasing intake of food energy, and by increasing activity to increase total energy expenditure. The practice of "eat less, do more" however, should not be applied to boys with DMD. Although obesity raises considerable parental concern, caution is needed when inducing a negative energy balance as this may increase loss of lean body mass which does not have the potential to regenerate. In adults on various energy restricted diets, it has been shown that weight loss comprises fat mass loss (around 70%) and lean mass loss (around 30%) (102). The issue of activity prescription also remains uncharted territory. There is a risk of increased muscle breakdown from overuse which must be balanced against the increased risk of muscle weakness from disuse (103). Activity prescription should be guided by physiotherapists. Probably due to these factors, there is only one relatively old publication on successful weight loss through dietary intervention in just one boy with DMD and another with BMD (104).

These two case studies demonstrated that weight loss was possible and led to an increase in mobility and self-esteem without deleterious effects on total body nitrogen (a surrogate measure of lean mass). However, the authors cautioned that weight loss should be carefully monitored due to the potential loss of lean tissue that usually results during periods of negative energy balance. To achieve this weight loss in the boy with DMD, energy intake was restricted to 2,600 kilojoules/day (55g protein, 30g fat, 40g carbohydrate) from January to December, 1981, leaving one to query micronutrient adequacy and patient satisfaction. Intake was then increased to 4,200 kilojoules/day (40% protein) and then gradually to 7,100 kilojoules/day. Total weight loss was 37.8 kg (from an initial weight of 86.6 kg). Whilst this

reports an extreme weight loss program, success is more likely to be achieved with family orientated, lifestyle interventions.

A recent case series investigated the feasibility and efficacy of a parent-focused intervention in three families with sons with DMD (105). The intervention aimed to improve eating and physical activity habits in the parents, and to subsequently improve weight in their sons with DMD. Although weight changes in boys with DMD following the intervention were variable; other favourable outcomes were observed. Specifically, the parents' weight decreased and there was an increase in the amount of healthy foods available in the home and in the children's perceived quality of life.

Pharmaceutical therapy may complement dietary and lifestyle interventions. This may firstly involve adjusting GCS dose. The recent practice guidelines recommend that GCS should only be titrated to a weight of 40 kg (maximum of 30 mg/day prednisone or 36 mg/day deflazacort) (3). If weight gain is unmanageable and intolerable, GCS dose may be reduced by 25-50% over several visits, ensuring that the dose is above the minimum effective level (0.3mg/kg/day). Alternatively, deflazacort could be trialled, or the GCS dosing regimen may be changed to a high dose weekend or an intermittent schedule (10 days on with 10 or 20 days off). Other pharmaceutical interventions may work in conjunction with GCS treatment.

The use of metformin, an insulin sensitiser, has been examined in a double blinded randomised controlled trial in a population of children with a primary muscle disorder or neural tube defect who were clinically obese or who had gained excessive weight in the previous year (106). The cohort included 13 boys with DMD, 12 of who were treated with deflazacort. Children treated with metformin gained significantly less weight during the treatment period, and also reduced their BMI. Other outcomes included an improvement in

insulin sensitivity, fasting glucose and fasting insulin, and a reduction in visceral fat. Although metformin therapy warrants further investigation, these results suggest that boys treated with GCS who have gained excessive weight should be evaluated for impaired glucose metabolism (35). Similarly, in 2005, Carter and colleagues published a letter reporting anecdotal evidence of weight loss in 2 boys with DMD using the anti-epileptic drug, topiramate (Topamax, Janssen-Cilag) (107). The patients lost 26% and 41% of their initial body weight, respectively. The authors concluded that further study investigating the use of pharmaceutical weight loss agents is warranted.

The most constructive advice in the management of overweight boys with DMD boys is given by Griffiths and Edwards in 1988: “prevention of excess weight gain in the younger patient is preferable to severe dietary restriction in the already obese subject” (97). The modern manifestation of this advice would be to provide anticipatory guidance to boys commencing GCS, and to follow up with regular monitoring of growth, in particular of weight gain. Given the devastating effects of excess weight on mobility, muscle loss associated with negative energy balance needs to be weighed up against the detriments of fat gain in the ambulatory DMD population.

To enable the provision of anticipatory guidance, height and weight should be measured at a minimum of every six months and plotted on growth charts (15, 82). As discussed in section 1.6.4 standard growth charts such as the ‘UK 90’ or the ‘CDC 2000’ can be used in clinical practice to monitor longitudinal growth and development in neuromuscular disorders.

1.8.2 Malnutrition

Dietary modification is an important strategy to manage feeding difficulties. Modifications include cutting food into smaller pieces, and with older individuals, changing texture to

smooth foods (24). A multidisciplinary team should be involved in the management of feeding difficulties with individual preference being paramount (64, 82). Gonzalez-Bermejo et al. analysed dietary intake in an older group of 20 DMD adults aged 22-33 years, using home mechanical ventilation (60). Despite using ventilator support nocturnally (n =13) or continuously (n = 7), no subjects had a gastrostomy and/or used oral supplements. However, all subjects reported eating slowly and with difficulty, but stated that these inconveniences were largely compensated for by the pleasure of eating a normal meal.

The nutritional benefits of enteral feeding have now been demonstrated by several authors (108-110). Collectively, gastrostomy feeding improved nutritional status across all cohorts and was well tolerated. In the literature, the incidence of gastrostomy feeding tubes in older boys with DMD ranges from nil to 50% (60, 111). Indications include difficulty in swallowing leading to aspiration, and under-nutrition (112). As with other conditions, multiple factors should be considered before gastrostomy placement. When the disease has reached a stage where alternate feeding options need to be considered, many patients have severe respiratory insufficiency, putting them at high risk of major complications when they are anesthetized or sedated for the gastrostomy procedure (113). Despite the severe risks, Birnkrant et al. have detailed successful gastrostomy placements in a 22 year old male (weight 25.2kg, forced vital capacity 20%); and a 19 year old male (weight 30.5kg; forced vital capacity 2 years prior to procedure 17%) (113). At 6 and 4 weeks following their procedure, serum pre-albumin had risen from 134 to 197 mg/L and from 172 to 255 mg/L, respectively. The authors encourage physicians to consider whether providing enteral nutrition support should be proactively planned at earlier stages of the disease process, before the risks associated with anaesthesia become high in those with compromised respiratory capacity.

In the past, gastrostomy placement in the DMD population was rare, most likely due to ethical issues surrounding this procedure (24). Hilton et al. presents an excellent discussion on these ethical considerations: "the 'can do' versus the 'should do'… the ability to act does not justify the action" (111). In the face of such confronting ethical decisions in medicine, and in this case dietetics, the authors suggest four considerations to professionals: medical indications; patient preference; quality of life; and contextual features (social and medical support). Increased carer time is another issue when considering gastrostomy feeding; an analysis of an adult DMD population concluded that inadequate and poorly directed social and medical support was an area of experienced difficulty (114). Heyman et al. demonstrated that use of a gastrostomy tube for enteral nutrition support in children is associated with significant increased care time by the primary caregiver (115).

The dietitian has an integral role in monitoring nutritional status as boys with DMD progress through the stages of the disease process. Cano et al. explored nutritional markers in patients on long term oxygen therapy and/or home mechanical ventilation (116). Of 744 participants, 13% had a neuromuscular disease. Fat free mass was determined to be the most sensitive marker for detecting malnutrition when compared to BMI, serum albumin and serum transthyretin (pre-albumin). Alternatively, Gonzalez-Bermejo et al. suggest that boys with DMD receive standard nutritional education and be told to eat *ad libitum* in order to satisfy hunger, whilst monitoring body weight precisely (60). If weight should alter in the absence of obvious factors (eg. infection) a prompt investigation of fat free mass and REE should be used in order to guide nutritional advice.

1.8.3 Predicting weight status

The variability in weight status has been identified anecdotally (112, 117). Some boys are relatively thin and remain so throughout childhood even during GCS treatment, whereas other

boys may gain weight excessively. Identifying individuals with DMD who are at risk of over- or under-nutrition would be of great benefit because it would support the provision of anticipatory nutritional counselling. A recent retrospective multicentre audit provides some evidence for predicting patients at risk of under nutrition (83). In this steroid-naïve cohort (N = 70), obesity at age 13 years was associated with later obesity. Conversely, normal or underweight at 13 years of age was associated with later underweight status. Using receiver-operating characteristic curves, weight for age ≥ 151% at age 13 years predicted later obesity; and weight for age ≤ 126.5% predicted later underweight status. The authors concluded that mild obesity (weight-for-age ≥126.5% and ≤ 151%) should not be discouraged because of its potential protective effect on malnutrition in the advanced stages of DMD. Although this study provides useful information on how to predict which boys will swing from overweight to underweight during their lifespan; one key question remains. How can we identify individuals who are likely to become overweight in late childhood/early adolescence?

1.9 Nutriceuticals

There are four randomised controlled trials, summarised in a recent Cochrane review providing high quality evidence for the use of specific dietary supplements to enhance muscle function (118). Because many families seek alternative or naturopathic supplements, dietitians need to be aware of their potential nutritional effects or interactions. The most promising of these is creatine monohydrate (CrM). Creatine is an amino acid found in muscle that stores energy as phosphocreatine for immediate use (45). Several studies have supported the use of CrM for increasing athletic performance and strength in healthy populations (119). A recent Cochrane review concluded that evidence from randomised trials shows that short and medium term CrM treatment improves muscle strength in people with neuromuscular disorders, and is well tolerated (118). In 138 participants treated with CrM, there was a significant increase in maximum voluntary contraction in CrM group compared with placebo, with a weighted mean difference of 8.47% (95% confidence interval (CI) 3.55, 13.38). There was also an increase in lean body mass during CrM treatment compared with placebo (weighted mean difference 0.63kg, 95% CI0.02, 1.25). The supplement doses included for children were 5 g/day (two trials), 3 g/day, and 100 mg/kg/day; and for adults 10 g/day (120-123). Another interesting finding from these studies was the effect on bone health in boys with DMD; treatment increased bone mineral density by 3% (121), and reduced levels of N-telopeptides, a marker of bone degradation (122). Since the Cochrane review, a further randomised controlled trial investigating CrM in boys with DMD has been published (124). The results presented from this study support the reviews findings; strength measures improved in the CrM treated group following 8 weeks of CrM supplementation at 5 g/day in a steroid-naïve population.

Of the studies included in the Cochrane review, all bar one were conducted in steroid-naïve dystrophic males (122). The use of synergistic therapies could possibly increase the positive

effect on muscle function. Using the *mdx* mouse model, Payne et al. tested several compounds (CrM, conjugated linoleic acid, α-lipoic acid, and β-hydroxy-β-methylbutyrate) alone, in combination, and in combination with prednisolone (125). Although each treatment alone and in combination showed some benefit, combination therapy with prednisolone provided the most consistent evidence of efficacy; there was increased peak grip strength, and a decrease in grip strength fatigue. Similar complementary effects have been observed between prednisone and CrM treatment *in vitro* (126).

A further clinical trial supporting the concept of nutriceutical therapies explored the effect of Coenzyme Q10 on strength measures in twelve GCS treated boys with DMD (127). Boys were dosed with individualised amounts of coenzyme Q10 sufficient to rise blood levels of the nutriceutical to >2.5µg/ml for the intervention period of 6 months. This pilot study demonstrated an 8.5% increase in muscle strength, and larger controlled trials are now warranted to confirm these results.

Glutamine has also been tested in clinical trials in boys with DMD. Studies in non-dystrophic humans have shown that doubling plasma glutamine concentration has stimulated protein synthesis, whilst an experimental decrease in plasma glutamine using a glutamine chelator has produced protein wasting (128, 129). Trials investigating the effect of oral glutamine supplementation in males with DMD on whole body protein kinetics have reached several conclusions. Initially, Hankard et al. concluded that glutamine may have a protein-sparing effect in children with DMD, decreasing estimated whole body protein degradation (130). Hankard et al. went on to demonstrate a significant decrease in glutamine availability in the post-absorptive state, leading the authors to question whether glutamine is “a conditionally essential amino acid in DMD?” (131). More recently, Mok et al. supplemented 26 boys with DMD aged 7-15 years with either oral glutamine or an amino acid supplement (132). The

results showed that oral glutamine or amino acid supplementation over 10 days inhibits whole body protein degradation. Despite promising results in these studies, a randomised control trial was unable to demonstrate a statistically significant effect of therapy based on manual and quantitative measurements of muscle strength. Glutamine was dosed at 0.6 g/kg/day (120). In this study, statistical power was reduced because the placebo group did not experience the physical decline that the investigators had expected; the authors concluded that a disease-modifying effect of glutamine in younger dystrophic males cannot be excluded. Following on from their earlier work, Mok et al. conducted a randomized cross-over design trial investigating the effects of glutamine supplementation at 0.5 g/kg/day on functional outcomes (walking speed) and body composition measured by DXA (133). Although no difference was found between glutamine and placebo treatment, subjects did not deteriorate over the nine months intervention period. Of the 30 boys recruited for this trial (aged 2 to 10 years), only five were being treated with GCS. Interestingly, a subgroup analysis revealed a significant effect of glutamine on functional measures in boys taking GCS, suggesting again that GCS and nutriceuticals may act synergistically.

The majority of other investigations in nutritional interventions have used the *mdx* mouse model. L-arginine is being investigated for its ability to increase utrophin levels. Utrophin is described as a 'dystrophin paralog' due to its structure, and its ability to perform similar functions at the sarcolemma, suggesting that increased levels of utrophin may compensate for the absence of dystrophin (5). Chaubourt et al. and Voisin et al. demonstrated an increase in utrophin levels in the muscle of *mdx* mice (134, 135). Voisin et al. (135) then explored the effect of this on muscle function. Isometric tension was found to increase by 30% in the diaphragm – the most affected muscle in the *mdx* mouse. The efficacy of green tea extract has also been examined in *mdx* mice, owing to its antioxidant properties which can decrease muscle necrosis (136, 137).

Finally, reviews on therapeutics in DMD have discussed the potential value of β-hydroxy-β-methylbutyrate (HMB) because of its potential value to increase lean body mass as demonstrated in patients with human immunodeficiency virus (40, 45, 138, 139). To date, use of HMB has only been investigated in the *mdx* mouse (125).

Despite the increasing number of clinical trials in recent years exploring the use of nutriceuticals for boys with DMD, the evidence is still not sufficient to upgrade the evidence level. Many studies still exist at the bench level, and there is inconsistent evidence for any one nutriceutical. Also, there is a lack of investigation into functional outcome measures – what is most meaningful to boys and their families. Similarly, the practice guidelines made no recommendations for the use of supplements due to an absence of supportive data or expert opinion consensus (3). The concept of complementary therapies warrants further attention.

1.10 Future nutritional issues

The multidisciplinary management of boys with DMD has progressed significantly and now involves the use of GCS therapy, ventilator support, spinal management and improved therapies such as physiotherapy. As a result, the life expectancy of boys with DMD is steadily increasing. Eagle et al. reviewed 197 patient notes from 1967 to 2002, concluding that the chances of survival to 25 years have increased from 0% in the 1960s, to 4% in the 1970s, 12% in the 1980s, and 53% in the 1990s; and this was before the advent of GCS treatment (140).

Perhaps the largest impact on survival has been the use of both invasive and non-invasive ventilator support. A cross-sectional survey of a Danish population of 65 DMD patients aged 18-42 years examined several aspects of this intervention (16). With an older DMD population emerging, it is unclear what nutritional issues will emerge in the adulthood life of DMD males. With increased body fat mass and an inability to exercise are these young men at risk of developing a cluster of cardiovascular risk factors such those described in the Metabolic Syndrome? Aitkens et al. and Kilmer and Zhao suggest this is possible and will provide a new level of complexity to dietetic management (141, 142).

1.11 Summary

There is little high quality evidence to support the key practice points highlighted throughout this paper (Table 1-3). Reviewing the evidence base for nutrition in DMD reveals consistent limitations across all areas. Overall, there is little research into nutrition and DMD. Papers report robust results, but few other studies support the findings; small sample sizes are common as recruiting from such a sparse patient population can be difficult; randomised controlled studies are scarce; and too many promising projects remain at the bench stage. It is for these reasons that many clinical recommendations are based on extrapolations from non-DMD studies or are based on expert opinion. Such recommendations are inevitably ranked as low level evidence. However, they are not without merit. Given the obstacles potential DMD researchers face, expert opinion based on clinical expertise and fundamental nutrition principles may provide the strongest and most accurate evidence as yet available.

The clinician needs to be aware of limitations when using expert opinion to guide practice. Such limitations may include the selective use of evidence, inappropriate interpretation of the evidence due to personal experience, and the impact of external influences including professional expectations and business interests. Challenges may also arise when expert opinions are conflicting and there is insufficient evidence to support either argument.

Management of boys with DMD is best provided through a multidisciplinary clinic. The two main nutritional concerns identified in DMD are early onset obesity and malnutrition during the advanced stages of the disease. The evidence presented in this review has provided key practice points for dietitians to be aware of when managing a boy with DMD.

The review has also highlighted many gaps in the literature specifically in the areas of energy requirements, bone health, micronutrient requirements (vitamin D), growth monitoring, and

use of novel amino acids and nutritional supplements. More research is urgently needed to develop these areas as they are essential tools of dietetic practice. Until the findings from contemporary research become available, expert opinion based on current research and fundamental knowledge of nutrition principles should be used to fill these gaps. The goal of dietetic management in DMD remains clearly to optimise nutritional status across the lifespan.

Table 1-3 Key nutrition practice points and supporting literature

Key Practice Point	Supporting Studies	Level of Evidence	Grade of recommendation	Limitations
Nutritional Requirements				
Predictive energy equations do not exist for boys with DMD.	Gonzalez -Bermejo et al. (2005) (60) Zanardi et al. (2003) (73) Satomura et al. (2001) (72) Hankard et al. (1996) (71) Okada et al. (1992) (59) Berardinelli et al. (2001) (26) Hogan (2008) (74) Shimizu-Fujiwara et al. (2011) (61)	3b 4 4 3b 4 3b 4 3b	D	Contradictory results. Small sample sizes. Varying age range. Only three studies have examined resting energy requirements in ambulatory children. Total energy expenditure has not been measured.
Total energy expenditure = REE (measured if possible) x physical activity level	Extrapolation from studies listed above.	5	D	Expert opinion
Monitor weight to guide energy prescription.	Almond et al. (2007) (64)	5	D	Expert opinion
Ensure adequate intake of micronutrients as per nutrient reference values.	nil	5	D	Expert opinion. No studies have investigated micronutrient requirements.
Provide Vitamin D (1000 IU daily) and calcium (750 mg daily) supplements, especially in those receiving GCS treatment.	Extrapolation from: Biggar et al. (2006) (39) Douvillez et al. (2005) (68) Söderpalm et al. (2007) (66)	 2b 4 3b	D	Expert opinion extrapolated from a few studies only. No intervention study has trialled Vitamin D (1000 IU daily) and calcium (750mg daily) supplementation and examined the effect on bone mineral density.
Monitor serum 25-hydroxyvitamin D.	Extrapolation from: Douvillez et al. (2005) Söderpalm et al. (2007)	 4 3b	D	Expert opinion Frequency of monitoring is required unclear.
Nutrition Assessment				
Short stature is a characteristic of boys with DMD.	Eiholzer et al. (1988) (78) Nagel et al. (1999) (79) Willig et al. (1993) (80)	2b 2b 2b	B	Eiholzer et al. and Nagel et al. have small sample sizes (25 and 34, respectively). Accuracy of height measurements in these retrospective studies.
Measure standing height if the boy is able to stand.	Stewart et al. (2006) (82)	3a	D	Not specifically focused on DMD. No supporting studies.
Upper arm length, tibial length, or knee height can be measured as surrogates for height in DMD.	Stewart et al. (2006) (82)	3a	D	Not specifically focused on DMD. No supporting studies.
Weight is disproportionately distributed in DMD.	Willig et al. (1993) (80) McDonald et al. (1995) (81)	2b 1b	B	

Key Practice Point	Supporting Studies	Level of Evidence	Grade of recommendation	Limitations
Measure weight with appropriate scales to match a boy's physical ability	Stewart et al. (2006) (82)	3a	D	Not specifically focused on DMD. No supporting studies.
Body composition is characterised by a decreased lean body mass and increased intramuscular fat mass. Various tools can be used to measure body composition.	Leroy-Willig et al. (1997) (84) Griggs et al. (1983) (91) Edmonds et al. (1985) (92) Hankard et al. (1996) (71) Franciotta et al. (2003) (95) Huang et al. (1994) (93) Gong et al. (2000) (94) Berardinelli et at. (2001) (26) Zanardi et al. (2003) (73) Palmieri et al. (1996) (88) Douvillez et al. (2005) (68) Söderpalm et al. (2007) (66) McDonald et al. (2005) (90) Gonzalez-Bermejo et al. (2005) (60) McCrory et al. (1998) (143)	3b 2b 3b 3b 3b 3b 3b 3b 4 3b 4 3b 3b 3b 3b	C	Small sample sizes (n = 8-26) Not all studies focused on DMD. Varying age ranges and methodology. No randomisation. No review papers available.
There are limitations to using height, weight or BMI alone as a screen for obesity in boys with DMD.	Leroy-Willig et al. (1997) (84) Pichiecchio et al. (2002) (85) Mok et al. (2006) (86) Pessolano et al. (2003) (96)	3b 3b 3b 4	C	
Of the various tools available to measure body composition in DMD, DXA and MRI are the most accurate, appropriate and non-invasive.	Leroy-Willig et al. (1997) (84) Franciotta et al. (2003) (95) Huang et al. (1994) (93) Gong et al. (2000) (94) Berardinelli et at. (2001) (26) Zanardi et al. (2003) (73) Palmieri et al. (1996) (88) Douvillez et al. (2005) (68) Söderpalm et al. (2007) (66) Pichiecchio et al. (2002) (85) Skalsky et al. (2009) (89) Mok et al. (2010) (87)	3b 3b 3b 3b 3b 4 3b 4 3b 3b 3b 3b	C	
Standard or national growth charts are appropriate for use when assessing growth in DMD	Almond et al. (2007) (64) Bushby et al. (2010) (15)	5 5		Expert opinion

Key Practice Point		Supporting Studies	Level of Evidence	Grade of recommendation	Limitations
Nutritional Management and monitoring					
Early onset obesity	Prevent excess weight gain by providing anticipatory guidance around energy balance prior to commencement of GCS.	Griffiths and Edwards, (1988) (97)	3b	D	Expert opinion
	Advise reduction in energy intake with caution. Follow up with regular measurement of fat free mass to monitor progress where available.	Edwards et al. (1984) (104)	4	D	Case study of two participants. No controls. Out-dated. Heavily restrictive dietary regimen used.
	Measure height and weight at six monthly intervals and plot on standard growth charts.	Bushby et al. (2010) (15) Stewart et al. (2006) (82)	5 3a		Expert opinion/consensus
Malnutrition in advanced stages of the disease	In advance stages of DMD, monitor individuals for weight loss.	Gonzalez-Bermejo et al. (2005) (60)	3b	D	Small sample size. Expert opinion.
	Dietary texture modifications may be required to accommodate eating difficulties.	Pane et al. (2006) (24)	4	D	No intervention studies. Only 1 observational study.
	Gastrostomy feeding is well tolerated and improves nutritional status.	Diamanti (2011) (109) Martigne et al. (2010) (108) Goldstein et al. (1989) (110)	4 4 4	C	Consistent studies but only level 4 evidence
	Support patients and families in decisions regarding enteral feeding and/or gastrostomy tube placement.	Hilton et al. (1993) (111)	5	D	Expert opinion
	A multidisciplinary team should be involved in the management of feeding difficulties.	Almond et al. (2007) (64) Stewart et al. (2006) (82)	5 3a	D	Expert opinion

Key Practice Point		Supporting Studies	Level of Evidence	Grade of recommendation	Limitations
Nutriceuticals	There is a limited evidence base to support the use of nutriceuticals in boys with DMD.	Extrapolation from: Chaubort et al. (1999) (134) Voisin et al. (2005) (135) Payne et al. (2006) (125) Hankard et al. (1998) (130) Hankard et al. (1999) (131) Mok et al. (2006) (132) Dorchies et al. (2006) (137) Buetler et al. (2002) (136) Fenichel et al. (1988) (144) Escolar et al. (2005) (120) Mok et al. (2009) (133) Banerjee et al. (2010) (124) Spurney et al. (2011) (127)	 5 5 5 3b 3b 4 5 5 1b* 1b 1b 1b 1b	D	Many studies conducted only in the *mdx* mouse. Translational studies in humans are required. * Double-blinded randomised control trial with 106 participants showed no effect of penicillamine and Vitamin E supplementation.
	Short and medium term creatine treatment improves muscle strength in people with NMD.	Kley et al. (2007) (118)	1a	A	Long term effects not reported.

2 Sub-phenotypes in Duchenne muscular dystrophy

A retrospective longitudinal analysis of the relationship between genotype, phenotype and growth in steroid treated and steroid-naïve boys with Duchenne muscular dystrophy

2.1 Introduction

DMD is caused by mutations in the dystrophin gene (*DMD-G*)[a], located at Xp21.2 and rendering the body unable to produce the protein, dystrophin. This gene was first described in 1986 (145). Subsequently, its great extent and complexity has been revealed. As the largest gene in the human genome, *DMD-G* spans ~2.5Mb of genomic sequence and contains 79 exons (5). Due to this very large size, a great many different types of mutational change are possible varying both in location and in type of functional disruption. To date, > 4,700 different mutations in *DMD-G* have been documented (11). As seen in Box 1, these can be grouped into three main categories: deletions, duplications (or triplications), or point mutations (small changes within a single exon). Point mutations are further classified as: nonsense, frame shift, missense or splice site mutations.

Mutation		Definition
Deletion		A deletion removes a piece of DNA from *DMD-G*. Deletions can be small, removing one or a few exons, or large removing many exons from the gene.
Duplication or triplication		A duplication occurs when a piece of DNA or exon is copied once (duplication), twice (triplication) or more.
Point mutation	Frame shift	A point mutation changes the reading frame. A reading frame consists of a group of three purine or pyrimidine bases in the DNA, each coding for a single amino acid. A frame shift mutation that shifts the grouping of these bases will change the code for amino acids.
	Nonsense	A point mutation in the DNA code that when translated into RNA signals the cell to prematurely stop building the dystrophin molecule, resulting in a shortened protein.
	Missense	A point mutation in which a change in the DNA code causes the substitution of one amino acid for another in the dystrophin protein. The presence of this amino acid substitution results in a non-functional protein
	Splice site	A point mutation that inserts or deletes a number of nucleotides in the specific site at which splicing of an intron takes place.

Box 1 Definition of common types of mutation found in the *DMD-G*

Definitions obtained from National Institutes of Health (146).

[a] *DMD* is the correct symbol of the dystrophin gene. *DMD-G* is used here to avoid confusion with the abbreviation for Duchenne muscular dystrophy (DMD).

Initially, genetic analysis was only capable of detecting deletions in *DMD-G*. Current technology has allowed the entire gene to be sequenced, and at much lower cost, so that many small novel point mutations can be identified (33). Such advances in genetic analysis mean that disease diagnosis can now draw on the results of gene sequencing data as well as on the traditional muscle biopsy (3). As gene therapies progress, mutation analysis is critical so that young men who may benefit from gene therapy can be identified for upcoming clinical trials. Promising therapeutic agents currently being tested include: ataluren (PTC124®), an orally administered drug that promotes ribosomal read-through of nonsense mutations; and antisense molecules which can skip targeted exons (11). Accurate mutation analysis is also essential so that informed genetic counselling can be provided to families.

Whilst there is observed variability amongst males with DMD in the rate of disease progression and response to GCS, little is understood about this heterogeneity which can be a significant confounder in clinical trials (147). Genetic influences could provide a possible explanation. Within the dystrophinopathies, a phenotype distinction can be made between DMD and the milder BMD in which some degree of dystrophin expression still remains. In cases of deletions within the *DMD-G*, this phenotype can be predicted by the "reading frame" hypothesis whereby males with BMD will produce a semi-functional, internally deleted dystrophin protein in comparison to males with DMD who produce a severely truncated dystrophin protein that is unstable (148, 149). Functionality persists in males with BMD due to the presence of the terminal domains within the dystrophin protein (11). When 258 independent deletions were examined, the 'reading frame' hypothesis predicted phenotype in 92% of cases (148).

Increasing the complexity of the genetic profile of DMD is the existence of various dystrophin isoforms. These are proteins of varying lengths containing different segments of

the basic dystrophin sequence. Isoforms are classified by their length and the tissue that they are commonly found in. For example, the full length isoform Dp427m spans 427 kilo Daltons in length and is found primarily in muscle. All of the full length isoforms (Dp427) are derived from a promoter at Exon 1. Shorter isoforms exist (Dp140, Dp260, Dp70, Dp116) and these are derived from more distal promoters. The location of the isoform promoters within the *DMD-G* is illustrated in Figure 2-1. Understanding the role of individual isoforms and their derivation may help to identify relationships between genotype and sub-phenotypes in DMD.

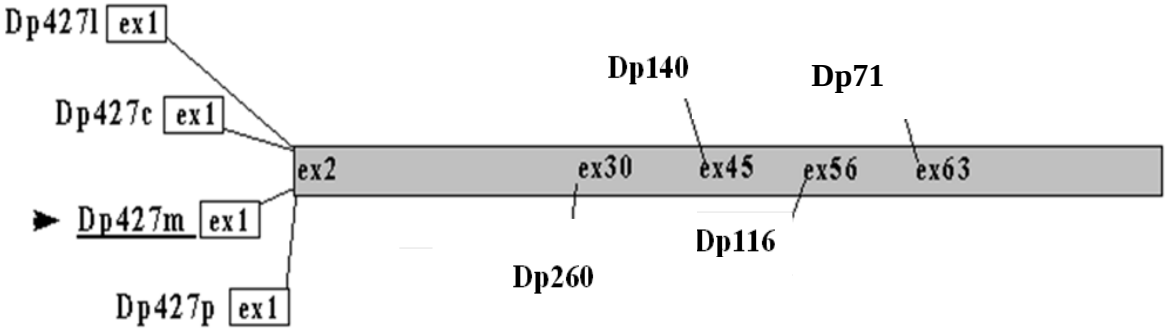

Figure 2-1 Location of dystrophin isoform promoters within the *DMD-G*

Figure obtained from the Leiden Muscular Dystrophy pages (150).

Isoforms Dp140 and Dp70 have been found to be abundant in foetal brain, and are hypothesised to be related to cognitive impairment in DMD (151, 152). Subsequently, distal mutations in the *DMD-G* have been associated with cognitive function in boys with DMD. For example, deletions in exon 52 of *DMD-G* have been described as functionally significant in the manifestation of mental retardation. In an early study, 70% of participants with a deletion at exon 52 had diagnosed mental retardation, whereas only 38% of participants with mental retardation had deletions at other exons (153). Other groups have further explored this association. Intellectual impairment was found to be more prominent in males with a deletion distal to exon 30 (154). A second more recent study confirms these results (152). In this

second study, cognitive impairment was measured by Full Scale Intelligence Quotients in 62 subjects. Cognitive impairment was found not to be associated with mutation type or size; but with the *site* of the *DMD-G* mutation. The boundary of proximal and distal groups was set at exon 30. Subjects with mutations distal to exon 30 had greater cognitive impairment. A similar relationship was observed when the proximal/distal boundary was extended to exon 45. Stronger correlations between cognitive impairment and mutation location were determined using a classification system based on mutations affecting the Dp140 promoter, providing evidence for the role of isoforms in clinical heterogeneity.

The relationship between dystrophin mutation and the onset of cardiomyopathy has also been explored. In males with BMD, deletions in exon 2-9, which affect the amino-terminal domain were found to be associated with early onset dilated cardiomyopathy (occurring in males in their mid-20s), compared to deletions in exon 50 and/or 51 that remove or disrupt hinge 3 in the dystrophin protein (in which cardiomyopathy onset is not seen until boys are in their mid-40s) (155).

Desguerre et al. have attempted to classify clinical heterogeneity across intellectual and motor outcomes in four distinctive sub-phenotypes: *A* severe intellectual and motor outcomes; *B* intermediate intellectual and poor motor outcome; *C* normal intelligence and delayed motor impairment; and *D* normal intelligence and poor motor outcomes (156). Some associations were evident between these four sub-phenotypes and particular mutated *DMD-G* regions. The frequency of mutations proximal to exon 30 increased from sub-phenotypes A to D; mainly due to the relationship with the intelligence component. Whilst cognitive function in this cohort was significantly associated with mutation site, similar relationships were not evident between genotype and motor function. It is of interest however, that boys classified in sub-phenotype C were more likely to be ‘thin’ based on BMI than those in the other groups.

Heterogeneity in growth patterns in DMD has been noted by several authors; particularly the identification of a ‘thin’ phenotype resistant to weight gain throughout GCS treatment (112, 117). Group *C* could thus represent a useful clinical sub-phenotype of boys with lower BMI and delayed motor impairment. Identifying individuals with DMD who are at risk of over- or under- nutrition is of great benefit in supporting the provision of anticipatory nutritional counselling.

The role of the dystrophin mutation in the clinical variability in DMD is of great interest, but to date few disease variants can confidently be linked to differences in genotype. As genetic analysis becomes a part of best practice, it becomes possible to explore more of the relationships between genotype and sub-phenotypes (3).

2.2 Aims and objectives

2.2.1 Aim

The primary aim of this analysis was to investigate possible relationships between genotype and sub-phenotypes in boys with DMD. A secondary aim was to investigate whether there were any regional differences in the clinical characteristics of boys with DMD living in Queensland compared to Victoria.

2.2.2 Objectives

1. To describe physical and clinical characteristics at one clinical time point in boys with DMD, through the investigation of:
    a. anthropometry: height, weight and BMI
    b. lung function: forced expiratory volume as a percentage of predicted (FEV1%) and forced vital capacity as a percentage of predicted (FVC%)
    c. bone health: bone mineral density, vitamin D (25(OH)D)
    d. GCS use: dose and duration
    e. ambulatory status, and in those who cannot walk, the age at loss of ambulation (LOA)
    f. age of diagnosis
    g. age at commencement of GCS
2. To determine if there are differences in the physical and clinical characteristics of boys with DMD living in Queensland compared to boys living in Victoria.
3. To describe:
    a. the range of genetic mutations in the *DMD-G* and the location of affected exons in the combined cohort
    b. how height and BMI change over time

4. To explore relationships between age at LOA, BMI or lung function and clinical and genetic characteristics in boys with DMD.

2.2.3 Hypotheses to be tested

I. Boys with DMD living in Queensland will not differ from those in Victoria in physical or clinical characteristics.

II. Age at LOA will not relate to clinical or genetic characteristics.

III. BMI will be unrelated to clinical or genetic characteristics.

IV. Lung function will not be related to clinical or genetic characteristics.

2.3 Methods

2.3.1 Study design

This is a retrospective case series, utilizing longitudinal data on physical, clinical and genetic characteristics collected during routine clinical care.

2.3.2 Ethics approval

Ethics approval was obtained from The Royal Children's Hospital, Melbourne (#29075B) and Royal Children's Hospital, Brisbane (HREC/09/QRCH/86) (Appendix 3and Appendix 4, respectively). The project was supported by MontroseAccess, Brisbane. As per the approved protocol, parental consent was waived. All data were stored in a non-identifiable format.

2.3.3 Clinics

Medical histories were examined from two Australian neuromuscular clinics: The Royal Children's Hospital, Victoria, and MontroseAccess, Queensland. Each clinic is the major centre where boys with DMD receive specialist medical care within their respective states. Families are referred to these centres upon diagnosis, hence our sample should include the majority of, if not all boys with DMD in Queensland and Victoria. Whilst the boys attend these neuromuscular clinics, their primary care giver is usually a local paediatrician or general practitioner in what constitutes an informal 'shared care' scenario.

2.3.3.1 The Royal Children's Hospital, Melbourne, Victoria

Boys with DMD living in Victoria attend the Neuromuscular Clinic, based within the Children's Neurosciences Centre at the Royal Children's Hospital, Melbourne. This is a multidisciplinary centre where boys can see a range of medical specialists including neurologists, respiratory physicians, cardiologists, orthopaedic surgeons, genetic counsellors, and allied health professionals, including a dietitian, within the one clinic. All medical notes

and correspondence as well as investigations for the boys attending the clinic are filed in their hospital medical history. These were examined for this analysis.

2.3.3.2 MontroseAccess, Brisbane

MontroseAccess is an independent, not-for-profit community organisation that provides therapy, respite and recreation services to children and young adults with physical disabilities living in Queensland. The organisation is governed by its own Board of Directors. Boys with DMD attend a neuromuscular clinic and receive physical therapy through MonstroseAcccess. The clinic is staffed by visiting neurologists from the Royal Children's Hospital, and the Mater Hospital, Brisbane. A range of allied health services including physiotherapy, occupational therapy, speech pathology and social work are available through MontroseAccess; although dietetic services are not included. Therapy and medical notes and correspondence for boys attending the clinic are filed in MontroseAccess patient files. These were examined for this analysis.

2.3.4 Data collection

Current clinic lists were provided by coordinators at each centre, and corresponding medical histories where obtained. Charts were examined from front to back. All available data on the following variables were collected:

- Date of birth
- Genotype: mutation type and location of affected exons
- Age at diagnosis
- Ambulatory status and age at loss of ambulation
- Height (cm)
- Body weight (kg)
- GCS use: type of steroid (Prednisolone or Deflazacort), dose, age at commencement

- Lung function (FEV1% and FVC%)
- Bone mineral density of lumbar spine (z-score)
- Serum 25(OH)D (nmol/L)
- Use of nutritional supplements
- Nutritional investigations eg. iron studies
- Dietitian consults (occasions of service)
- Number of brothers and their clinical status

The date as which the variable was measured was recorded for all data points. These dates were subsequently converted to age in years.

Age of diagnosis was determined as the date at which the neurologist documented a discussion with parents regarding the diagnosis. If this was not available, age at diagnosis was taken as the date of the first genetic report or as the date of muscle biopsy.

Age at commencement of GCS was determined as the date at which the neurologist documented the commencement of GCS. Similarly, age at loss of ambulation was determined as the date of the first neurologist entry stating that the patient no longer had the ability to walk.

From collected anthropometry data, BMI (weight (kg)/height $(m)^2$) was calculated. Weight, height and BMI z-scores were determined using the CDC reference values for children (157). Weight was also used to calculate GCS dose in mg/kg.

With regard to genotype, if no mutation was detected during genetic analysis, the mutation was classed as ‘unknown’. This occurred more frequently in older males, where dystrophin

sequencing may not have been available at the time of genetic analysis. In these cases, it was assumed that the mutation was a point mutation as this is highly likely.

If there was no genetic information in the chart, the patient was excluded from the audit. This was done because missing genetic information is quite different from an unknown mutation. A patient with missing information could have a common and easily identifiable mutation, thus biasing the unknown mutation group.

As a medical record is a legal and true representation of subject's medical history, all data was recorded exactly as it was documented in the medical record.

2.3.5 Exclusions

Subjects were excluded from the analysis if on chart examination, the boy had BMD; or if a genetic report could not be located within the medical history. As identifying changes in growth over time was a key objective, charts were excluded if no anthropometric measures were documented.

2.3.6 Statistical analysis

Statistical analysis was conducted primarily using SPSS, Version 18.0 (SPSS Inc., Chicago, IL). Microsoft Excel was also used for construction of graphs. The statistical methods used are summarised in the sections below in conjunction with the corresponding objectives and hypotheses. For all analyses, significance was considered to be obtained when $p \leq 0.05$. Results are displayed as mean ± standard deviation (SD), or median (interquartile range (IQR)) for non-normally distributed variables.

2.3.6.1 Testing for normality

The distribution of each variable was examined visually using histograms. Normality of data distribution was tested using the Shapiro-Wilk Test. This test was chosen as it more appropriate for small sample sizes but can also handle sample sizes as large as 2,000 (158).

2.3.6.2 Description of physical and clinical characteristics (Objectives 1)

Cross-sectional, descriptive analyses of the two cohorts (Queensland and Victoria) both separately and combined were undertaken. For patients with several observations for each characteristic, the last data point recorded for each variable was used. Weight, height and BMI z-scores and FEV1% were used in the analysis to adjust for age.

2.3.6.3 Comparison of physical and clinical characteristics between the Victorian and Queensland cohorts (Objective 2 and Hypotheses I)

When comparing means, a two sample Student's *t*-test or the non-parametric equivalent (Mann-Whitney U) was used to detect differences between physical and clinical characteristics, and age at key points between the Victorian and Queensland cohort. A chi squared test was used to compare proportions.

2.3.6.4 Mutation Distribution (Objective 3, 4)

Data collected on genetic mutations from the two clinics was explored using frequency (expressed as percentage) and histograms. Frequency of affected exons was expressed per size of the exon in base pairs (exon sizes described in Appendix 5). Types of mutations as well as exon location of the mutation were investigated. The data was also split by state (QLD/VIC) to see if the spread of mutations differed in the two clinics. The chi-squared test was used to look at differences in proportions of mutation types across the two cohorts.

2.3.6.5 Description of changes in BMI and height over time (Objectives 5, 6)

All height and BMI records collected from the chart were converted to z-scores and grouped by year of age. For each subject, height and BMI measures were examined individually and allocated to the appropriate age. Only one measure per subject was allocated to each year of age. Where a subject had several measures in the one age group, the measure taken closest to mid-year was used. Bar graphs were used to graphically summarise the mean height and BMI for each year of age. Within each year of age, BMI z-scores were classified as underweight, appropriate weight, overweight, and obese. These classifications were made based on the Clinical Practice Guidelines for the Management of Overweight and Obesity in Children and Adolescents produced by National Health and Medical Research Council (159). The corresponding percentiles and z-scores are summarised in Box 2. A bar graph was used to display the proportion of subjects in each BMI category at each age.

BMI Category	Percentile	z-score
Underweight	$\leq 5^{th}$	≤ -1.645
Appropriate weight	$> 5^{th}$ and $< 85^{th}$	> -1.645 and < 1.036
Overweight	$\geq 85^{th}$ and $< 95^{th}$	≥ 1.036 and < 1.645
Obese	$\geq 95^{th}$	≥ 1.645

Box 2 BMI categories and corresponding percentiles and z-scores

2.3.6.6 Investigation of relationships between age at LOA and clinical and genetic characteristics (Objective 7, Hypothesis II)

Multiple regression analysis was used to explore the relationship between age at LOA and the clinical and genetic characteristics of boys with DMD. Clinical characteristics entered into the model included age at chart audit, duration of GCS treatment, and BMI z-score (taken as the BMI z-score closest to LOA). For the purpose of the analysis, mutation type was expressed as

a dichotomous categorical variable: *deletion* versus *other* (including unknowns). Similarly, location of affected exons was dichotomised as: *within exon 1-44* versus *within exon 45-79*. These categories were chosen so that each group incorporated proximal (exon 2-20) or distal (exon 45-55) known hotspots, while also providing approximately equal numbers of exons in each group (160).

Standard multiple regression in which all independent variables are entered into the equation simultaneously was used in the analysis. Before proceeding with the model, assumptions were tested as summarised in Box 3. Logistic regression was also used to predict LOA < 10 years of age, given that the mean age at LOA was found to be 9.9 years. Possible predictive factors were first explored in univariable analyses. These included: duration of GCS treatment, mutation type, and location of mutation. BMI at specific ages was also examined as an independent predictor. To ensure adequate numbers in compared categories, age groups were combined (6.00-7.99 years and 8.00-9.99 years). BMI was entered as absolute z-scores or was classified as BMI $\geq 85^{th}$ (yes/no) and BMI $\geq 95^{th}$ (yes/no). Independent variables that were found to be significant predictors of LOA at < 10 years of age were then examined together in a multivariable analysis. For each model, the goodness of fit was examined using the Omnibus Test of Model Coefficients and the Hosmer-Lemeshow Goodness of Fit Test (values > 0.05 indicate a 'good' fit, in each case).

2.3.6.7 Investigation of relationships between BMI z-score and clinical and genetic characteristics (Objective 8, Hypothesis III)

Multiple regression was used to explore relationships between BMI z-scores and clinical and genetic characteristics of boys with DMD. Clinical characteristics entered into the model included age at which BMI was measured, duration of GCS treatment, ambulatory status (yes/no), and lung function (FEV1%, taken as the last FEV1% measure for each subject).

Genetic variables were grouped as described in 1.2.6.6. Standard multiple regression was used and assumptions were seen to be met (Box 3) before full modelling proceeded.

The relationship with BMI z-score overtime and genetic variables was also explored. Standard multiple regression was used to determine associations between BMI z-score at each age group (2-17 years) and type of *DMD-G* mutation and site of mutation separately. No other variables were entered into the regression model.

Assumption	Check
Multicollinearity Strong relationships existing between independent variables entered in the model. Multicollinearity decreases the quality of the regression model.	Correlations between dependent variables were reviewed to check that no strong relationships were evident. Collinearity diagnostics were also examined. Tolerance was accepted at > 0.1 and variance inflation factor (VIF) at < 10.
Normality & Linearity Residuals should be normally distributed about and have a straight line relationship with predicted dependent variable scores.	The normal probability plot was inspected to ascertain whether a reasonably straight line was evident.
Homoscedasticity The variance of the residuals about predicted dependent variable scores should be the same for all predicted scores.	The scatterplot of standardised residuals was inspected to ensure it was roughly distributed in a rectangular pattern.
Outliers Regression is sensitive to extreme outliers.	Outliers were identified using Mahalanobis distances above critical points, or in casewise diagnostics. If outliers were found the maximum Cook's distance was examined to determine whether the outlier would affect the data. Maximum Cook's distances < 1.0 were accepted.

Box 3 Assumptions examined when performing multiple regression

Summarised from Pallant (161)

2.3.6.8 Investigation of relationships between lung function clinical and genetic characteristics (Objective 9, Hypothesis IV)

Multiple regression was used to explore the relationship between lung function (FEV1%) and clinical and genetic characteristics. Clinical characteristics entered into the model included age at which lung function was measured, duration of GCS treatment, ambulatory status (yes/no), and BMI z-score (taken as BMI closest to FEV1% measure for each subject). Genetic variables were grouped as described in 1.2.6.6. Standard multiple regression with checking of assumptions was undertaken (Box 3).

2.4 Results

2.4.1 Subjects

The chart audit occurred between August and November, 2009. A total of 193 medical records were examined with 144 (75%) subject records included in the analysis. The primary reason for record exclusion in Victoria or Queensland is detailed in Table 2-1. The birth dates of excluded participants fell between 29/12/1975 and 13/07/2009. Of the included medical records, 80 (56%) were from the clinic in Victoria while 64 (44%) were from the clinic in Queensland. This represents 92% and 60% of these clinic populations, respectively. Birth dates for included participants fell between 02/06/1987 and 25/07/2005. Fourteen sets of two brothers were in the included group (Appendix 6); no sets of three brothers were identified. One boy was documented as deceased (DOB: 29/06/1991).

Table 2-1 Reason for exclusion of subjects' medical records in Victoria and Queensland

	n (% of all records excluded)		
Reason for exclusion	Victoria	Queensland	Total
Subject did not have DMD	0 (0.0)	3 (7.1)	3 (6.1)
No anthropometric data recorded	2 (28.6)	36 (85.7)	38 (77.6)
Genetic report not located	4 (57.1)	3 (7.1)	7 (14.3)
Other	1 (14.3)	0 (0.0)	1 (2.0)
Total of medical records excluded	**7 (100.0)**	**42 (100.0)**	**49 (100.0)**

2.4.2 Description and comparison of physical and clinical characteristics between Victorian and Queensland cohorts

The cross-sectional analysis was completed using the most up-to-date measure documented in the medical chart for each variable. Date at which measures were documented fell between 10/07/1997 and 18/11/09. Table 2-2, Table 2-3, and Table 2-4 provide a summary of clinical,

and anthropometric and other physical characteristics, respectively for the combined cohort, with comparisons made between the Victorian and Queensland cohorts. Information on GCS use is summarised separately in Table 2-5.

The Victorian and Queensland cohorts were very similar in most physical and clinical characteristics. Significant differences were observed between the cohorts in weight z-score (median (IQR): VIC 0.21 (-0.66, 1.15) vs QLD 1.22 (-0.08, 1.70), p=0.004); and consequently in BMI z-score (VIC 1.21 (0.19, 1.87) vs QLD 1.61 (0.93, 2.22), p=0.025). Also, boys attending the neuromuscular clinic in Brisbane commenced GCS therapy over a year earlier than boys in Melbourne; although the later received a higher dose of GCS both in absolute amount and as mg/kg/day (Table 2-5).

Approximately half of the combined cohort was wheelchair bound at the time of the chart review; age at LOA was documented in 69 of 73 (95%) of non-ambulatory patients.
Figure 2-2 displays the proportion of non-ambulatory boys who lost the ability to walk at each age group. More than 50% of the cohort became wheelchair bound between the ages of 9 – 11 years. Extreme values were observed for many variables. The minimum values recorded for FEV1%, lumbar spine z-score, vitamin D, and height z-score were 9%, -3.9, 16nmol/L, and -4.0 respectively. The maximum BMI z-score recorded was 3.4.

Table 2-2 Summary and comparison of clinical characteristics between the Victorian and Queensland cohorts

	All	Victoria	QLD	*p* (VIC vs QLD)
Age at review of record				
Observations (n)	144	80	64	
Age at review of record (years)	11.9 ± 4.0	12.0 ± 4.5	11.8 ± 3.4	0.694
Diagnosis				
Observations (n)	140	78	62	
Age at diagnosis (years)	4.4 ± 2.3	4.42 ± 2.00	4.3 ± 2.7	0.731
Ambulatory Status				
n (%) wheelchair bound	73 (50.7%)	38 (47.5%)	35 (54.7%)	0.391
Age at loss of ambulation				
Observations (n)	69	36	33	
Age at loss of ambulation (years)	9.9 ± 1.9	9.8 ± 1.9	10.0 ± 2.0	0.644

Data are presented as the mean ± SD, or as number (proportion as a percentage); comparisons were made using Student's t test or chi squared, respectively.

Table 2-3 Summary and comparison of anthropometric characteristics between the Victorian and Queensland cohorts

	All	Victoria	QLD	*p* (VIC vs QLD)
Weight				
Observations (n)	144	80	64	
Age at assessment (years)	10.7 (8.4, 13.9)	10.8 (8.0, 14.9)	10.6 (8.5, 12.7)	0.395
Weight (kg)	38.7 (28.1, 55.0)	36.0 (25.1, 55.0)	41.8 (30.0, 53.0)	0.257
Weight z-score	0.59 (-0.39, 1.55)	0.21 (-0.66, 1.15)	1.22 (-0.08, 1.70)	**0.004**
Height				
Observations (n)	139	78	61	
Age at assessment (years)	10.7 (8.5, 13.9)	10.8 (8.0, 14.8)	10.7 (8.5, 12.7)	0.392
Height (cm)	133.0 (122.6, 152.0)	133.5 (120.6, 152.8)	133.0 (125.3, 152.0)	0.763
Height z-score	-1.07 (-2.08, 0.02)	-1.3 (-2.15, -0.35)	-0.80 (-2.08, 0.47)	0.082
BMI				
Observations (n)	139	78	61	
Age at assessment (years)	10.7 (8.5, 13.9)	10.8 (8.0, 14.8)	10.7 (8.5, 12.7)	0.390
BMI (kg/m^2)	21.0 (17.6, 25.6)	20.3 (17.2, 24.7)	21.7 (18.4, 27.3)	0.094
BMI z-score	1.35 (0.29, 2.03)	1.21 (0.19, 1.87)	1.61 (0.93, 2.22)	**0.025**

Data are presented as the median (IQR); comparisons were made using the Mann-Whitney U-test

Table 2-4 Summary and comparison of physical characteristics between the Victorian and Queensland cohorts

	All	Victoria	QLD	*p* (VIC vs QLD)
FEV1				
Observations (n)	112	61	51	
Age at assessment (years)	11.1 (9.0, 14.3)	11.4 (9.0, 15.2)	10.9 (9.8, 12.8)	0.228
% FEV1	77 (55, 93)	86 (61, 93)	68 (52, 93)	0.149
FVC				
Observations (n)	113	61	52	
Age at assessment (years)	11.1 (9.0, 14.2)	11.4 (9.1, 15.4)	10.8 (8.8, 12.8)	0.167
% FVC	75 (51, 90)	81 (62, 90)	69 (48, 89)	0.284
Bone Mineral Density				
Observations (n)	28	19	9	
Age at assessment (years)	9.26 ± 2.89	9.06 ± 3.06	9.68 ± 2.63	0.607
Lumbar spine z-score	-1.4 ± 1.1	-1.5 ± 1.2	-1.0 ± 0.8	0.250
Serum 25 hydroxyvitamin D				
Observations (n)	33	32	1	-
Age at assessment (years)	10.24 ± 3.81	10.25 ± 3.87	10.01	-
nmol/L	50 ± 18	50 ± 19	54.0	-

Data are presented as the median (IQR) or mean ± SD; comparisons were made using the Mann-Whitney U-test or Students *t* test, respectively.

Table 2-5 Summary and comparison of GCS use between the Victorian and Queensland cohorts

	All	Victoria	QLD	*p* (VIC vs QLD)
GCS Use				
n (%) ever used GCS	103 (71.5%)	58 (72.5%)	45 (70.3%)	0.773[a]
GCS Duration				
Observations (n)	96	56	40	
Age at commencement (years)	6.9 (5.5, 8.0)	7.4 (6.2, 8.4)	6.1 (5.0, 7.5)	**0.004**
Duration GCS therapy (years)	2.4 (1.3, 3.9)	2.4 (1.0, 3.8)	2.7 (1.9, 4.7)	0.181
Prednisolone				
n (%) treated with Prednisolone	93 (90.3%)	51 (87.9%)	42 (93.3%)	0.358
Age at last recorded dose (years)	9.99 ± 2.44	10.35 ± 2.49	9.56 ± 2.33	0.119
Last recorded dose (mg/day)	17.5 (15, 21.4) [a]	20.0 (17.5, 22.5)	15.0 (12.5, 18.8) [b]	**0.002**
Last recorded dose (mg/kg/day)	0.48 ± 0.17 [c]	0.53 ± 0.16 [d]	0.44 ± 0.16 [e]	**0.019**
Deflazacort				
n (%) treated with Deflazacort	10 (9.7%)	7 (12.1%)	3 (6.7%)	0.507
Age at last recorded dose (years)	8.81 ± 1.93	9.02 ± 2.32	8.32 ± 0.42	0.631
Last recorded dose (mg/day)	27.0 (24.0, 30.8)	27.0 (24.0, 33.0)	30.0 (24.0, 30.0)	0.833
Last recorded dose (mg/kg/day)	0.83 ± 0.12 [f]	0.85 ± 0.1 [g]	0.80 ± 0.17	0.668

[a] n=92; [b] n=41; [c] n=78; [d] n=38; [e] n=40; [f] n=8; [g] n=5. GCS glucocorticosteroids. Data are presented as number (proportion as a percentage), median (IQR), or mean ± SD; comparisons were made using chi squared test, Mann-Whitney U-test or Student's t test, respectively.

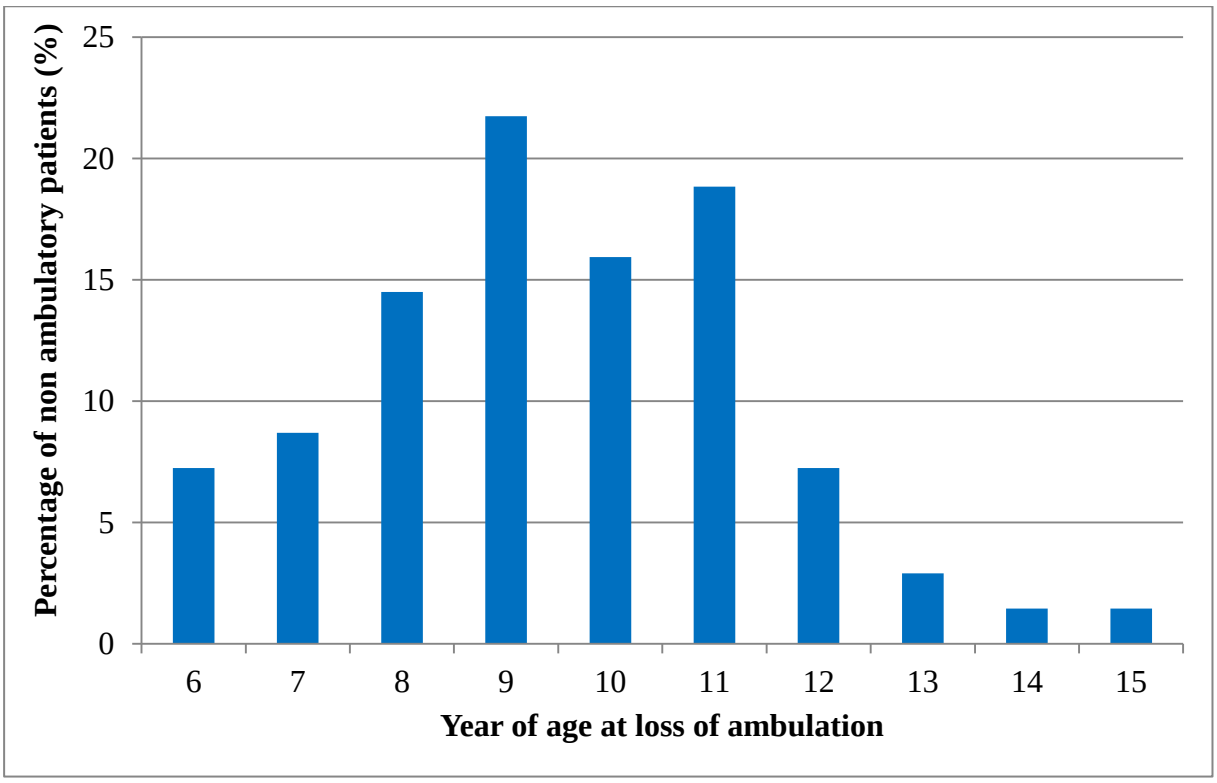

Figure 2-2 Proportion of non-ambulatory DMD patients who lost ambulation at each year of age

2.4.3 Types of mutations

The proportion of each type of mutation associated with DMD in the cohorts studied is detailed in Figure 2-3. Deletions were the most common mutation type, accounting for 60% of all mutations across combined cohorts. The difference in the proportion of deletions observed in Victoria and Queensland (65% vs 55%, respectively) was not of statistical significance (p=0.209). Across combined cohorts, duplications, nonsense and unknown mutations were equally reported at approximately 10% of all mutations. Differences in the proportion of duplications found in Victoria as compared to Queensland approached statistical significance (5.0% vs 15.6% respectively; χ^2=3.44, p=0.064). The proportion of nonsense and unknown mutations was similar in both cohorts (10%). Remaining mutation types accounted for approximately 10% of mutations across the combined cohorts. Point mutations were not further categorised in the genetic reports.

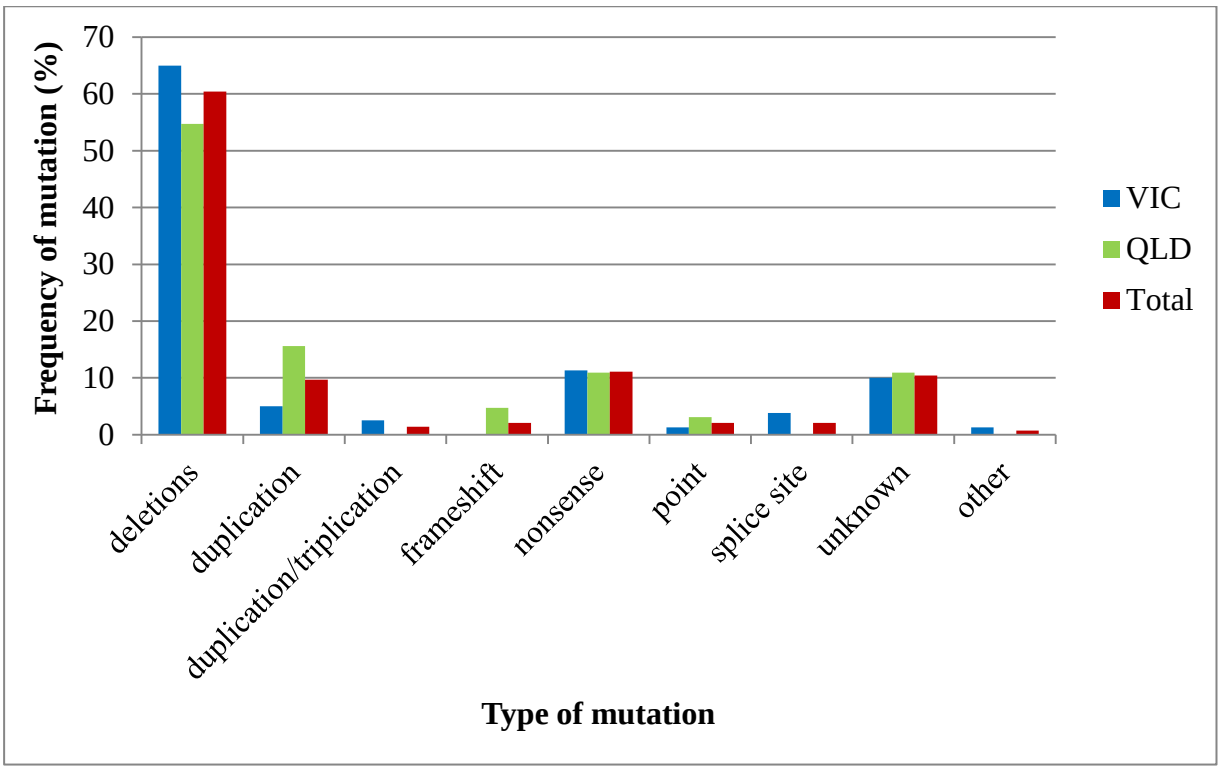

Figure 2-3 Proportion of documented mutation types in boys with DMD living in Victoria and Queensland

The mutation described as 'other' was a duplication with a premature stop codon.

2.4.4 Location of affected exons

The frequency of affected exons across the cohort is described in Figure 2-4, whilst Figure 2-5 and Figure 2-6 illustrate the frequency of affected exons across the different mutations and in each state, respectively. A clear 'hot spot' is identifiable between exon 47 and exon 52 and to a lesser degree between exon 12 and exon 15 of *DMD-G*. No mutations were documented after exon 65. Visually, deletions appear more common after exon 26 while duplications are more common between exon 1 and exon 24 (Figure 2-5). Similarly, the proportion of affected exons between exon 1 and exon 30 appears higher in Queensland (Figure 2-6). The number of observations per affected exon was too small to test these differences statistically.

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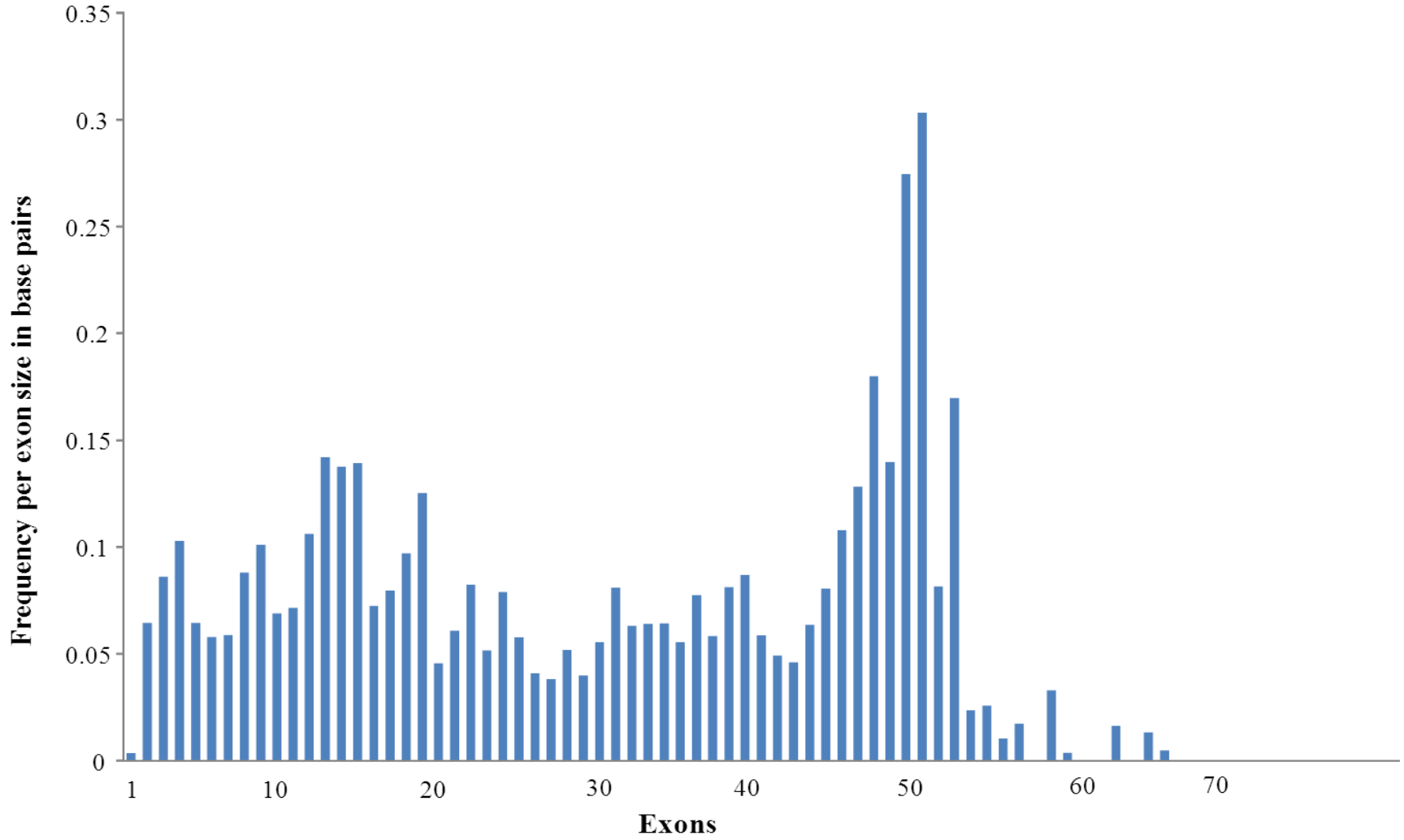

Figure 2-4 Frequency of affected exons in the dystrophin gene expressed per exon size in base pairs in boys with DMD living in Victoria and Queensland

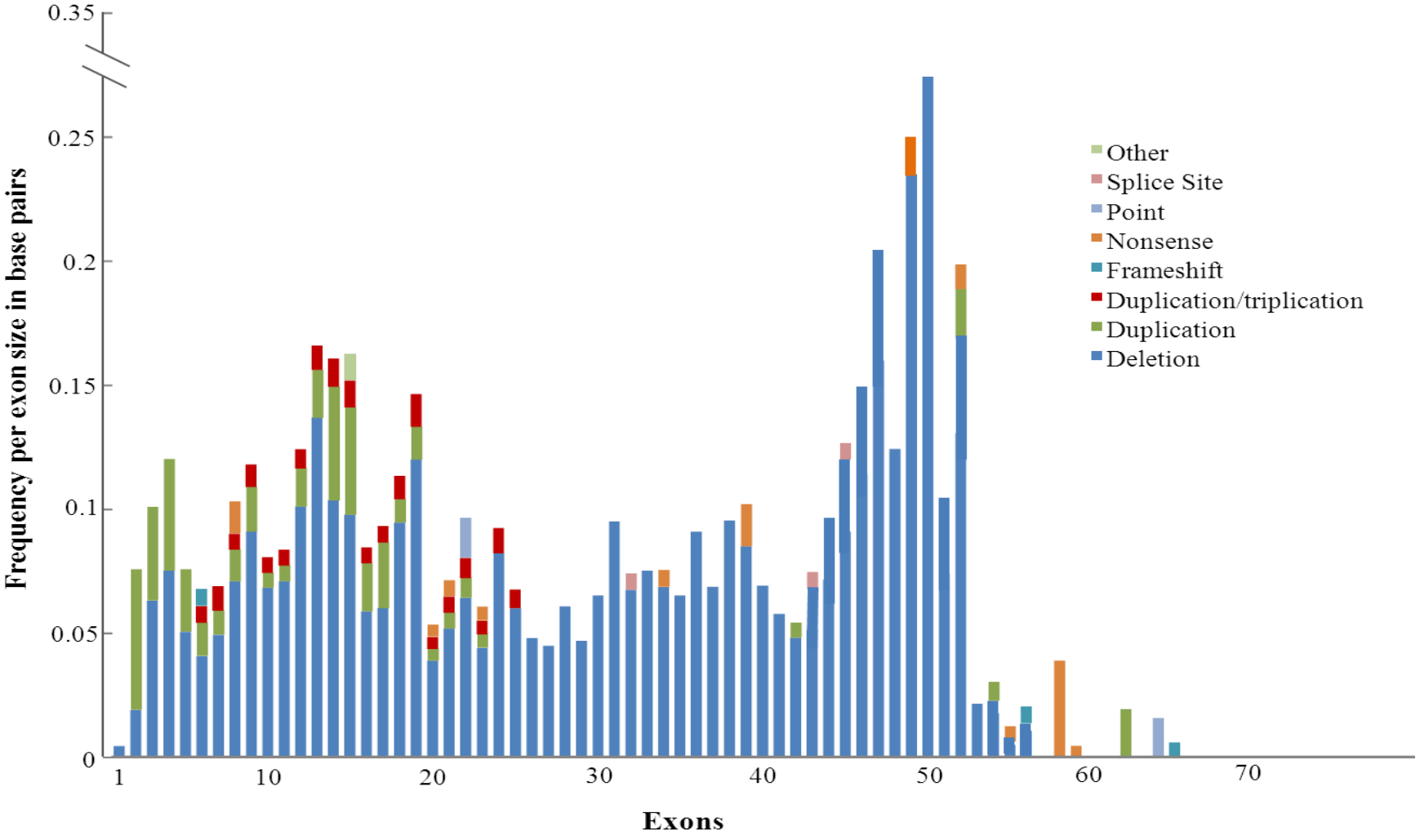

Figure 2-5 Frequency of affected exons in the dystrophin gene expressed per exon size in base pairs and split by mutation type in boys with DMD living in Victoria and Queensland

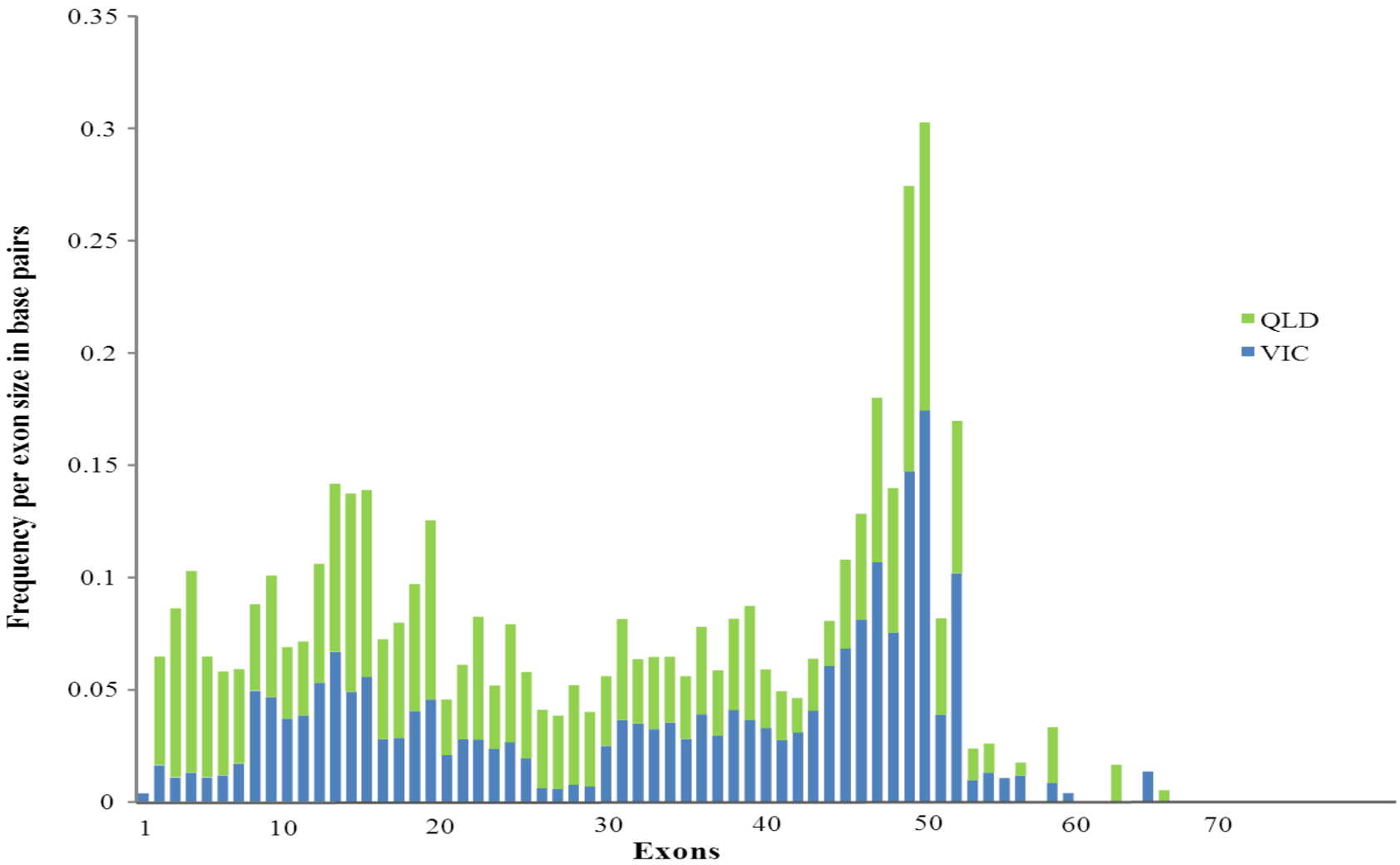

Figure 2-6 Frequency of affected exons in the dystrophin gene expressed per exon size in base pairs and split by cohort location (Victoria and Queensland)

2.4.5 Description of changes in BMI over time

Longitudinal data for change in BMI z- score is presented in Figure 2-7. A variable number of observations were made for each age group (n=7 at age 2 and 17 years; n=56 at age 7 years). BMI z-scores hold steady at approximately 1 SD above the mean until 12 years of age after which there is a marked and progressive decline. Despite the visual decline observed, this trend was not statistically significant (Freidman Test, p=0.671). The visual decline in BMI z-score with age is also evident in Figure 2-8. The proportion of patients with BMI < 5th percentile steadily increases with age, whilst there is a concomitant, but less obvious, decline in the proportion of patients with BMI > 85th percentile.

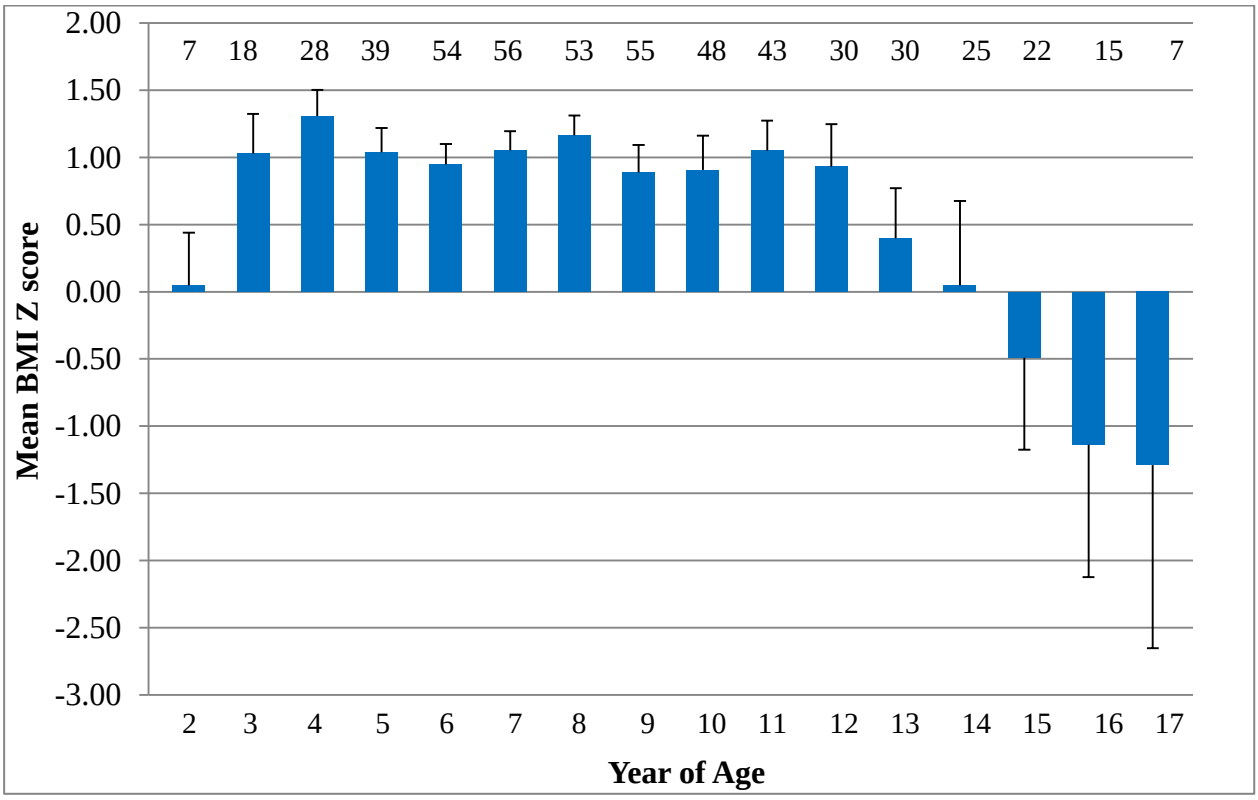

Figure 2-7 Mean BMI z-score by year of age in boys with DMD in combined cohorts living in Queensland and Victoria

Solid bars represent the mean BMI z-score for each age group while the number above each bar represents the number of observations. The thin vertical lines represent the standard error of the mean.

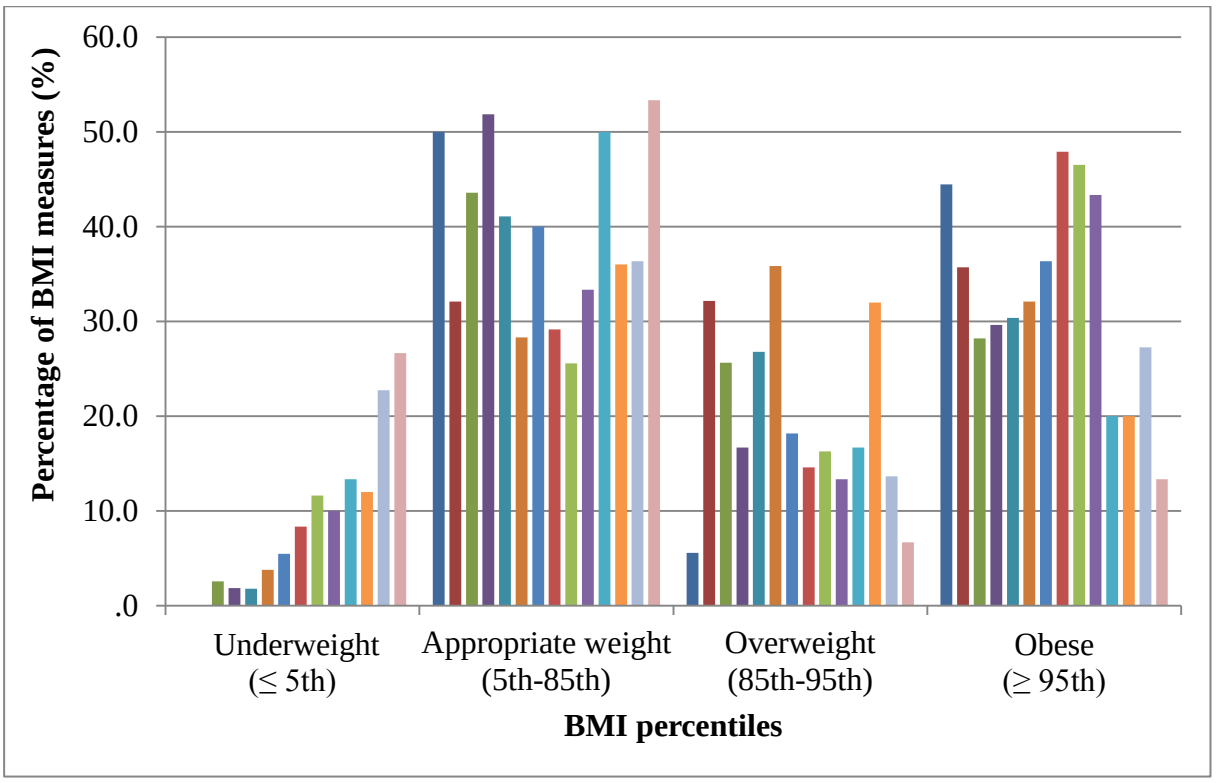

Figure 2-8 Classification of BMI percentiles by year of age and BMI status in boys with DMD living in Queensland and Victoria

Each coloured bar represents a yearly age increment from age 3 years to age 16 years progressing from left to right. Refer to Box 2 for classification of BMI measures.

2.4.6 Description of changes height over time

Longitudinal data for change in height is represented in Figure 2-9. Again, there were a variable number of observations for each age group. Between the ages of 2 and 13 years, height z-scores fluctuate between -0.5 and -1.0 standard deviations below the mean. From ages 14 to 17 years there is a marked decline in height z-scores representing a slowing in height growth.

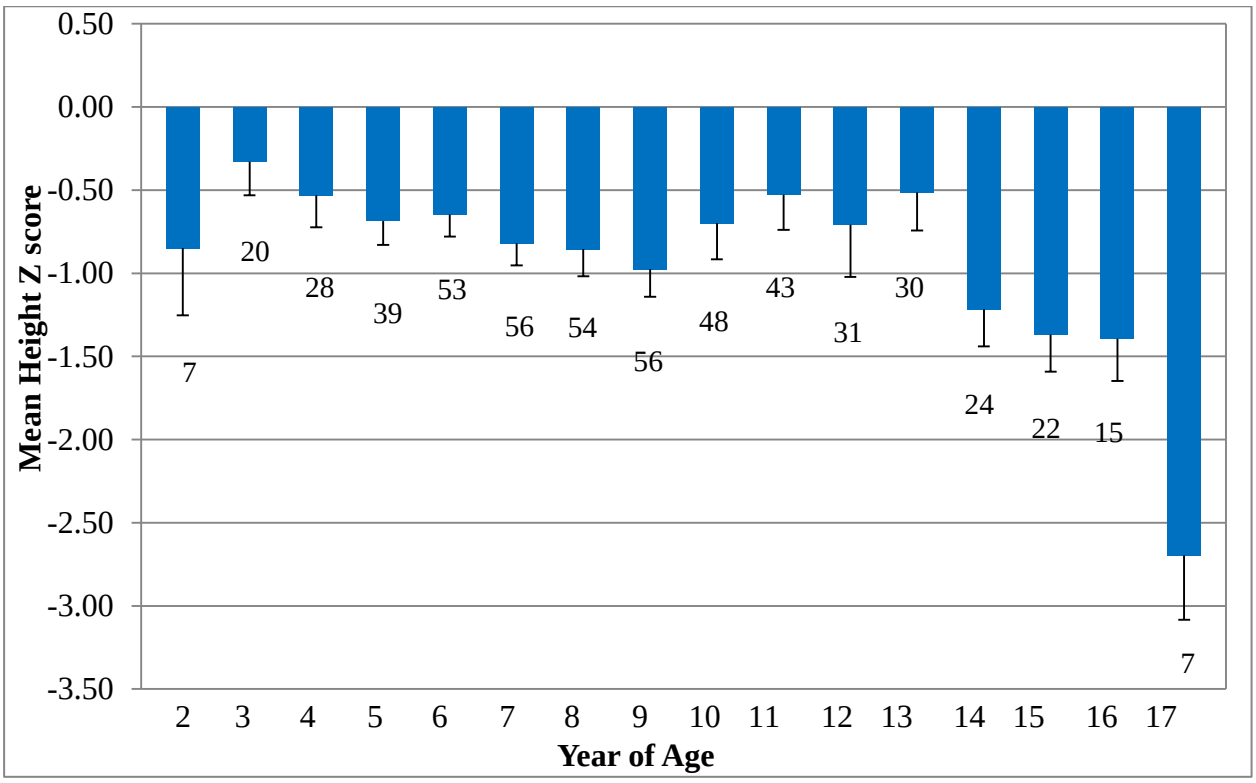

Figure 2-9 Mean height z-score by year of age

Solid bars represent the mean height z-score for each age group while the number below each bar represents the number of observations. The thin vertical lines represent the standard error of the mean.

2.4.7 Exploring the relationship between age at LOA and clinical and genetic factors

Standard multiple regression was used to explore relationships between age at LOA and clinical and genetic factors (Table 2-6). Clinical characteristics (age at chart audit, duration of GCS treatment and BMI z-score at closest to age of LOA) were entered into the model in two steps (Model A and B) before genetic factors were considered (Model C). The variance explained by each model was significant.

Age at audit and duration of GCS explained 33.1% of variation in age at LOA. When the BMI z-score closest to age at LOA was entered into the model, there was little change in the variance explained (32.3%) suggesting that BMI did not influence age at LOA. In the final model, BMI z-score was removed and mutation type (deletion or other) was entered,

increasing the variance explained to 46.3%. It proved important in this model to control for variability resulting from age at chart audit (explains 19.5% of variance) as well as for the duration of GCS therapy (9.2% of variance). With these variables entered, the effect of mutation type becomes evident, explaining 13% of the variance. The age at LOA was significantly lower in boys with a deletion compared to boys with duplications, point mutations and unknown mutations (9.30 ± 1.60 vs 10.97 ± 2.03 years, respectively, $p < 0.0005$).

Table 2-6 Summary of multiple linear regression models exploring the relationships between age at loss of ambulation and clinical and genetic variables

Independent variable	**Dependent variable: Age at Loss of Ambulation (n=64)**			
	B	*P*	Variation Explained (%)	95% CI
Model A[a]				
Age at audit (years)	0.458	**0.000**	20.7	0.143, 0.383
Duration of GCS treatment (years)	0.337	**0.002**	11.4	0.114, 0.489
Model B[b]				
Age at audit (years)	0.475	**0.000**	21.5	0.138, 0.365
Duration of GCS treatment (years)	0.351	**0.002**	11.7	0.113, 0.468
BMI z-score at LOA	-0.001	0.992	0	-0.172, 0.170
Model C[c]				
Age at audit (years)	0.442	**0.000**	19.5	0.144, 0.362
Duration of GCS treatment (years)	0.305	**0.002**	9.2	0.103, 0.443
Mutation type (deletion versus other)	0.364	**0.000**	13.1	0.693, 2.212

[a] explains 33.1% of variance; [b] explains 32.3% of variance; [c] explains 46.3% of variance.
LOA: loss of ambulation, GCS: glucocorticosteroids, BMI: body mass index, CI: confidence interval

There were a reduced number of observations for which to explore the relationship between age at LOA and location of the affected exons. Unknown mutations (classed as 'other' above) were excluded from this regression because the affected region had not been identified. In this multiple regression analysis with reduced numbers (n=50), location of the mutation was entered into the model with age at audit, duration of GCS treatment, BMI z-score closest to age at LOA, and mutation type. The model was significant ($p<0.0005$) explaining 49.8% of the variance in age at LOA. Location of the mutation had little influence on the model (0.1%); the significant independent variables remained age at audit, duration of GCS treatment and mutation type.

To explore predictive factors for age at LOA < 10 years, logistic regression was used. Univariable analyses were first conducted in which possible predictive factors were explored (Table 2-7). Significant predictors included duration of GCS treatment, mutation type, and BMI z-score at age 6.00-7.99 years ≥ 85th percentile. Because of the low number of observations available for BMI, only duration of GCS and mutation type were entered in the multivariable analysis.

The results from the multivariable analysis predicting LOA < 10 years are summarised in Table 2-8. The model explained between 23.3% (Cox and Snell R squared) and 31.2% (Nagelkerke R squared) of the variance in age of LOA. Both duration of GCS treatment and mutation type remained significant independent predictors of LOA at < 10 years. Mutation type was the strongest predictor of LOA; boys with deletions in the dystrophin gene are six times more likely to lose ambulation before age 10 compared to boys with duplications, point and unknown mutations. Shorter duration of GCS is also a predictor of early LOA; for every year of GCS treatment, boys with DMD are 0.65 times less likely to lose ambulation before the age of 10 years.

Table 2-7 Univariable logistic analysis predicting age at loss of ambulation below 10 years of age in boys with DMD

		Dependent variable: LOA < 10 years (Y/N)			
Independent variable	**n**	**B**	***p*[b]**	**Odds Ratio**	**95% CI**
Duration GCS treatment	64	-0.372	**0.014**	0.690	0.513, 0.928
Mutation type (deletion or other)	69	1.604	**0.003**	4.971	1.701, 14.533
Location of mutation	56	-0.357	0.517	0.700	0.238, 2.060
BMI z-score age 6-7.99	22	0.870	0.097	2.386	0.854, 6.664
BMI z-score age 6-7.99 ≥ 85th %-ile	22	1.897	**0.050**	6.667	1.004, 44.284
BMI z-score age 6-7.99 ≥ 95th %-ile	22	0.223	0.806	1.250	0.211, 7.414
BMI z-score age 8-9.99	34	0.218	0.429	1.243	0.725, 2.132
BMI z-score age 8-9.99 ≥ 85th %-ile	34	1.269	0.122	3.556	0.712, 17.763
BMI z-score age 8-9.99 ≥ 95th %-ile	34	0.724	0.303	2.062	0.520, 8.175

p for independent variable, LOA: loss of ambulation, GCS: glucocorticosteroids, BMI: body mass index, CI: confidence interval

Table 2-8 Multivariable logistic analysis predicting age at loss of ambulation below 10 years of age in boys with DMD

		Dependent variable: Age at LOA < 10 years (n=64)		
χ^2		17.014		
P		<0.0005		
Cox & Snell R Square		0.233		
Nagelkerke R Square		0.312		
Independent variables	**B**	***P***	**Odds Ratio**	**95% CI**
Duration GCS treatment	-0.428	**0.017**	0.652	0.460, 0.925
Mutation type (deletion or other)	1.819	**0.004**	6.165	1.780, 21.356

LOA: loss of ambulation, GCS: glucocorticosteroids, CI: confidence interval

In summary, this analysis demonstrates that a deletion mutation in the *DMD-G* may negatively impact on functional ability, resulting in earlier loss of independent ambulation. Neither the mutation site, nor BMI had influence on LOA. Conversely, a longer duration of GCS treatment may prolong independent walking.

2.4.8 Exploring the relationship between BMI z-score and clinical and genetic factors

Standard multiple linear regression was used to explore relationships between BMI z-score and clinical and genetic factors (Table 2-9). Clinical characteristics (age, duration of GCS treatment, ambulatory status and FEV1%) were entered into the model in two steps (Model A and B) before genetic factors were considered (Model C). The variance explained by each model was significant.

Age and duration of GCS explained 22.2% of variation in BMI z-score. When ambulatory status and lung function were added to the model, the variance in BMI z-score explained increased to 31.3%. Genetic characteristics had very little effect. The strongest other relationship seen was between BMI and age explaining 8.6% of variation. This was an inverse relationship whereby as age increases, BMI decreases. Lung function, ambulatory status and duration of GCS treatment were also significantly related to BMI z-score. While the relationship between BMI z-score and ambulatory status was evident in this multivariate regression model, difference in BMI z-score was not evident when boys who were ambulatory were directly compared to males who could no longer walk (BMI z-score: 1.19 ± 1.12 vs 0.50 ± 2.68, respectively, p=0.700). The relationship between BMI z-scores over time and genetic variables was also explored (data not shown). No relationship could be found between BMI z-score at any given age and mutation type or mutation location.

In summary, the BMI of boys with DMD is influenced by age, duration of GCS treatment, lung function and ambulatory status. A higher BMI is associated with younger age, longer duration of GCS treatment, and higher lung function; a lower BMI is associated with older age, shorter duration of GCS and lower lung function. BMI appeared unrelated to genetic variables.

Table 2-9 Summary of multiple regression models exploring the relationships between BMI z-score and clinical and genetic variables

Independent variable	Dependent variable: BMI z-score (n=94)			
	B	P	Variation explained (%)	95% CI
Model A[a]				
Age (years)	-0.368	**0.000**	13.5	-0.267, -0.089
Duration of GCS treatment (years)	0.317	**0.001**	10.0	0.103, 0.386
Model B[b]				
Age (years)	-0.397	**0.001**	9.0	-0.305, -0.081
Duration of GCS treatment (years)	0.223	**0.030**	3.8	0.017, 0.326
Ambulatory status (Y/N)	0.322	**0.011**	5.2	0.247, 1.819
FEV1(%)	0.367	**0.003**	7.2	0.008, 0.038
Model C[c]				
Age (years)	-0.391	**0.001**	8.6	-0.303, -0.076
Duration of GCS treatment (years)	0.221	**0.034**	3.7	0.014, 0.327
Ambulatory status (Y/N)	0.325	**0.011**	5.3	0.245, 1.844
FEV1(%)	0.375	**0.003**	7.4	0.008, 0.039
Mutation type (deletion or other)	-0.007	0.942	0.0	-0.677, 0.629
Location of mutation	-0.051	0.587	0.2	-0.761, 0.433

[a] explains 22.2% of variance; [b] explains 31.3% of variance; [c] explains 31.6% of variance.
BMI: body mass index, GCS: glucocorticosteroids, FEV1(%): forced expiratory volume as percentage of predicted, Y/N: yes/no.CI: confidence interval.

2.4.9 Exploring the relationship between lung function and clinical and genetic factors

Standard multiple linear regression was used to explore relationships between lung function (FEV1%) and clinical and genetic factors (Table 2-10). Clinical characteristics (age, duration of GCS treatment, ambulatory status and BMI z-score at lung function) were entered into the model in two steps (Model A and B) before genetic factors were considered (Model C). The variance explained by each model was significant.

Age and duration of GCS explained 44.8% of variation in FEV1%. When ambulatory status and BMI z-score were added to the model, more than half (53.3%) of the variance in FEV1% was explained. Again, genetic characteristics had very little effect on FEV1% in the total model or as independent variables. All other independent variables were significantly related to FEV1%. The strongest relationship was between lung function and ambulatory status, explaining 6.6% of the variance. FEV1% was significantly lower in non-ambulatory than in ambulatory boys (60 ± 25% vs 92 ± 14% respectively, $p<0.0005$). An inverse relationship was evident between lung function and age, and a directly proportional relationship with BMI z-score and duration of GCS treatment.

In summary, in this cohort lung function did not relate to genetic variables. Rather it associated with age, duration of GCS treatment, ambulatory status and BMI z-score. An older age and non-ambulatory status are associated with decreased lung function; whereas a longer duration of GCS and higher BMI are associated with increased lung function.

Table 2-10 Summary of multiple regression models exploring the relationships between forced expiratory volume in 1 sec as a per cent of predicted and clinical and genetic variables

Independent variables	**Dependent variable: FEV1% (n=87)**			
	B	*P*	Variation explained (%)	95% CI
Model A[a]				
Age (years)	-0.531	**0.000**	28.2	-5.350, -2.857
Duration of GCS treatment (years)	0.403	**0.000**	16.2	2.996, 6.994
Model B[b]				
Age (years)	-0.257	**0.016**	3.5	-3.589, -0.386
Duration of GCS treatment (years)	0.274	**0.001**	6.3	1.355, 5.443
Ambulatory status (Y/N)	-0.340	**0.001**	6.4	-27.711, -7.053
BMI z-score at lung function	0.208	**0.015**	3.5	0.681, 6.213
Model C[c]				
Age (years)	-0.262	**0.015**	3.5	-0.365, -0.405
Duration of GCS treatment (years)	0.268	**0.002**	5.9	1.261, 5.389
Ambulatory status (Y/N)	-0.350	**0.001**	6.6	-28.331, -7.388
BMI z-score at lung function	0.214	**0.013**	3.7	0.758, 6.327
Mutation type (deletion or other)	-0.011	0.892	0.0	-9.520, 8.296
Location of mutation	0.084	0.291	0.7	-3.774, 12.424

[a] explains 44.8% of variance; [b] explains 53.3% of variance; [c] explains 54.1% of variance.
BMI: body mass index, GCS: glucocorticosteroids, FEV1(%): forced expiratory volume as percentage of predicted, Y/N: yes/no.CI: confidence interval.

2.5 Discussion

This analysis is unique. Here, for the first time, the genetic profile of boys with DMD in two Australian states has been documented and a relationship between genotype and function elucidated; boys with a deletion in *DMD-G* are more likely to stop walking before age 10. Whilst the relationship between growth and walking ability remains unclear, a higher BMI may be protective against a decline in lung function. The study also provides evidence for commencing GCS earlier, as a longer duration of GCS treatment is associated with walking past age 10 years, and increased lung function and BMI.

In accordance with the null hypothesis, the Victorian and Queensland cohorts were very similar across most physical, clinical and genetic factors, with the exception of weight, BMI z-score and commencement and dose of GCS therapy. Boys from Queensland with DMD were heavier and consequently had a higher BMI than those from Victoria. Differences in GCS commencement and dose may explain these observed differences in weight. Regression analysis (Section 2.4.8) demonstrates that longer duration of GCS treatment is associated with higher BMI. Boys in Queensland commenced GCS therapy over a year earlier than boys in Victoria. Although the duration of GCS use between the two cohorts was not statistically different, the IQR was higher in the Queensland group (1.9-4.7 years vs VIC 1.0-3.8 years) suggesting that boys in Queensland may indeed receive GCS therapy for longer. Observed differences in body weight could also relate to the absence of a dietitian in the Queensland clinic, suggesting more limited availability of nutritional therapy. These data might therefore be useful in the development of a business case to support the addition of dietetic staff to the Queensland clinic.

The cohort in Victoria received significantly higher doses of prednisone in both absolute and relative terms (mg/kg/day) compared to the Queensland cohort. This did not impact on

function as both groups lost ambulation at a similar age. Notably, both groups received much less GCS (mean dose 0.48 mg/kg/day) than the dose determined by meta-analysis to be the most effective for maintenance of muscle strength and function (0.75mg/kg/day) (37). Lower doses of GCS were perhaps given in order to manage weight gain. Use of deflazacort (mean dose: 0.83mg/kg/day) was closer to the recommended therapeutic dose (0.9mg/kg/day) (3). Across the combined cohort, a lack of monitoring for GCS induced osteoporosis was also evident.

Current practice guidelines for the management of GCS induced osteoporosis recommend serial monitoring of bone mineral density and annual monitoring of serum vitamin D (162, 163). In this cohort, only 20% of participants had a documented dual energy X-ray absorptiometry (DXA) scan for measurement of bone mineral density, or had assessment of serum 25-hydroxyvitamin D. These figures are disturbing given the highly deleterious effect of GCS therapy on bone strength; GCS induced osteoporosis contributes to fractures in as many as 30–50% of adults prescribed GCS, even when they receive 2.5- to 7.5-mg prednisone equivalents per day (164, 165). Improved protocols need to be implemented to ensure systematic monitoring for bone complications in boys with DMD who receive GCS therapy.

There is very little literature regarding current GCS use in DMD. Matthews et al. reported that only 51% of an American cohort of boys with DMD and BMD received GCS treatment (48), substantially less than in the present cohort (71.5%). These American boys with DMD received more prednisone (0.729mg/kg/day) than our Australian cohort but were given a similar dose of deflazacort (0.83mg/kg/day).

The genetic profile between the two cohorts was also very similar. In the combined cohort, deletions were the most common mutation, accounting for 60% of all mutations, followed by

duplications and point mutations equally reported at approximately 10%. The mutation profile documented here, specifically deletions and duplications, is very similar to other mutation profiles in DMD reported in the literature from a number of different countries and cultural backgrounds (Table 2-11). The proportion of point mutations seen in this group appears to be lower. With advances in *DMD-G* sequencing, the unknown mutations (10%) in this group would likely now be identified as point mutations, increasing the proportions of these to similar levels observed internationally. Similarly, the hot spot regions observed in this cohort (exons 12-15 and exons 47-52) are located within hot spot regions previously documented (exons 2-20 and exons 45-55) (160). This suggests that geographical differences have little influence on mutations affecting the *DMD-G*, a conclusion also supported by the lack of difference seen in the mutation profile between boys from Victoria and Queensland.

Table 2-11 Prevalence of mutation profiles in DMD cohorts as previously reported

Study	Country	Deletions	Duplications	Point Mutations
This study	**Australia**	**60%**	**10%**	**10%**
Todorova et al. (2008) (166)	Bulgaria	63%	22%	11%
Liechti-Gallati et al. (1989) (167)	Switzerland	66%	NR	NR
Brabec et al. (2009) (168)	Czech Republic & Slovakia	58.8%	9.8%	16.7%
Takeshima et al. (2010) (169)	Japan	60%	8%	32%
Tuffery-Giraud et al. (2009) (160)	France	61%	13%	26%
Basak et al. (2009) (170)	India	62%	NR	NR
Swaminathan et al. (2011) (171)	India	73%	NR	NR
Cunniff et al. (2009) (172)	USA	78.3%	11.3%	10.4%

NR not reported; USA United States of America.

This is the first study to report standard weight and BMI z-scores in GCS treated boys with DMD, and thus no comparison to other literature can be made. While comparisons of the proportion of under- and overweight boys presenting at different ages is possible, this is limited by the different classifications used and the absence of GCS treatment in other cohorts. In the present cohort the highest proportion of obese boys (47.9% based on BMI) was seen at age 10 years. This is similar to the findings of McDonald et al. and Willig et al. who reported that 44% and 54% of boys, respectively, aged 9-13 years had a body weight above the 90th percentile (80, 81). Use of the weight-for-age ratio rather than BMI has resulted in a higher proportion of boys being classified as obese around this same time (73%) (83). On the other hand, the proportion of boys found to be underweight during the more advanced stages of DMD was similar in the study by Martigne et al. based on weight-for-age ratio and in this present study (based on BMI) (34% at age 18 years vs 26.7% at age 16 years, respectively) (83). Other studies defining underweight as weight below the 10th percentile reported much higher proportions: 65% at age 17 years, and 54% at age 18 years, respectively (80, 81). The need for standard classification systems for defining overweight and underweight across all studies is obvious, and would allow for the prognostic implications of over- and under nutrition to be better explored.

Between the ages of 2 and 13 years, height z-scores in boys with DMD varied between -0.33 and -0.97 SD below the mean at 3 and 9 years, respectively (Figure 2-9). This reduced stature is consistent with that reported in steroid-naïve cohorts of similar age (eg: height z-scores: -1.0 and -0.936 (79, 80)). Whilst the aetiology of short stature in the absence of GCS treatment is unclear, it has been argued that it may provide some functional benefit due to the ‘favourable mechanics’ associated with a smaller size (35, 79). There is limited evidence suggesting short stature may be associated with increased strength and improved clinical outcomes (81, 173). In the present cohort, mean height z-scores decreased to less than -1.0 SD

below the mean between the ages of 14-17 years, at a time when boys should be reaching their adolescent growth spurt. This slowed growth may be indicate growth failure secondary to chronic GCS use, or it may reflect inaccurate estimation of body height due to the presence of severe scoliosis and/or contractures in non-ambulatory boys. Decreased decline in nutritional status could also be a contributing factor.

This analysis demonstrated that a deletion mutation in the *DMD-G* may negatively impact on functional ability, resulting in earlier loss of independent ambulation. In the present cohort, boys with deletions in the dystrophin gene were six times more likely to lose ambulation before 10 years of age compared to boys with duplications, point and unknown mutations. This is a novel finding, warranting further exploration within a larger cohort.

Deletions and duplications resulting in rearrangement of chromosomal sequence are thought to result in severe disease. This is because deletions or duplications, in many cases, can involve more than one gene (174). Or in this instance, because of the sheer size of the dystrophin gene, deletions and duplications can remove a great deal of the reading frame. Due to the size of our cohort, there was insufficient power to investigate duplications as a separate mutational entity. However, diseases caused by deletions are thought to more severe than those caused by duplications (174). Other genetic disorders characterised by deletions specifically include *cri du chat* Syndrome and Prader-Willi Syndrome. Within the neuromuscular disorders, a relationship between mutation class and phenotype has previously been observed.

Charcot-Marie-Tooth is a neuropathy that causes the slow degeneration of nerves in the extremities. It is caused by mutations in the gene coding for the Connexin32 protein (*Cx32*). Missense mutations in specific regions of the gene are associated with a milder clinical

phenotype; whereas deletion mutations in any location of the Connexin32 protein cause severe forms of the disease and onset in the first decade of life (175). In this example, the location of the missense mutations was associated with disease severity, yet in the present cohort, there was no relationship between location of mutation and disease severity. Similar results were observed by Desguerre et al. in their examination of relationships between phenotypes and sub-phenotypes; there was no correlation between age at LOA and site of mutation (156). In such a large gene, the potential to identify regions associated with function will be limited by the relatively small cohorts.

Based on the number of observations, the present cohort could only be split into two groups (mutations in exon 1-44 versus mutations in exon 45-79), although each group did incorporate known proximal (exon 2-20) or distal (exon 45-55) hotspots (160). A similar division was previously used to explore the relationship between mutation location and cognitive function, with the finding that distal mutations were associated with greater cognitive impairment perhaps because these distal mutations disrupt the production of dystrophin isoforms that are found primarily in the brain (152-154). The promoter regions for specific isoforms may therefore be a logical starting point to investigate genotype-phenotype relationships. Also, different mutational classes may be of importance in different regions of the dystrophin gene. Larger cohorts would be needed to explore whether deletions in specific locations are associated with more or less severe phenotypes.

This study only examined genetic factors relating to the *DMD-G* locus. Other genotypes may be involved in the observed heterogeneity in DMD. Recently, the osteopontin (*SPP1*) genotype was identified as a genetic modifier of disease severity in DMD; specifically mutations in the osteopontin gene promoter, causing low levels of osteopontin expression, were associated with shortened time to LOA and loss of muscle strength (147). Other possible

genetic modifiers may relate to the dystroglycan complex; a missense mutation in the gene encoding for the dystroglycan protein was identified in a women with limb-girdle muscular dystrophy (176).

Similar genotype-phenotype relationships have been identified in persons with cystic fibrosis. Homozygous ΔF508 mutations are associated with more severe disease including early onset of symptoms before 6 months, increased pancreatic enzyme replacement therapy, poorer lung function, increased yearly incidence of chronic *Pseudomonas aeruginosa* infection and increased yearly mortality rates when compared to heterozygous ΔF508 mutations (177). Identification of this phenotype has allowed for more directed care to be given, especially in the provision of pancreatic enzyme replacement therapy. In the same vein, the results cited here suggest that boys with a deletion in the *DMD-G* would most likely benefit from commencing GCS at a younger age.

The duration of GCS treatment was a significant predicator of age at LOA; for every year spent on GCS treatment, boys with DMD are 0.65 times less likely to lose ambulation before the age of 10 years. The optimal time to commence GCS treatment and the duration of treatment is still a matter of much debate (3). Whilst the negative effects of GCS treatment such as weight gain and decreased bone health need to be considered, early introduction of GCS may provide some functional benefits. Appropriate screening and prevention for weight gain and decline in bone health would need to be implemented.

Unexpectedly, BMI had little influence on age at LOA in the standard regression model. Whilst it is suspected that excess weight increases the burden on already weak muscles, there is little evidence to demonstrate that excess weight decreases walking ability in boys with DMD. Increased weight may in fact promote muscle strength. This is a contentious issue.

Absolute quadriceps strength has been demonstrated to be increased in obese males compared to normal weight males (178). Yet, in obese adolescent males, obesity has little or no effect on quadriceps muscle function (179). Functional measures may provide greater insight into the relationship between weight status and strength. In a larger study utilising data from the population-based Health 2000 Survey in Finland, long-term exposure to obesity was associated with poor hand grip strength later in life (180). Also, using the six minute walk test, an outcome of particular relevance to DMD, Hulens et al. demonstrated that obese women walked slower than normal weight women (181). Alternatively, there may be limitations with the use of BMI in this analysis.

The BMI measure used in the analysis was taken at a time closest to the age at LOA. As this might vary around the actual time of LOA by several years, obtaining clear relationships appeared difficult using continuous data. In univariable logistic regression, BMI z-score at age 6.00-7.99 years >85th percentile, was a significant predictor of age at LOA; those with a BMI $\geq$ 85th percentile were 6.6 times more likely to stop walking before age 10. Due to small numbers in this analysis (n=22), BMI as a predictor of age at LOA was unable to be taken into the larger multivariable analysis. There are also limitations inherent with BMI as a measure because it does not provide an indication of body composition. The proportion of muscle mass may be increased in boys with a higher BMI. Although Hankard et al. found no difference in muscle mass between obese and non-obese boys with DMD; an increase in in the proportion of muscle was observed when boys gained weight during GCS treatment (38, 71). The relationship between body composition, weight and function in boys with DMD needs further exploration.

The analyses in Sections 2.3.6.7 and 2.3.6.8 investigate the relationships between clinical and genetic variables. As hypothesised, there was no relationship between clinical characteristics and genetic factors. Yet, contrary to the null hypothesis, the clinical characteristics explored were interrelated. These analyses provide more information about advanced stages of DMD, highlighting factors associated with the decline. Importantly, age is still associated with decline in BMI and lung function, even in this heterogeneous population. In addition, an increased BMI and longer duration of GCS maybe beneficial in slowing the decline in FEV1%. Martigne et al. reported similar conclusions; mild obesity (weight-for-age ≥126.5% and ≤ 151%) should not be discouraged because it is associated with a protective effect against malnutrition in the advanced stages of DMD (83). Again, the beneficial effects of a higher BMI on FEV1% need to be considered against the detrimental effects of a higher BMI on walking ability. At present, there is insufficient evidence from this study and in the literature to support either argument.

To this author's knowledge, Martigne et al. is the only other group to document similar relationships between clinical variables (83). In their cohort, weight status was not associated with LOA or age of spinal surgery; however, at maximal follow up (mean age 18.3 years) 75% of subjects who did not require respiratory support were obese; compared to 30% of participants requiring respiratory support.

Although there was no relationship between genetic factors and clinical characteristics, a relationship between genotype and age at LOA was determined, as was a relationship between ambulatory status (not walking) and BMI and FEV1%. LOA may therefore act as a modifier between genotype and clinical factors. For example, a boy with a deletion mutation, who stopped walking before the age of 10 years, may experience a decline in lung function before a boy, with a duplication or other mutation type, who continues to walk past the age of 10

years. A proposed model of these relationships is presented in Figure 2-10. As the duration of GCS treatment is associated with both walking function and clinical factors, this has also been incorporated into the proposed model.

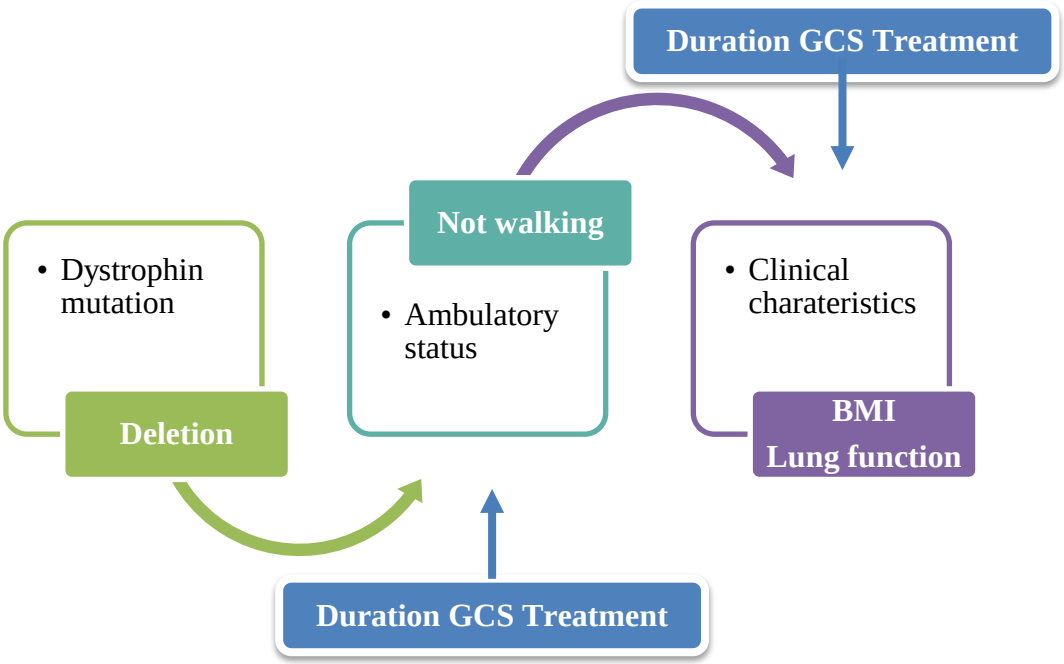

Figure 2-10 Proposed relationship between *DMD-G* mutation, ambulatory status and clinical characteristics.
Ambulatory status acts as a modifier between dystrophin mutation and clinical characteristics.

There are several limitations with this current study. Although interesting relationships were observed, as a retrospective case series, causation cannot be established. This study aimed to achieve a representative sample across both states. This was accomplished in Victoria (90%); however, only 60% representation was achieved in Queensland. There were many factors that contributed to this. Most significantly was the organisation of the Queensland clinic outside of Queensland Health, the major health body. Due to this, some boys in Queensland had several medical records, making documentation of care, including genetic information and anthropometric data, difficult to locate. Other limitations of this study include inaccuracies in

anthropometric data, especially height measures that may be influenced by contractures and scoliosis. Analyses of anthropometric data were also limited by reduced sample size. Anthropometric data was collected from occasions of clinical care; so for most subjects there was not a complete data set over time, that is, each boy included did not have a documented height and weight for each age group. Because of this, it was difficult to identify patterns of weight gain and relate these to genetic and clinical factors.

2.6 Future directions

This analysis provides enticing data that warrants further exploration in a larger cohort. Ideally, this would be collected with a prospective study design that would follow boys longitudinally from diagnosis. Age of LOA could then be more reliably obtained, and anthropometric could be collected at standardised intervals allowing for predictors of weight status and weight change to be explored more rigorously. The relationship between change in other clinical factors, such as lung function, and genotype could be investigated further. Alternatively, existing data from the current international natural history study conducted by the Cooperative International Neuromuscular Research Group (CINRG) could be utilised. This latter option is at present, being investigated by the author. Analysis of this existing data could be used to provide further support for prospective study exploring the role of growth, function and genetics in sub-phenotypes within DMD.

3 Exploring vitamin D in boys with Duchenne muscular dystrophy

This chapter relates to the published manuscript presented in Appendix 7:

Davidson ZE, Walker KZ, Truby H. Do Glucocorticosteroids Alter Vitamin D Status? A Systematic Review with Meta-Analyses of Observational Studies. J Clin Endocrinol Metab. 2012;97(3):738-44.

This chapter relates to the published abstracts:

Davidson ZE, Elliott SA, Sinclair K, Wallace G, Posselt H, Truby H. Oral Program: Vitamin D insufficiency in boys with Duchenne muscular dystrophy. Nutr Diet. 2009;66:A1-A24.

Davidson ZE, Kornberg AJ, Ryan MM, Kimlin M, Truby H. Conference Abstracts Nutrition Society of Australia and Nutrition Society of New Zealand 2011: I: The relationship between serum vitamin D and sun exposure in boys with Duchenne muscular dystrophy treated with corticosteroids compared with their healthy siblings. Australas Med J. 2011;4(12):789-813.

Davidson ZE, Walker KZ, Truby H. Conference Abstracts Nutrition Society of Australia and Nutrition Society of New Zealand 2011: IV: Serum 25 hydroxyvitamin D in patients treated with glucocorticosteroids: a systematic review with meta-analyses. Australas Med J. 2011;4(12):739-88.

3.1 Introduction

There has been little investigation into the micronutrient status and requirements of boys with DMD. Micronutrients of particular importance, particularly after the initiation of regular GCS therapy, are calcium and vitamin D. The functions of these are interconnected since a critical function of vitamin D is the regulation of calcium homeostasis by increasing calcium absorption from the intestine and calcium reabsorption from the kidneys. There is substantial evidence that boys with DMD have low bone mineral density and an increase in fracture risk when compared to healthy controls (66-70). As fractures will have a very significant effect on mobility (30-50% of boys sustaining a fracture in their lower limbs will never resume walking), it is essential to maintain optimal serum 25(OH)D levels in boys with DMD (69, 70). Vitamin D also has a role in maintaining muscle strength and function (182), an additional reason why in myopathies such as DMD, which cause loss of muscle mass, the maintenance of optimal vitamin D levels is of particular importance.

Although decreased levels of serum 25(OH)D have been previously reported in boys with DMD (66), the pathophysiology of this phenomenon remains unclear. The main aim of this chapter is thus to determine possible explanations for decreased serum 25(OH)D concentrations in boys with DMD. *Investigation 1* explores the role of sun exposure, whilst *Investigation 2* examines the prolonged use of GCS therapy as a contributing factor.

For the purpose of these investigations, vitamin D deficiency is defined as serum 25(OH)D < 20 ng/ml (<50 nmol/L) (183). At 25(OH)D concentrations of 10-20 ng/ml (25-50 nmol/L), parathyroid hormone is distinctly elevated, intestinal calcium absorption is reduced and deleterious effects on bone mineralization become evident (184). Even when serum 25(OH)D falls below 24-30 ng/ml (60-75 nmol/L), muscle and immune function can be compromised (185). The formation of the biologically active form of vitamin D requires two sequential

hydroxylation steps, one in the liver to produce calcidiol (25-hydroxyvitamin D (25(OH)D)) and the second in the kidney (under the influence of parathyroid hormone) to produce calcitriol (1,25-dihydroxy vitamin D3) (186). Calcidiol (25(OH)D) remains the best serum measure of vitamin D status and is the form in which oral doses are provided (183, 187). Conversion factors for measures of serum vitamin D concentrations and dietary intake or supplementation are provided in Box 4.

Conversion Factors	
1 ng/ml	= 2.496 nmol/L
1 µg	= 40 IU

Box 4 Vitamin D conversion factors

3.2 Investigation 1 – sun exposure

3.2.1 Background

Vitamin D is unique nutritionally, in that dietary sources contribute little to body stores. Instead the primary source of vitamin D comes from production of cholecalciferol in the skin after exposure to ultraviolet B radiation from sunlight. Cholecalciferol is subsequently converted to 25(OH)D in the liver (182). Despite an abundance of sunshine in Australia, suboptimal levels of Vitamin D have been reported in Australian adults (188). A recent national cross-sectional study determined 31% of Australian adults are deficient in vitamin D (182). This may have been compounded by the 'sun safe' message to avoid sun exposure targeted at reducing the risk of melanoma; and this health behaviour has been extensively adopted by the Australian population. Children with disabilities or chronic illness are often assumed to have decreased vitamin D levels because of inadequate sun exposure. Here, vitamin D levels in ambulant boys with DMD are compared to their healthy siblings who inhabited the same home environment with the objective of determining if a decreased sun exposure explained reduced serum 25(OH)D levels in boys with DMD.

3.2.2 Objectives

1. To describe serum 25(OH)D concentration in boys with DMD compared to their healthy siblings.
2. To describe the amount of sun exposure boys with DMD receive compared to their healthy siblings.
3. To examine the relationship between sun exposure and 25(OH)D concentrations in boys with DMD and in their healthy siblings.

3.2.3 Hypotheses to be tested

I. Boys with DMD will have significantly lower serum 25(OH)D concentrations than their healthy siblings.

II. Boys with DMD will spend less time in the sun than their healthy siblings.

III. There will be a directly proportionate relationship between sun exposure and serum 25(OH)D concentrations in both boys with DMD and their healthy siblings.

IV. The relationship between sun exposure and serum 25(OH)D concentrations will be of similar strength in both boys with DMD and their healthy siblings.

3.2.4 Subjects

Boys with DMD were recruited from the neuromuscular clinic at The Royal Children's Hospital, Melbourne, Australia. Diagnosis was defined by documentation of a deletion or duplication in the dystrophin gene, or absence of dystrophin on muscle biopsy, in conjunction with phenotypic evidence based on characteristic clinical symptoms or signs by nine years of age (ie, proximal muscle weakness, waddling gait, and Gower's' manoeuvre), an elevated serum creatine kinase, and ongoing difficulty with ambulation. Healthy siblings of participating boys were recruited as a comparator group thereby controlling for environmental factors such as latitude and weather patterns. Recruitment and assessments occurred during the month of September 2009. The study was approved by Human Research Ethics Committee at The Royal Children's Hospital (HREC# 29075B, Appendix 3), and informed consent was obtained from parents/guardians for boys with DMD (Appendix 8) and their health siblings (Appendix 9).

3.2.5 Methods

Venous blood samples were collected from all children by a trained paediatric phlebotomist. Serum 25(OH)D was measured by Pathology Queensland with a Liaison Auto analyser (DiaSorin, Stillwater, MN) using a chemiluminescent technique which has a coefficient of variability < 10% (189). Information on nutritional supplement use was collected; but dietary intake was not examined as in Australia, where few foods are fortified with vitamin D, most foods provide negligible amounts of this vitamin. For each child separately, parents completed an adapted version of a sun exposure questionnaire, developed specifically for personal UV exposure assessment (190). The questionnaire was modified for boys with DMD and for sibling participants (Appendix 10). This questionnaire was provided for use by Professor Michael Kimlin, AusSun Research Laboratory, Queensland University of Technology, Brisbane. Although the tool was not validated in Australia, the group has used this questionnaire in several Australian cohorts (190-192). The questionnaire uses time spent outdoors as a proxy for sun exposure. Parents were asked to identify the amount of time their child spends outdoors during each hour between 5am and 7pm. For each hour slot, parents could choose never; <15 minutes, 15-30 minutes, 30-45 minutes, 45-60 minutes. This was completed for a typical Monday-Sunday period for the previous month.

3.2.6 Statistical analysis

Total minutes per day spent outside was calculated by summation of the minutes/hour, rounding up to the nearest 15 minute segment. The average time spent outside in minutes/day was calculated across the seven day period for each individual, as well as the average minutes/hour on any given day. Data were first examined for normality using the Shapiro-Wilk test for normality. An independent Student's t-test was used to compare differences in mean time spent outside between boys with DMD and their siblings. Serum 25(OH)D data was split into quartiles and the mean sun exposure per day for boys in each quartile was

compared using a one-way analysis of variance. Pearson's correlations were applied to explore the relationship between mean time spent outside exposure and serum 25(OH)D. Data are reported as mean ± SD and significance was accepted at $p \leq 0.05$. The statistical package used in this analysis was SPSS, Version 18.0 (SPSS Inc., Chicago, IL).

3.2.7 Results

Six boys with DMD, each with at least one healthy sibling (n = 9), were recruited. Blood samples could not be obtained from two sibling participants. All children attended regular school or kindergarten. Five of the boys with DMD were receiving GCS treatment (prednisolone 0.51 – 0.64mg/kg/day or deflazacort 0.83mg/kg/day). All boys with DMD were independently ambulant although one regularly required a motorised chair. Vitamin D supplementation was reported by one boy with DMD (1000 IU/day).

The mean age of the boys and their siblings was similar (8.2 ± 2.1 vs. 8.3 ± 2.6; p=0.902). Serum 25(OH) D was significantly lower in boys with DMD compared to their siblings (52.2 ± 14.2 nmol/l vs. 77.9 ± 11.4 nmol/l; p=0.004) with no difference in mean time spent outside /day (251 ± 85 minutes/day vs. 300 ± 83 minutes/day; p=0.288). Within the DMD group, four of six boys had low serum 25(OH)D levels (≤ 50nmol/L) (75). Of note, serum 25(OH)D in the single steroid-naïve participant was 42 nmol/L. Despite the significant difference in serum 25(OH)D levels there was no difference in mean daily sun exposure between boys with DMD and their healthy siblings. The cohort as a whole, spent more time outside on weekend days compared to week days (336 mins/weekend day vs. 257 mins/week day; p=0.001). Further analysis revealed no difference between the two groups when comparing mean sun exposure for each day of the week (Mon-Sun); mean sun exposure/day in children with serum 25(OH)D above and below the normal levels 50nmol/L; and mean sun exposure/day in quartiles of serum 25(OH)D.

Although not significant, the relationship between mean daily sun exposure and serum 25(OH)D (Figure 3-1) was considerably stronger in healthy siblings (r = 0.642, p=0.12) than in boys with DMD (r = 0.146, p=0.782).

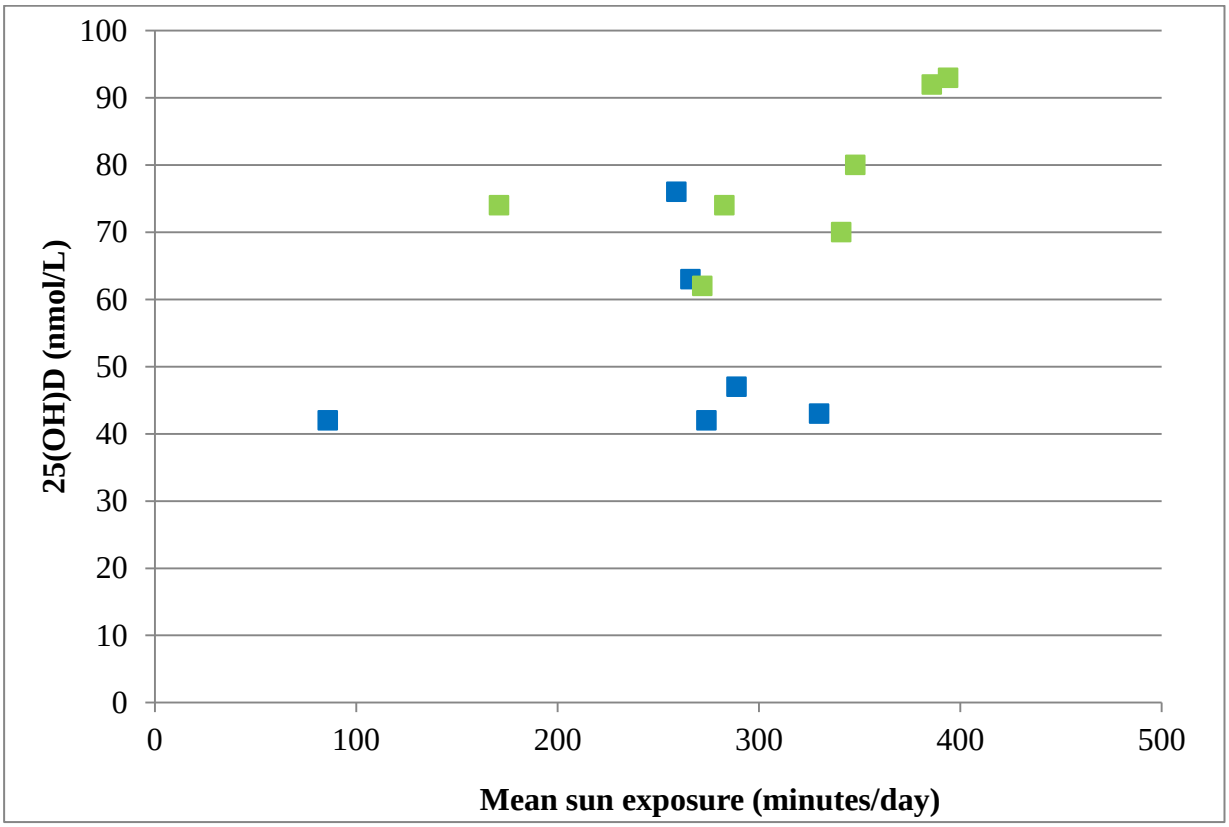

Figure 3-1 Relationship between mean sun exposure and serum 25(OH)D in boys with DMD and healthy siblings

The blue squares represent boys with DMD; green squares represent sibling participants.

3.3 Investigation 2 – corticosteroid use

3.3.1 Background

GCS are used as the primary medical management strategy in DMD as well as in a large range of conditions including inflammatory bowel disease, rheumatoid arthritis, and autoimmune disease. Whilst GCS are efficacious in managing these inflammatory conditions, their use can be accompanied by substantial side effects (162). Important among these is glucocorticoid-induced osteoporosis (GIO), the most widespread form of secondary osteoporosis (193). GIO contributes to fractures in as many as 30-50% of adults prescribed GCS even when they receive 2.5-7.5 mg prednisone equivalents per day (164, 165).

Sustained use of GCS increases bone loss by reducing intestinal calcium absorption and increasing renal excretion, so that the ensuing negative calcium balance leads to increased bone resorption (194). To combat this, vitamin D supplementation has been explored as a potential adjunct therapy, since vitamin D regulates calcium homeostasis by increasing intestinal calcium absorption and reabsorption from the kidneys (195).

Several systematic reviews indicate the efficacy of vitamin D plus calcium supplementation in maintaining bone mineral density and reducing vertebral fracture risk in patients treated with GCS (196-198). The 2010 recommendations for the prevention and treatment of GIO from the American College of Rheumatology advise supplementation with 800-1000 IU/day (20-25 µg /d) 25(OH)D although it is conceded that more may be necessary if GCS adversely affects vitamin D absorption (162). Recommendations from the Endocrine Society suggest higher levels of supplementation, proposing that patients using GCS require two to three times more vitamin D than healthy people of the same age (183). For a 30 year old individual, this equates to a dose of 3,000-6,000 IU 25(OH)D per day (75-150 µg /d). Conversely, the Royal

College of Physicians of London recommend vitamin D supplementation only in cases of vitamin D deficiency (163).

Whilst it is evident that vitamin D supplementation is beneficial in the prevention and treatment of GIO, the extent to which GCS compromises vitamin D status remains unclear. This is a critical gap in knowledge. Historically, prednisone was thought to decrease the plasma half-life of vitamin D, although this half-life is most likely to be that of 25(OH)D (199). The long term effects of chronic GCS use on plasma 25(OH)D levels are uncertain. Some authors suggest that 25(OH)D levels remain normal, others report reduced levels long-term (200-204). Importantly, since vitamin D deficiency is in itself a risk factor for osteopenia, osteoporosis and increased fracture risk, vitamin D insufficiency or deficiency may compound the effects of GIO and attenuate the effectiveness of a standard vitamin D supplementation at 800-1,000 IU/d (20-25 µg /d) since achievement of optimal plasma levels of 25(OH)D are known to be highly dependent on the initial starting value (205, 206).

3.3.2 Objectives

1. To determine serum 25(OH)D concentrations in patients treated with GCS
2. To compare serum 25(OH)D concentrations in patients treated with GCS and steroid-naïve controls.

3.3.3 Hypotheses

I. Serum 25(OH)D levels will be in the deficient range (< 20 ng/ml (50nmol/L)) in patients treated with GCS
II. Serum 25(OH)D is decreased in patients treated with GCS compared to steroid-naïve controls

3.3.4 Methods

A systematic review with meta-analyses was used to address these objectives. Specifically, these questions have been investigated using three methods: calculation of the weighted mean serum 25(OH)D concentration in patients treated with GCS; comparison of serum 25(OH)D in patients treated with GCS compared to controls who are steroid-naïve and are either healthy or who have active disease; and comparison of serum 25(OH)D before and after GCS administration.

3.3.4.1 Systematic review

The search strategy was determined by two investigators (ZD and HT) and conducted by one (ZD). The systematic review was originally conducted May 2010, and updated in August 2011 through a search of two databases (MEDLINE via OVID and CINAHL) using the following keywords: prednisolone, glucocorticoids, adrenal cortex hormones, vitamin D, vitamin D deficiency, vitamin D insufficiency. In addition the MEDLINE search incorporated the terms: hydroxycholecalciferols and dihydroxycholecalciferols. In the CINAHL search added terms were: 25-hydroxyvitamin D, cholecalciferols and calcitriol. All search terms were ‘exploded’. Each search was limited to human studies published in English between January 1970 and August 2011. No attempt was made to access unpublished communications. All recovered abstracts were reviewed (by ZD) and where eligibility was unclear, the full article was also reviewed by HT. Articles were retrieved where studies measured 25(OH)D in any population aged >18 years treated with GCS. All experimental study designs were included, but case studies, commentaries, narrative reviews, conference abstracts and editorials were excluded. Studies were coded as included or excluded. Reasons for exclusion were: not a study, not a relevant population, not a relevant outcome, duplicate, or unable to be retrieved. To be eligible for data extraction, retrieved studies were re-examined against further

criteria (Table 3-1). Retrieved studies were also hand searched for further references meeting inclusion criteria for this review. Contact with study authors was found not to be required.

Table 3-1 Reasons for exclusion of studies in patients treated with glucocorticosteroids (GCS)

Excluded	• vitamin D supplementation ≥ 400 IU/day (10 µg /d) • GCS treatment <2 weeks duration • duration of GCS treatment unclear • serum 25(OH)D not measured • >50% study population on GCS for renal or hepatic disease • >50% the study population on GCS post-transplant • study population includes a patient with Cushing's syndrome • vitamin D supplementation study without a measure of 25(OH)D at baseline[†] • 25(OH)D in users versus non-users of GCS not clearly defined • treatment status with GCS unclear in some subjects

[†]Except where patients commenced GCS and vitamin D supplementation at the same time and there was no placebo arm (GCS alone)

Review of all included studies (by ZD) led to the extraction of the following information: affiliation and source of funds, study design, location, sample size, population characteristics and potential confounding variables (age, gender, ethnicity, vitamin D supplementation, sun exposure, cumulative GCS dose, daily GCS dose), type of 25(OH)D assay, and mean serum 25(OH)D. Any studies providing insufficient information for the meta-analysis were now excluded; at a minimum a sample size and a mean serum 25(OH)D level were required.

Study quality was assessed as positive, neutral or negative according to criteria developed by the American Dietetic Association (207). Levels of evidence were assigned according to an accepted hierarchy (208). Whenever the measure of 25(OH)D taken came from a single point within an intervention or cohort study (eg. baseline measure), the level of evidence was considered equivalent to a cross sectional study. Study quality and level of evidence were not considered when assessing eligibility for inclusion.

3.3.4.2 Meta-analyses

Analyses were undertaken using STATA (Version 11, StataCorp, 2009). The relationship between GCS treatment and serum 25(OH)D was explored by meta-analysis. Three key elements were examined: the weighted mean 25(OH)D concentrations; comparison of 25(OH)D concentrations in GCS users and non-users; and comparison of 25(OH)D before and after GCS administration. All measures of 25(OH)D have been reported as ng/ml.

Weighted mean 25(OH)D

The weighted mean was calculated firstly, in accordance with sample size and secondly in relation to the standard deviation (SD) with consideration of random effects principals (209). Studies were included in these analyses if they provided the mean serum 25(OH)D concentration plus either the sample size or a SD or standard error of the mean (SEM), respectively.

Comparison of 25(OH)D between GCS users and non-users

Studies allowing comparison of 25(OH)D concentrations between GCS users and non-users reported mean ± SD (or SEM) serum 25(OH) D concentrations for a cohort of GCS users and a cohort of steroid-naïve controls (either healthy or with a disease). Where 25(OH)D was reported for several GCS treated groups versus one control group, the measure with the least

variation was used in analyses. Effect sizes were calculated using Cohen's *d* (210). A negative *d* value indicates that serum 25(OH)D is decreased in GCS users compared to non-users. Effect sizes are taken as small: *d*=0.2, medium: *d*=0.5, and large: *d*=0.8 (210).

The STATA *metan* command was used to calculate overall weighted mean difference in serum 25(OH)D between GCS users and all controls (healthy *and* diseased); between GCS users and healthy controls; and between GCS users and controls with active disease (211). A random effects model was also used whereby the standardised mean difference (SMD) was used in place of mean 25(OH)D. Heterogeneity was examined both qualitatively and quantitatively using the I^2 statistic: low heterogeneity is defined as < 25%, medium as < 50%, and high as < 75% (212).

Comparison of 25(OH)D before and after GCS administration

Studies were included for comparison of 25(OH)D before and after GCS administration if they reported 25(OH)D, and SD or SEM in a group of subjects before commencing GSC and following at least one month of treatment. Studies were excluded if GCS administration was accompanied by supplementation with vitamin D. Again Cohen's *d* was used to estimate effect size and the overall SMD was calculated.

3.3.5 Results

The process of study selection for the meta-analyses is illustrated in Figure 3-2. From the search 1,309 studies were received for classification. Of these, 171 studies, including ten studies identified via a hand search, were retrieved, of which 38 were found eligible for data extraction. During data extraction, a further 13 studies were excluded because they provided insufficient data points for inclusion in any meta-analyses. Of the 25 included studies, 12 were cross sectional studies, two were cohort studies, six studies had either a single arm

intervention or were a non-randomised control trial, and five were randomised control trials. One additional study became available after the search was completed. This could not be included in the meta-analysis due to the lack of mean values for vitamin D in GCS treated patients (213).

The studies included in the meta-analyses are summarised in Table 3-2, Table 3-3, Table 3-4. Most (92%) were assessed as of neutral quality only; one paper was assessed as positive quality (214) and one as negative quality (215). Participants included patients treated with GCS for inflammatory bowel disease, sarcoidosis, rheumatoid arthritis and other rheumatic disorders, chronic obstructive pulmonary disorder, asthma, connective tissue disorders, and systemic lupus erythematosis.

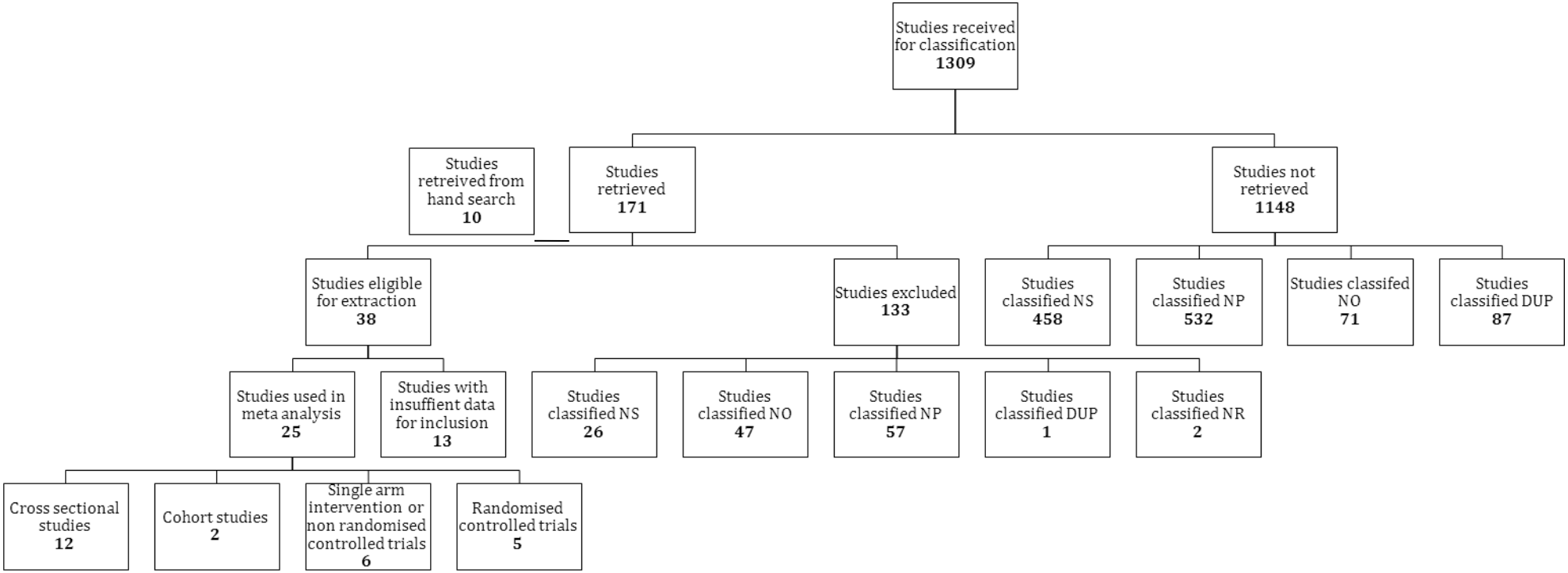

Figure 3-2 Flow chart illustrating the process of study selection in the systematic review for the meta-analyses

DUP: duplicate paper; NO: not a relevant study outcome; NP: not a relevant population; NR: not able to be retrieved; NS: not a relevant study.

Thirteen of the 25 included studies were conducted in the United States, others were from Europe (n=10), New Zealand (n=1) and Canada (n=1). Participants were aged 23-69 years; 0-100% male, and were given 5-150 mg/d GCS. Serum 25(OH)D was generally determined by competitive protein binding assay or radioimmunoassay.

3.3.5.1 Weighted mean serum 25(OH)D

A total of 25 studies with 867 participants allowed the determination of a mean 25(OH)D weighed by sample size as 22.4 (95% CI: 19.4, 25.3) ng/ml, while 23 studies with 640 participants provided data allowing estimation of a mean 25(OH)D weighed by SD as 21.0 (95% CI: 13.5, 28.5) ng/ml (Table 3-2).

Table 3-2 Summary of studies used to calculate a weighted mean 25(OH)D

Reference	Country	Design	25(OH)D Assay	n	Patient Group	Mean age (years)	% Male	Mean GCS dose (mg/d)	GCS duration (months)	Mean 25(OH)D (ng/ml)
Abitbol et al. 1995 (216)	France	CS	CPB	52	IBD	37.0 (12.0)		8.8 (9.7)	N/A[a]	13.2 (9.10)
Adler et al. 2003 (217)	USA	C	RB	10	Sarcoidosis					19.8 (10.5)
Aloia et al. 1974 (218)	USA	CS	RL	17	ID					33.9(17.9)
Als et al. 1987 (219)	Denmark	CS	RIA	31	RA	55.5 (12.8)				13.2 (10.0)
Bernstein et al. 1995 (220)	USA	CS	RR	15	IBD					36.0 (11.0)
Bijlsma et al. 1988 (221)	Netherlands	RCT	CPB	10	Mixed	50.3 (14.3)	30	N/A[b]	44.2 (2-161)	25.3 (15.8)
			CPB	11	Mixed	41.1 (15.7)	18	N/A[c]	32.4 (2 – 123)	18.9 (8.3)
Bikle et al. 1993 (222)	USA	CS	CPB	22	COPD	69.0 (9.0)		7.5-60	12-192	23.2 (10.4)
Bonadonna et al. 2005 (223)	Italy	CS	RIA	6	Mixed	52.0 (11.9)	100	10.0 (3.5)	17.5 (13.6)	20.5 (2.7)
Braun et al. 1983 (224)	Netherlands	RCT	RIA	7	Mixed	39.0 (11.6)	14	13.6 (4.4)	6-192	20.3 (9.8)
			RIA	7	Mixed	34 (13.6)	14	13.6 (4.2)	6-192	20.5 (14.5)
Colette et al. 1987 (215)	France	I	via radiocalcium	7		48.0 (13.2)	100	18.0 (18-25)	≥3	8.0 (7.4)
D'Angelo et al. 1985 (225)	Italy	CS	CPB	10	RA	23-69	30	≤ 10	4-6	19.5 (7.8)
			CPB	8	RA	31-67	25	≤ 10	12-36	21.6 (6.8)
			CPB	15	RA	32-61	27	> 15	≥10	35.6 (17.0)
Driscoll et al. 1982 (226)	USA	CS	CPB	6	IBD-C	43.2 (16.6)	33	18.3 (8.2)		14.2 (13.7)
Dykman et al. 1984 (214)	USA	RCT	CPB	10	RD	51.5 (14.9)	10	11.3 (13.28)	87.6 (60.7)	14.3 (4.7)
			CPB	13	RD	46.5 (13.7)	23	12.2 (9.4)	57.6 (69.2)	19.9 (8.3)
Grecu et al. 1991 (227)	USA	CT	CPB	12	BA	63.0 (56-72)	100	10-20	>12	29.8 (10.4)

Reference	Country	Design	25(OH)D Assay	n	Patient Group	Mean age (years)	% Male	Mean GCS dose (mg/d)	GCS duration (months)	Mean 25(OH)D (ng/ml)
Hahn et al. 1977 (200)	USA	CS	CPB	21	Mixed	43.7 (12.8)	52	22.3 (9.6)	58.8 (55.0)	14.5 (6.9)
Hahn et al. 1979 (201)	USA	CT	CPB	17	Mixed	47.2 (12.4)	24	17.4 (7.4)	51.6 (34.6)	15.4 (4.5)
Klein et al. 1977 (203)	USA	I	CPB	16	CTD	57.1 (12.8)	25	40.0 (15-100)	3.0 (10-15)	13.9 (6.5-24.8)
			CPB	5	CTD	48.0 (18.1)	0	9.5 (8-10)	3.0 (10-15)	19.4 (10.2-22.0)
			CPB	6	CTD	52.3 (19.3)	67	34.0 (15-50)	3.0 (10-15)	17.9 (15.4-27.3)
Lund et al. 1985 (228)	Denmark	CS	CPB	50	RA	63.0 (35-78)	4	5-10	96.0 (6-420)	17.0 (10.0)
Newman et al. 2006 (229)	USA	C		200	Mixed	62.7 (12.7)	32		70.8 (85.2)	21.4
Reid et al. 1990 (230)	New Zealand	CT	CPB	16	Mixed	48.0 (20.0)	50	16.2 (6.0)	60.0 (52.8)	20.5 (10.0)
			CPB	19	Mixed	52.0 (13.1)	58	12.3 (6.1)	78.0 (83.7)	23.9 (10.9)
Rickers et al. 1982 (231)	Copenhagen	RCT	HPLC	15	Mixed	63.2 (47-76)	40	19.9	6	13.8 (8.5)
Slovik et al. 1980 (202)	USA	CS	CPB	48	Asthma		44	13.0 (6.9)	59.0 (55.4)	35.0 (20.8)
			CPB	10	Mixed			150.0 (91.7)	0.4 (0.3)	30.0 (9.5)
Toloza et al. 2010 (232)	Canada	CS	RIA	124	SLE	48.3 (13.1)	0	N/A[d]	122.4 (110.4)	27.5 (12.4)
Worth et al. 1994 (233)	Germany	RCT	HPLC	14	Asthma	55 (41.2)	21	27.0 (44.9)	>9	21.0 (7.5)
			HPLC	19	Asthma	58.0 (52.3)	47	28.0 (52.7)	>9	23.6 (21.8)
Zerwekh et al. 1984 (234)	USA	CT	CPB	18	RA	54.9		5.0	6	30.0 (21.2)

Mean (standard deviation/range), GCS glucocorticosteroids, 25(OH)D 25 hydroxyvitamin D. *Country:* USA United States of America. *Design:* CS cross sectional, C cohort, RCT randomised controlled trial, I intervention study (single arm), CT controlled trial. *Assay:* CPB competitive binding assay, RB radio-binding assay, RL radioligand assay, RIA radio-immunoassay, RR radioreceptor assay, HPLC high performance liquid chromatography. *Patient group:* IBD inflammatory bowel disease, ID inflammatory diseases, RA rheumatoid arthritis, COPD chronic obstructive pulmonary disorder, IBD-C Crohns, RD rheumatic diseases, BA bronchial asthma, CTD connective tissue disorders, SLE systematic lupus erythematosis. Mean cumulative GCS dose (g): [a] 10.5 (13.0), [b] 22.9 (1.7-76.2), [c] 22.1 (0.5-99.1), [d] 41.5 (39.2).

Table 3-3 Summary of studies used in analysis comparing 25(OH)D between GCS users and non-users

Reference	Country	Design	25(OH)D Assay	GCS Tx	n	Patient Group	Mean age (years)	% Male	Mean GCS dose (mg/d)	Duration GCS (months)	Mean 25(OH)D (ng/ml)	Effect size (Cohen's *d*)
Abitbol et al. 1995 (216)	France	CS	CPB	GCS	52	IBD	37.0 (12.0)		8.8 (9.7)	N/A[a]	13.2 (9.1)	-0.025
				No GCS	32	IBD	44 (15)				13.4 (7.0)	
Adler et al. 2003 (217)	USA	C	RB	GCS	10	Sarcoidosis					19.8 (10.5)	0.205
				No GCS	23	Sarcoidosis					17.7 (10.0)	
Als et al. 1987 (219)	Denmark	CS	RIA	GCS	31	RA	55.5 (12.8)		2.5-10	>6	13.2 (10.0)	-0.736
				No GCS	38	Healthy	42.5 (15.3)	55			24.7 (19.7)	
Bernstein et al. 1995 (220)	USA	CS	RR	GCS	15	IBD					36.0 (11.0)	0.364
				No GCS	4	IBD					32.0 (11.0)	
Bikle et al. 1993 (222)	USA	CS	CPM	GCS	22	COPD	69.0 (9.0)		7.5-60	12-192	23.2 (10.4)	0.259
				No GCS	14	COPD	65.0 (10.0)				20.4 (11.2)	
Colette et al. 1987 (215)	France	CT	via radiocalcium	GCS	7		48.0 (13.2)	100	18.0 (18-25)	≥3	8.0 (7.4)	-1.057
				No GCS	7	Healthy	65.0 (13.2)	71			14.8 (5.3)	
D'Angelo et al. 1985 (225)	Italy	CS	CPB	GCS	10	RA	23-69	30	≤ 10	4-6	19.5 (7.8)	
				GCS	8	RA	31-67	25	≤ 10	12-36	21.6 (6.8)	1.229

Reference	Country	Design	25(OH)D Assay	GCS Tx	n	Patient Group	Mean age (years)	% Male	Mean GCS dose (mg/d)	Duration GCS (months)	Mean 25(OH)D (ng/ml)	Effect size (Cohen's *d*)
D'Angelo et al. 1985 (225) (cont)				GCS	15	RA	32-61	27	> 15	≥10	35.6 (17.0)	
				No GCS	8	RA	20-61	25			13.6 (6.2)	
Hahn et al. 1977 (200)	USA	CS	CPB	GCS	21	Mixed	43.7 (12.8)	52	22.3(9.6)	58.8 (55.0)	14.5 (6.9)	-0.144
				No GCS	19	Mixed	47.9 (10.03)	53			15.6 (8.3)	
Hahn et al. 1979 (201)	USA	CT	CPB	GCS	17	Mixed	47.2 (12.4)	24	17.4 (7.4)	51.6 (34.6)	15.4 (4.5)	-0.268
				No GCS	19	Healthy	45.7 (16.6)	32			16.9 (6.5)	
Slovik et al. 1980 (202)	USA	CS	CPB	GCS	48	Asthma		44	13.0 (6.9)	59.0 (55.4)	35.0 (20.8)	-0.399
				No GCS	12	Asthma					44.0 (24.2)	
				GCS	10	Mixed			150.0 (91.7)	0.4 (0.3)	30.0 (9.5)	-1.188
				No GCS	47	Healthy					44.0 (13.7)	
Zerwekh et al. 1984 (234)	USA	CT	CPB	GCS	18	RA	54.9		5	6	30 (21.2)	-0.174
				No GCS	16	RA	53.9				33.0 (12.0)	

Mean (standard deviation/range), GCS glucocorticosteroids, 25(OH)D 25 hydroxyvitamin D. *Country:* USA United States of America. *Design:* CS cross sectional, C cohort, I intervention study (single arm), CT controlled trial. *Assay:* CPB competitive binding assay, RB radio-binding assay, RIA radio-immunoassay, RR radioreceptor assay. *Patient group:* IBD inflammatory bowel disease, RA rheumatoid arthritis, COPD chronic obstructive pulmonary disorder. Mean cumulative GCS dose (g): [a] 10.5 (13.0).

Table 3-4 Summary of studies used in analysis comparing 25(OH)D before and after GCS administration

Reference	Country	Design	25(OH) D Assay	n	Patient Group	Mean age (years)	% Male	Mean GCS dose (mg/d)	Mean 25(OH)D (ng/ml) *Baseline*	Mean 25(OH)D (ng/ml) *6 months*	Effect Size (Cohen's *d*)
Rickers et al. 1982 (231)	Copenhagen	RCT	HPLC	15	Mixed	63.2 (47-76)	40	19.9	17.1 (8.52)	13.8 (8.52)	-0.388
Zerwekh et al. 1984 (234)	USA	CT	CPB	18	RA	54.9		5	30 (12.7)	30 (21.2)	0

Mean (standard deviation/range), GCS glucocorticosteroids, 25(OH)D 25 hydroxyvitamin D. *Country:* USA United States of America. *Design:* RCT randomised controlled trial, CT controlled trial. *Assay:* HPLC high performance liquid chromatography, CPB competitive binding assay. *Patient group:* RA rheumatoid arthritis.

3.3.5.2 Comparison of serum 25(OH)D between GCS users and non-users

Twelve studies including a total of 271 GCS users and 251 steroid-naïve controls (123 healthy, 128 with disease) were available to compare serum 25(OH)D between GCS users and non-users (Table 3-3). A meta-analysis (Figure 3-3) indicated that the SMD was not significant (SMD = -0.2 (95% CI: -0.4, 0.1) ng/ml). A moderate degree of heterogeneity was evident between studies (53.9%).

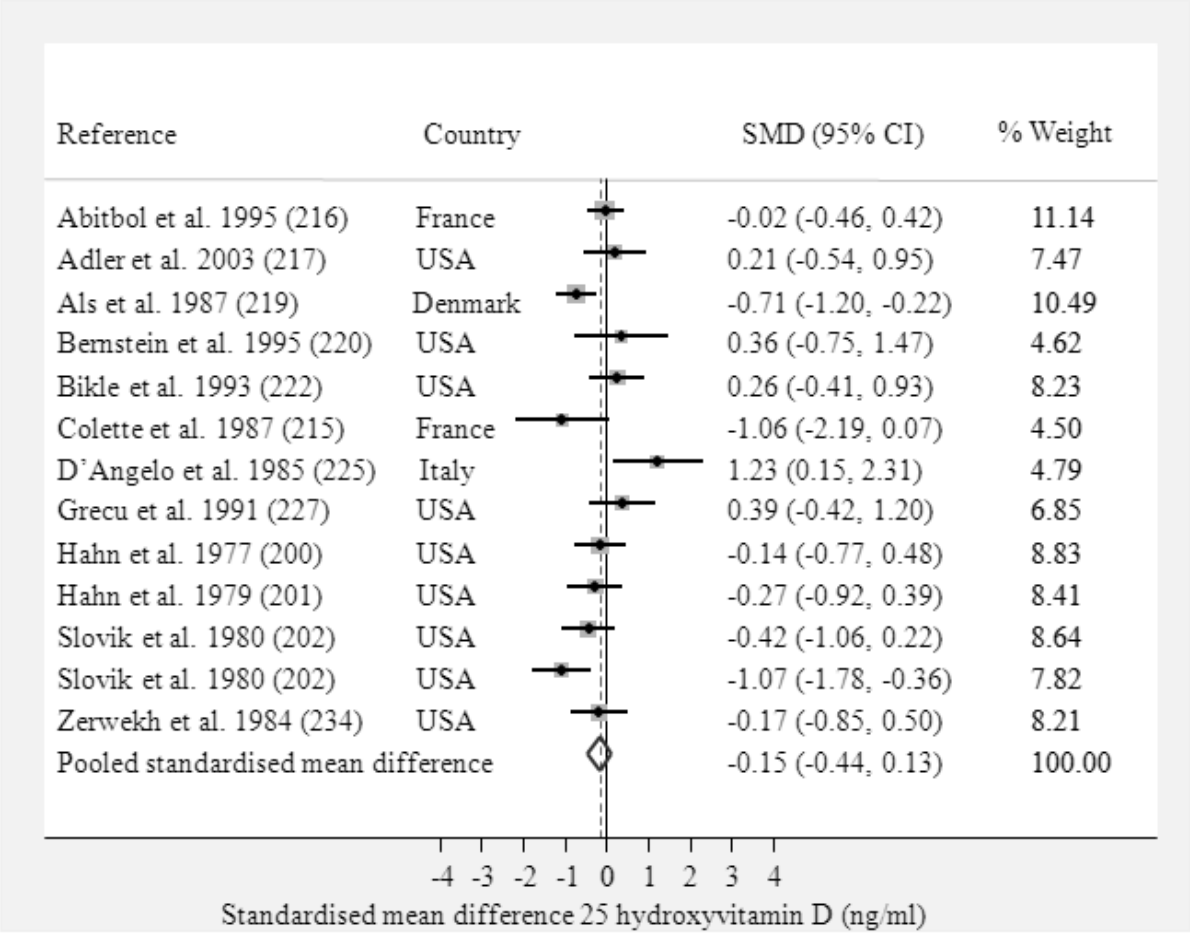

Figure 3-3 Random effects meta-analysis comparing 25(OH)D concentrations between GCS users and non-users (both healthy and disease controls)

I^2=53.9%, p 0.011; test of SMD=0: z=1.06, p=0.289

Meta analyses also examined relationships between GCS users and healthy controls; and between GCS users and disease controls (Figure **3-4** and Figure 3-5). Serum 25(OH)D in GCS users was significantly below that of healthy controls (SMD = -0.5 (95% CI: -1.0, -0.1) ng/ml, *p*=0.03). Heterogeneity (I^2=56.4%) was moderate in this analysis. In contrast, there was no difference in serum 25(OH)D between GCS users and disease controls (SMD = 0.0 (95% CI: -0.2, 0.3) ng/ml). There was a low degree of heterogeneity between these studies (I^2=16.2%).

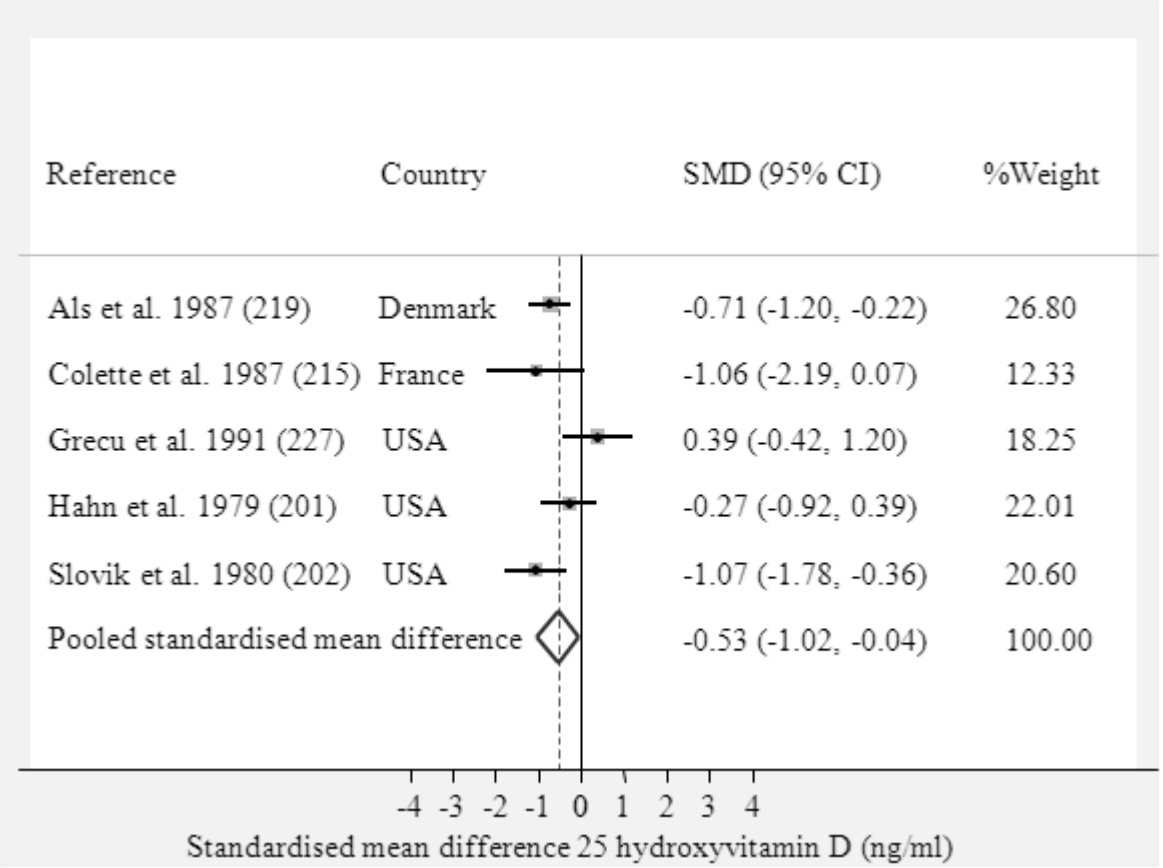

Figure 3-4 Random effects meta-analysis comparing 25(OH)D concentrations between GCS users and healthy control non-users

I^2=56.4%, p 0.057; test of SMD=0: z=2.13, *p*=0.033

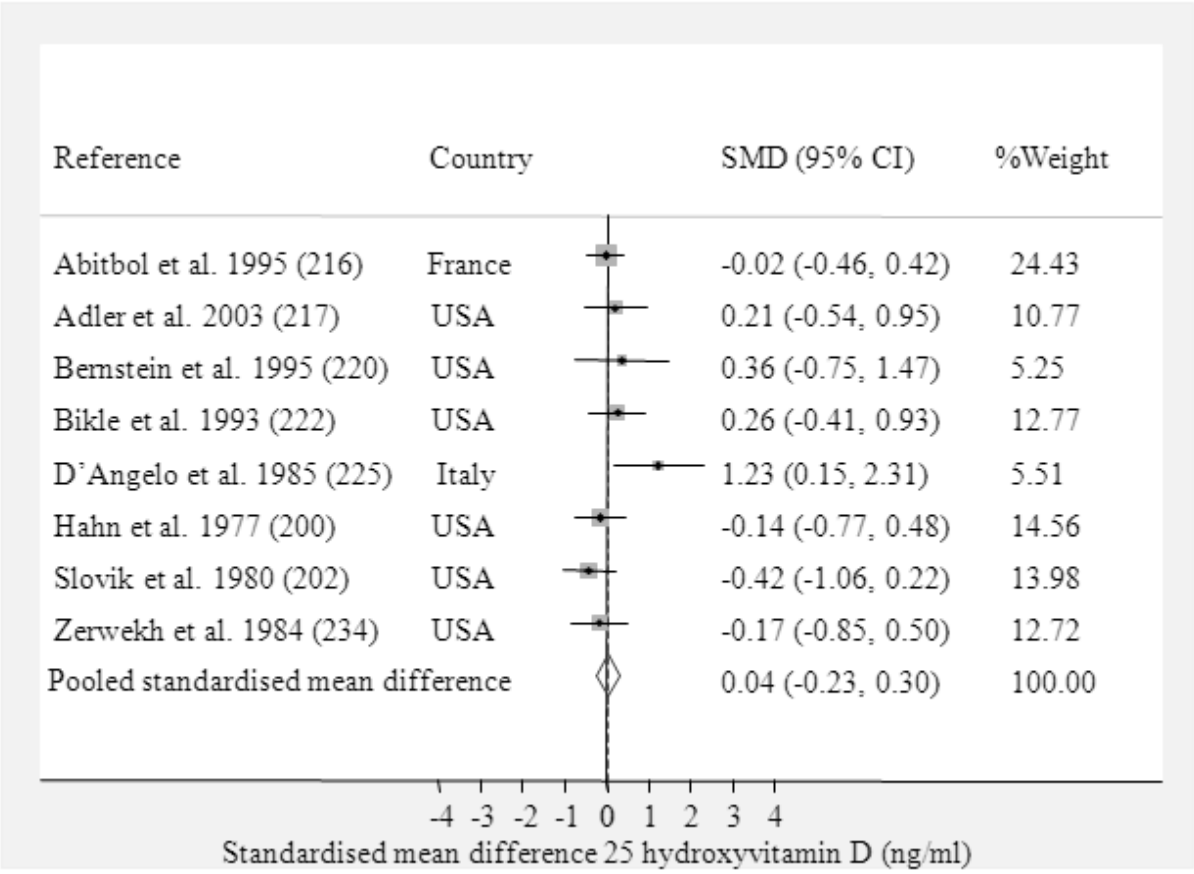

Figure 3-5 Random effects meta-analysis comparing 25(OH)D concentrations between GCS users and non-users with active disease

I^2=16.2%, p 0.302; test of SMD=0: z=0.26, p=0.798

3.3.5.3 Comparison of serum 25(OH)D before and after GCS administration

Only two studies with a total of 33 participants could be analysed to examine the difference in serum 25(OH)D concentration before and after GCS administration (Table 3-4). No significant change in serum 25(OH)D occurred after six months (SMD -0.174 (95 % CI: -0.659, 0.311) ng/ml). The mean daily GCS dose given in each study however was very different. Where the mean GCS dose was 19.9 mg/day , the effect size was small to medium, whilst with a mean GCS dose of only 5mg/day no change in serum 25(OH)D concentrations was observed (231, 234).

3.4 Discussion

These two streams of investigation address the question of the aetiology of low 25(OH)D concentrations in boys with DMD. The first investigation demonstrates that whilst boys with DMD have significantly lower serum 25(OH)D levels, they receive similar sun exposure to their healthy siblings. Based on the finding from the second investigation, prolonged used of GCS therapy may provide an explanation for this observed discrepancy.

Adults receiving GCS therapy appear frequently to have concentrations of serum 25(OH)D that are suboptimal for the prevention or management of GIO. Whilst neither weighted mean statistic (by sample size: 22.4 (95% CI: 19.4, 25.3) ng/ml; by SD: 21.0 (95% CI: 13.5, 28.5) ng/ml) fell below 20 ng/ml, the size of CIs suggest that the true weighted mean statistic could indeed indicate deficiency. Furthermore, serum 25(OH)D in GCS users was on average -0.5 (95% CI: -1.0, -0.1) ng/ml lower than found in healthy controls. This finding is supported by the difference in 25(OH)D found between boys with DMD and their healthy siblings. A similar relationship was not evident between GCS users and disease controls, indicating that disease status may explain the difference in 25(OH)D between GCS users and healthy controls. These results are consistent with findings from a recent population study using data from NHANES III; the authors determined that those using
GCS were two times more likely to be vitamin D deficient (defined at 25(OH)D <10ng/ml) compared to those not using GCS (213).

Prednisone not only increases the catabolism of 25(OH)D but may also induce significant weight gain (183, 199, 235). Obesity may then compound the deleterious effects of GCS on vitamin D status since it is known that vitamin D sequesters in body fat, decreasing its bioavailability (183, 236). The prevalence of obesity thus complicates assessment of the effects of GCS on vitamin D status. Given the increased intramuscular fat mass and obesity

that develops as a part of DMD pathology, a sequestering effect could be contributing to the decreased levels of 25(OH)D observed. Nonetheless, it is clear that 25(OH)D was suboptimal in the GCS treated cohorts examined here, suggesting that recommendations for vitamin D supplementation in people treated with GCS may need revision.

Based on our weighted means in adults which are in the range 16-24 ng/ml, 1,800 IU/d (45µg/d) per day of vitamin D supplementation would be required to achieve serum concentrations of 25(OH)D ≥ 32ng/ml (206). In comparison to this calculated requirement, the Endocrine Society recommends a higher daily dose for adult GCS users (3000-6000 IU/day (75-150 µg/d)) whilst the recommendations of the American College of Rheumatology are considerably lower (800-1000 IU/d (20-25 µg/d)) (162, 183). Misra et al. (75) recommends supplementation with > 5000 IU/day for children > 12 months old who are vitamin D insufficient or deficient; however, at present there are no specific recommendations for paediatric populations using GCS nor are there specific recommendations for boys with DMD. Although the meta-analyses presented here used data from adult populations, the calculated weighted mean 25(OH)D was similar to the observed mean 25(OH)D in the cross-sectional study (52.4 nmol/L vs 52.2 nmol/L, respectively). Thus it is reasonable to generalise the calculated requirement from the meta-analyses to the DMD population instead of using the broader paediatric recommendations.

It is essential to measure 25(OH)D concentrations in individuals with DMD at the time that they first commence GCS therapy as there may be considerable variation in their vitamin D status, and hence their requirement for supplementation. Yet this review found several studies reporting a lack of assessment and/or treatment for bone health in patients commencing or receiving GCS therapy (237-240). Similarly, the lack of assessment of bone health in boys with DMD has been highlighted in Chapter 2. In practice it is probable that many patients

commencing GCS do not have their 25(OH)D measured. As bone is lost rapidly during the first few months of GCS treatment, it would be prudent to set vitamin D recommendations at or above 2,000 IU/d (50 µg/d) to achieve optimal levels of 25(OH)D during this critical time (193). Exemptions would only be appropriate where an individual is at risk of hypercalciuria and hypercalcaemia, for example in patients with sarcoidosis (241). An intake of 2,000 IU/d (50 µg/d) of vitamin D is well below the recognised safe upper limit of vitamin D intake (3,000 IU (75ug) per day for children 4-8 years of age, and 4,000 IU (100µg) per day for persons above 9 years of age) as determined by the Institute of Medicine (IOM) (242). The safe upper limit of vitamin D intake has however, been a matter of much contention and for many patients at risk of deficiency, an upper limit as high as 10,000 IU (250 µg) has been proposed (183). A dose at this level has no detrimental effects on serum calcium or urinary calcium excretion (243). Moreover, Vitamin D toxicity appears rare. Severe adverse effects are not seen until serum 25(OH)D rises above 700 nmol/L (244). Very high bolus doses however, are not advised. A recent Australian study noted adverse effects when elderly women were given 500,000 IU (12,500 µg) of vitamin D in a single oral annual dose, a single dose 10x higher than that established as safe in previous trials (245). This non-physiological dose increased basal 25(OH)D from a median of 49 nmol/L to 120 nmol/L within one month. Although no serious toxicity was reported at these serum concentrations, a significant increase in the rate of falls and fractures was detected. The increase in falls appeared to be related to the single, very large dose given (500,000 IU (12,500 µg)) rather than to the serum 25(OH)D concentrations achieved. This study questions the safety of high-dose vitamin D supplementation, even in the absence of clinical toxicity.

The potential relationship between Vitamin D and muscle function warrants attention. In the elderly, dietary vitamin D supplementation has been found to lower the risk of falling by as much as 22% (246). In the Longitudinal Aging Study of Amsterdam, a population-based

study in men and women aged 65 and over, researchers determined that lower 25(OH) D and higher parathyroid hormone levels increased the risk of sarcopaenia in older men and women (247). Lower serum 25(OH) levels have also been associated with improved functional outcomes in women aged 55 and older (191); and with decreased muscle power and force in females aged 12-14 (248). Also, of interest are body composition changes related to vitamin D status. In healthy females vitamin D insufficiency is associated with increased fat infiltration in muscle as measured by computed tomography (249). Although a causal relationship between vitamin D and muscle function is yet to be established, the question lingers: if we can optimise vitamin D status, will it improve muscle function for boys with DMD?

There were several strengths and limitations to both streams of investigation. The first investigation was limited by small numbers due to the need to find sibling controls. However, recruiting a sibling cohort was advantageous because it effectively controlled for other environmental determinants of 25(OH)D levels and was a novel strategy. The sun exposure diary used in this investigation has been previously validated in several groups in the United States of America. In a cohort of adolescent females UV radiation exposure measured using a polysulfone dosimeter was significantly correlated with minutes spent outdoors (249). The tool was more recently validated by Chodick et al in a cohort of 124 men and women (250). In this validation study, participants completed a daily activity diary recording time they spent on major indoor and outdoor activities. Personal solar UV radiation exposure was again simultaneously measured using a polysulfone dosimeter. Self-recorded daily time spent outdoors was found to be significantly associated with personal UV radiation exposure. The validity of the amended questionnaire used in this study is supported by its sensitivity to expected differences in sun exposure between week and weekend days. However, the use of time spent outdoors as a proxy for UV radiation exposure does not account for the child's use

of sun safe practices, such wearing a hat and applying a sunscreen. It was assumed that such sun safe practices would be similar within families. Due to the exploratory nature of the protocol used in *Investigation 1*, the biochemical panel was limited to measurement of serum 25(OH)D. In expanding the study population, future investigations should include a broader range of biochemical markers such as 1,25-dihydroxyvitamin D, parathyroid hormone and bone alkaline phosphatase to gain an understanding of the effect of reduced serum 25(OH)D on bone metabolism.

This chapter provides the first meta-analysis of serum 25(OH)D concentrations in patients receiving GCS. Here, the use of a weighted mean 25(OH)D statistics increases the clinical significance of this research and provides evidence for recommendations for vitamin D supplementation during GCS treatment. Although unique, the meta-analysis provides Level III quality evidence at best as it mainly draws on cross-sectional measures of 25(OH)D. Non English publications were excluded, which may overestimate the effect by 2% (251). Unpublished communications were also not reviewed as advised by Schlesselman (252).

With the exception of one analysis a moderate degree of heterogeneity, both qualitatively and quantitatively was evident among reviewed studies. This is inevitable as GCS are used widely across a diverse range of cohorts varying in age, disease types, and locality. Although this level of heterogeneity is held statistically to reduce the generalisability of a meta-analysis; it can conversely be argued that from a clinical viewpoint the diverse range of populations included in the analysis should increase generalisability. It can be noted that heterogeneity was low (I^2=16.2%) in the analysis comparing 25(OH)D concentrations in GCS users vs non users with disease. Overall, the quality of studies utilised in the meta-analyses were assessed as ‘neutral’. This rating appeared more reflective of study reporting than of study design and methods since, cross sectional studies reviewed often provided insufficient description of their

methods. Another key limitation of the second investigation was the dearth of information in studies regarding sun exposure and seasonal variation in serum 25(OH)D. These factors were often not measured as reviewed studies addressed very different research questions to our own. Only two studies were designed to assess the effect of GCS on 25(OH)D with a high level of evidence; these were studies where 25(OH)D concentrations were measured before and after GCS administration. Moreover in studies where 25(OH)D was not the primary outcome measure, other key confounders were not adjusted for. Therefore, this investigation highlights the need for appropriately designed, longitudinal clinical studies to investigate the true effect of GCS on 25(OH)D concentrations.

3.5 Summary

These investigations build a clearer picture of vitamin D in boys with DMD. Firstly, young, ambulatory boys with DMD who attend regular school have low serum 25(OH)D levels, and this most likely cannot be attributed to a reduction in sun exposure. GCS therapy is associated with suboptimal 25(OH)D concentrations, and may potentially explain the reduction of 25(OH)D observed in DMD cohorts.

Regular monitoring of 25(OH)D and supplementation of vitamin D and calcium should have a high priority as a part of routine care for boys with DMD. Based on the weighted mean 25(OH)D value obtained by meta-analysis, recommendations for vitamin D supplementation should at a minimum, be 1800 IU (45 µg)/d for boys receiving GCS therapy. The response to vitamin D supplementation at 2,000IU/day is explored in Chapter 5.

4 Investigating outcome measures for use in Duchenne muscular dystrophy

4.1 Introduction

Selecting appropriate measurement tools to accurately determine outcomes is an essential issue to consider when designing randomised controlled trials. This has proven to be a complex issue for the neuromuscular community when seeking to determine whether treatments improve muscle strength and/or functionality (253). Historically, manual muscle tests have been used to measure muscle strength, but these tests are plagued with issues of unreliability (254). Instead, improved ambulatory capacity has been identified as a more meaningful objective measurement outcome and has been used in clinical trials for boys with DMD (255). Functional measures that assess ambulatory capacity, such as the six minute walk test (6MWT) have been the focus of much recent attention. The 6MWT is a highly reliable assessment tool that measures the distance a subject can walk in a specific time frame, providing an objective and reliable measure of functional capacity which can be compared over time (253, 254).

Quantitative measurement of ambulation patterns outside of a hospital setting can also provide important information regarding an individual's day-to-day functional capacity. Although there are a range of options, the StepWatch™ activity monitor is an accelerometer that has been investigated for use in DMD research because of its sensitivity in accurately recording step counts in individuals who have a widely varied gait (256).

Accurate measurement of daily energy intake also presents methodological difficulty. In free living children the accuracy of self-reported measures of energy intake are regarded as unreliable since in children mis-reporting is often an even greater issue than in adults (257). In particular, food diaries are associated with significant underreporting ranging from 19-41% of estimated energy intake (258). Yet, the reliance on food diaries to measure or track changes in dietary intake is common in clinical settings. In children with a chronic disease where

monitoring nutrition and growth is at the crux of treatment, parents are often asked to report diet to health professionals as part of a routine clinic visit. Lack of motivation has been identified as a potential contributor to underreporting; however, parents of children with a chronic disease may well be more motivated to report accurately (259). Alternatively, parents may be better trained to be able to report on their child's dietary intake, as parent report of dietary intake has shown to have a good level of agreement with energy expenditure by measured by doubly labelled water (DLW) (259). Seven day weighed food records have been demonstrated to provide valid estimations of energy intake in children aged 7 and 9 years (257). However, with families having to cope and comply with many other home based therapies, increased participant burden for multiple days precludes this more intensive measurement instrument (260). The DLW method for measuring energy expenditure in humans is considered the 'gold standard' for measurement of total energy expenditure (TEE) and validation of energy intake measures (258, 261). Because it is not influenced by self-reported intake error, DLW can be used to validate dietary energy measurement tools by detecting true reporting bias (261).

This chapter serves to introduce and justify the functional outcome measures chosen in the pilot randomised controlled trial (Chapter 5), specifically StepWatch™ activity monitoring and the 6MWT; and to provide evidence to substantiate their use in an Australian cohort. In addition, the validity of self-reported energy intake from diet diaries used in the pilot study is examined against the gold standard measure TEE using DLW.

4.2 Aims and objectives

4.2.1 Aims

The aim of these investigations was to explore possible outcome measures for use in a dietary intervention study in boys with DMD. Specifically, StepWatch™ activity monitoring and the 6MWT as functional outcome measures, and a three day food record as a measure of energy intake.

4.2.2 Objectives

1. To compare community ambulation as measured by StepWatch™ activity monitoring between boys with DMD and healthy controls, specifically examining:
    a. Total steps
    b. Time spent inactive
    c. Time spent at low activity
    d. Time spent at medium activity
    e. Time spent at high activity
2. To compare distance walked in a 6MWT between boys with DMD and healthy controls.
3. To explore relationships between distances walked in a 6MWT and parameters of community ambulation in boys with DMD and healthy controls.
4. To determine if parents of ambulatory boys with DMD can accurately report their child's dietary energy intake.

4.2.3 Hypotheses to be tested

I. Boys with DMD will walk shorter distances in a 6MWT compared to healthy controls.

II. Boys with DMD will be less active in the community compared to healthy controls as measured by StepWatch™ activity monitoring. This will translate into more inactivity, and a lower step count, as well as less time spent at low, medium and high activity.

III. There will be a directly proportionate relationship between the distance walked in a 6MWT and total step count in both boys with DMD and healthy controls.

IV. There will be a directly proportionate relationship between the distance walked in a 6MWT and time spent at low, medium and high activity in both boys with DMD and healthy controls.

V. There will be an inverse relationship between inactivity and the distance walked in a 6MWT in both boys with DMD and healthy controls.

VI. Consistent underreporting will be evident when parents report their son's energy intake using a three day food diary.

4.3 Methods

4.3.1 Functional outcome measures

4.3.1.1 Participants

Boys with DMD were recruited through neuromuscular clinic at The Royal Children's Hospital, Melbourne, as a part of a randomised controlled trial (Chapter 5). Pre-treatment measures were utilised. Eligible participants were ambulatory boys aged 5 to 13 years with a definite diagnosis of DMD defined as documentation of a deletion or duplication in the dystrophin gene, or absence of dystrophin on muscle biopsy, in conjunction with phenotypic evidence based on characteristic clinical symptoms or signs by 9 years of age (ie, proximal muscle weakness, waddling gait, and Gower's' manoeuvre), an elevated serum creatine kinase, and ongoing difficulty with ambulation. Ethics approval was obtained from The Royal Children's Hospital, Melbourne and Monash University Human Research Ethics Committees (HREC# 30022 and 2010001692, respectively, Appendix 11 and Appendix 12). Informed consent was obtained from parents or guardians (Appendix 13).

Healthy control males, aged 5-13 years were recruited through Monash University via the staff intranet website, global staff emails and flyer advertisements around the Monash Clayton campus. Ethics approval was obtained from Monash University Human Research Ethics Committees (HREC# 2010000344, Appendix 14) and informed consent was obtained from parents or guardians (Appendix 15).

4.3.1.2 Anthropometry

Height and weight were determined with a portable stadiometer and digital weight scales (both Seca, Deutschland). All boys were able to stand for the height measure, and no severe curvatures were observed. BMI was calculated as body weight (in kilograms) divided by height (in metres squared). All participants had their shoes and any bulky items of clothing

removed before measurements were taken. Weight, height and BMI z-scores were determined using the CDC reference values for children (157).

4.3.1.3 Six minute walk test

The 6MWT is a simple test that requires a marked course and a stopwatch. The test measures the distance that a subject can walk in a period of six minutes and is a measure of sub-maximal functional capacity, as participants exercise at their desired intensity and are allowed to stop and rest during the test. Two people are required to perform the test: a clinical evaluator and a 'safety chaser'. Members of the research team (ZD and Aimee Segman (AS)) received training in both roles from a neuromuscular research physiotherapist at The Royal Children's Hospital (Katy de Valle). The tests were conducted (rotating these roles) by ZD, AS and research physiotherapists at The Royal Children's Hospital (Katy de Valle, Kate Carroll, and Rachel Kennedy).

Boys with DMD completed the 6MWT at the Children's Neurosciences Centre at The Royal Children's Hospital, Melbourne, whilst healthy control subjects completed the test at Monash University Clayton campus.

The 6MWT was set up according to the guidelines of the American Thoracic Society (ATS) (262), and was performed on an indoor course in an area chosen as straight, flat and rarely used as a thorough fare (Figure 4-1). A 25 metre course length was used in accordance with DMD-specific protocols, rather than the 30 metre course recommended by the ATS (253). Visible tape was used to mark a starting line, the centre line of the course, five metre intervals, and the direction of travel around cones placed at the end of the course. Several cones were positioned on either side of the track so that participants did not veer out of the course when walking.

A stopwatch was used to time the walk. Time taken for each lap was recorded as the subject crossed the horizontal taped line at the end of each 25 metre lap. Six numbered pieces of tape (one to six) were used to mark the distance at each minute-split on the course, and four sequentially lettered pieces of tape (A to D) were used to mark points where the participant had fallen, if at all.

Figure 4-1 Six minute walk test course set up at Monash University Clayton Campus

The test sequence was conducted in accordance with DMD-specific protocols (253). The clinical evaluator acted as the study timer and scribe and was assisted by the ‘safety chaser' who walked behind the participant. The role of the chaser was to provide encouragement, place the numbered markers at minute intervals, and to assist in the event of a fall.

Participants were not required to fast, and performed the test at varying times of the day. The participant was provided with a chair to rest for 5-10 minutes prior to commencing the test. As the participant was resting, the clinical evaluator explained the test procedures. Specifically:

- This is a test to see how far the participant can walk in six minutes.
- The participant can set their own pace; they may walk fast, but cannot run or jog.
- The participant should walk close to the taped course, but should not walk on the tape or cross over to the other side of the course.
- The participant may rest if needed during the test.
- If the participant falls, they should rest if needed, but may still continue if able.
- The clinical evaluator then demonstrates the process of walking around the cones.

The timer commenced the test by calling "ready, set, go". The safety chaser walked 1-2 metres behind the participant as they completed the course. Standard encouragement was provided at approximately 15 sec intervals. At the end of each minute, the timer counted down "56, 57, 58, 59, 1 minute" and the chaser placed a minute marker on the floor. At the end of the test, the timer counted down and called "stop walking". The participant was provided with a chair or wheelchair to rest if necessary. In the event of a fall, the safety chaser ensured that the participant was unharmed and assisted him to standing and to continue walking if able. The safety chaser placed a marker on the course at the place of the fall. The clinical evaluator recorded the time and distances on the 6MWT assessment form (Appendix 16). Any notes about the test were also recorded. For example, the participant wore a knee brace during the walk test.

4.3.1.4 StepWatch™ activity monitoring

The StepWatch™ accelerometer (Orthocare Innovations, WA USA) is a small ankle-worn device that records the number of steps taken in real time (Figure 4-2). It is a research-grade instrument for assessment of ambulatory activity during day-to-day life. The accelerometer is waterproof and provides no visual feedback to the wearer.

Members of the research team (ZD, AS) were trained by a neuromuscular research physiotherapist at The Royal Children's Hospital (Katy de Valle) on how to accurately fit and calibrate the monitor, and how to advise parent/guardians on the use of the accelerometer at home. Subjects wore the StepWatch™ monitor for five consecutive days. Data from the first day was excluded as the time from when the device was worn was dependent on the subject's appointment time. Subjects were instructed to remove the monitor when they showered and slept.

Figure 4-2 The StepWatch™ activity monitor is placed just above the ankle on the left foot

The StepWatch™ settings were calibrated for each individual by the researcher (ZD, AS), with the use of a laptop and docking station. Subject's height was entered into the software and quick stepping, walking speed, and leg motion (dynamic, gentle or normal) selections

were programmed for each child individually. Pre-programmed activity levels in the StepWatch™ accelerometer were: *inactive* (zero step rate), *low activity* (one to fifteen steps per minute), *medium activity* (16-30 steps per minute), and *high activity* (greater than 30 steps per minute). An example of downloaded data from a monitor is presented in Figure 4-3.

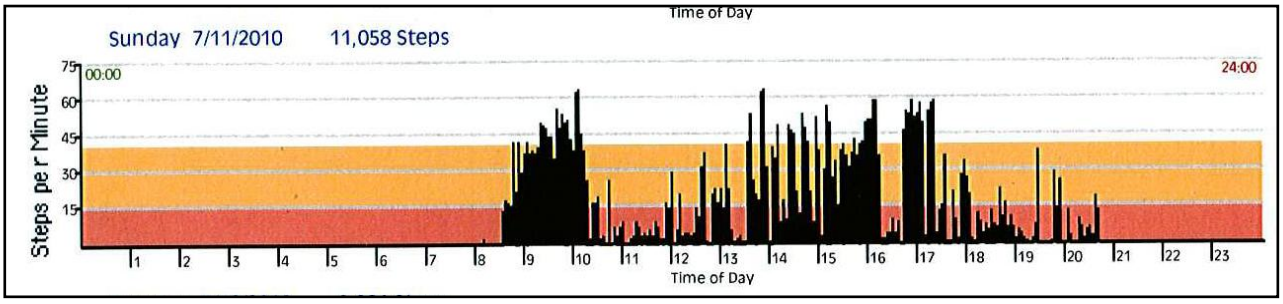

Figure 4-3 Display from a StepWatch™ activity monitor illustrating step rate per minute in actual time

4.3.2 Energy intake

4.3.2.1 Participants

Ambulatory boys with a definitive diagnosis of DMD (see section 4.3.1) were recruited from two neuromuscular clinics in Australia: "MontroseAccess" - a community centre providing therapies for boys with DMD, and The Royal Children's Hospital, Melbourne between January 2008 and September 2009. The protocol was approved by Human Ethics Committees at The Royal Children's Hospital, Melbourne, the Royal Children's Hospital, Brisbane, and the University of Queensland (29075B, 2007/119, and 2007000797, respectively, Appendix 3, Appendix 17 and Appendix 18). Written informed consent was obtained from parents or guardians and prior to the study assessments (Appendix 8 and Appendix 19).

4.3.2.2 Anthropometry

Anthropometry measures were collected as described in section 4.3.1.2.

4.3.2.3 Food diaries

A food dairy (Appendix 20) was provided to parents or guardians to record all food and liquid consumed by their child over three consecutive days (two week days and one weekend day) concurrently with measurement of TEE. Week and weekend days were included to represent 'usual' dietary intake (263). A three day collection period limits subject burden but provides adequate data for comparison between groups and also to nutrient references values (recommended dietary intake and estimated average requirements) (264). Details requested regarding food and drink items, included brand name, flavour, cooking method, and amount eaten provided as either a weight (e.g. packaged food), common portion size (e.g. matchbox size piece of cheese) or as a household measure (e.g. one cup). The food diaries were analysed using FoodWorks v 6.0 nutritional analysis software (Xyris Software, Brisbane, Australia, 2009) based on Australian food composition tables from NUTTAB 2006 and AUSNUT 2007. Nutritional information for food and drink items not present in these databases were located on the Calorie King Australia website (265).

4.3.2.4 Total energy expenditure

The DLW method for measuring energy expenditure uses water enriched with two naturally occurring stable isotopes, deuterium (hydrogen) (2H2) and oxygen 18 (^{18}O) (266). Over time, the deuterium is eliminated from the body in the form of water and the ^{18}O is eliminated as both water and carbon dioxide (267). The differences in the elimination rates of the two isotopes is a measure of carbon dioxide production, which is used to determine TEE (267). It takes the isotopes approximately ten days to be eliminated from the body, during which a small amount of urine is required to be collected each day. Deuterium and oxygen 18 are non-toxic and have no taste.

Boys with DMD were provided with a standard dose of DLW calculated based on total body water, determined by bioelectrical impedance (267). A ten ml urine sample was collected prior to and five hours after the DLW was consumed. Additionally, a ten ml urine sample was collected daily from participants for ten consecutive days following the dose of DLW. The urine samples were frozen at the subject's home in sterile containers until they were collected by the researcher. Samples were analysed and TEE determined by Ms Sarah Elliott (The University of Queensland, Brisbane).

4.3.3 Statistical analysis

Normality of the data was confirmed using the Shapiro-Wilk test for normality. Independent Student's t-tests were used to compare differences in anthropometric and functional measures between boys with DMD and control participants, and estimated energy intake and TEE in boys with DMD. Pearson's correlations were applied to explore the relationship between six minute walk distance and anthropometric and StepWatch™ outcome variables. Bland-Altman analysis was used to assess the agreement between estimated energy intake and TEE (268). Goldberg cut-offs were not used to identify under-reporters. In small studies ($n<100$) where energy expenditure can be directly compared to energy intake, these cut-offs are irrelevant (269). Data are presented as mean ± SD and significance was accepted at $p \leq 0.05$. The statistical package used in this analysis was SPSS, Version 18.0 (SPSS Inc., Chicago, IL).

4.4 Results

4.4.1 Functional outcome measures

Sixteen boys with DMD and thirteen healthy controls completed the functional measures. Clearly erroneous StepWatch™ readings were received from two participants (DMD=1, control=1). These inaccuracies appeared more likely to be due to incorrect usage rather than equipment failure. Most boys with DMD were receiving GCS treatment (prednisolone, n=9, 0.45-0.78 mg/kg/day; and deflazacort, n=5, 0.53-0.79 mg/kg/day). Anthropometric data are summarised in Table 4-1. Although weight z-scores were similar between the two cohorts, boys with DMD were significantly shorter than control participants. This was reflected in significantly higher BMI z-scores in the DMD cohort.

Table 4-1 Anthropometric characteristics of boys with DMD and control participants

Outcome	DMD (n=16)	Controls (n=13)
Age (years)	9.0 ± 2.1	9.0 ± 2.4
Weight z-score	0.71 ± 1.4	0.48 ± 0.76
Height z-score	-1.29 ± 1.07	0.60 ± 1.05*
BMI z-score	1.44 ± 1.08	0.32 ± 0.88*

Data presented at mean ± SD. BMI body mass index. * DMD vs control $p<0.005$.

A comparison of outcome measures between boys with DMD and control participants are presented in Figure 4-4. Boys with DMD walked significantly shorter distances in the 6MWT compared to healthy controls (387 ± 86 vs. 598 ± 63 metres, respectively, $p<0.0005$). Data from the StepWatch™ activity monitors demonstrated that boys with DMD were more inactive (DMD 1103 ± 134 vs. control 1016 ± 62 minutes, $p=0.036$), took fewer steps each day (DMD 5138 ± 2500 vs. control 7239 ± 2621 steps, $p=0.044$), and spent less time in high activity (DMD 25 ± 17 vs. control 53 ± 34 minutes, $p=0.018$). Conversely, there was no difference between the two cohorts in time spent at low activity (DMD 205 ± 74 vs. control 251 ± 44 minutes, $p=0.059$) or at medium activity (DMD 107 ± 51 vs. control 120 ± 33 minutes, $p=0.455$).

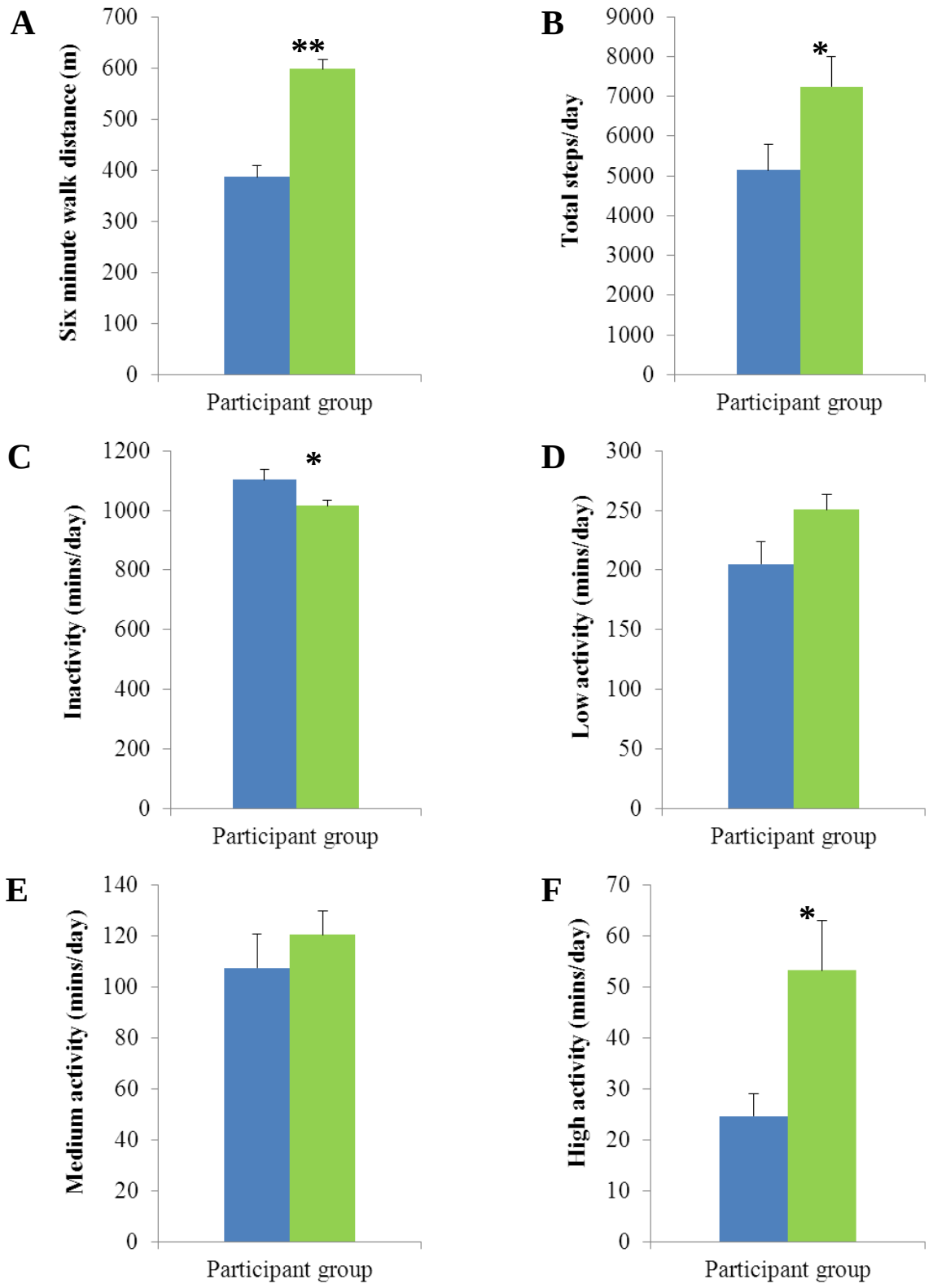

Figure 4-4 Differences in functional outcome measures between boys with DMD and control participants

Graph A presents data from the six minute walk test; graphs B-F present data from StepWatch™ activity monitoring. Solid bars represent the mean score for each cohort and the thin vertical lines represent the standard error of the mean. Boys with DMD are presented by the blue bars, and control participants by the green bars. * DMD vs control $p<0.05$, ** DMD vs control $p<0.005$.

Relationships between functional outcome measures and anthropometric data were also explored in both boys with DMD and control participants (Table 4-2). Strong significant relationships were observed between all StepWatch™ parameters and six minute walk distance in boys with DMD only. The inverse relationship between inactivity and six minute walk distance is illustrated in Figure 4-5. In control participants, six minute walk distance was only significantly correlated with age. No relationships were evident between anthropometric variables and six minute walk distance or StepWatch™ data (not presented) in either cohort.

Table 4-2 Relationships between six minute walk distance and StepWatch™ and anthropometric variables in boys with DMD and control participants

Dependent variables	**DMD n=16**	**Control n=13**
Age	-0.201	0.748*
Weight z-score	0.080	0.130
Height z-score	-0.005	0.225
BMI z-score	0.087	0.027
Total steps/day	0.776*	0.031
Inactivity (minutes/day)	-0.803**	-0.323
Time spent in low activity (minutes/day)	0.804**	0.514
Time spent in medium activity (minutes/day)	0.701*	-0.225
Time spent in high activity (minutes/day)	0.723*	0.134

Independent variable: distance walked in a six minute walk test. Pearson's correlation coefficient presented. * $p<0.005$, ** $p<0.0005$.

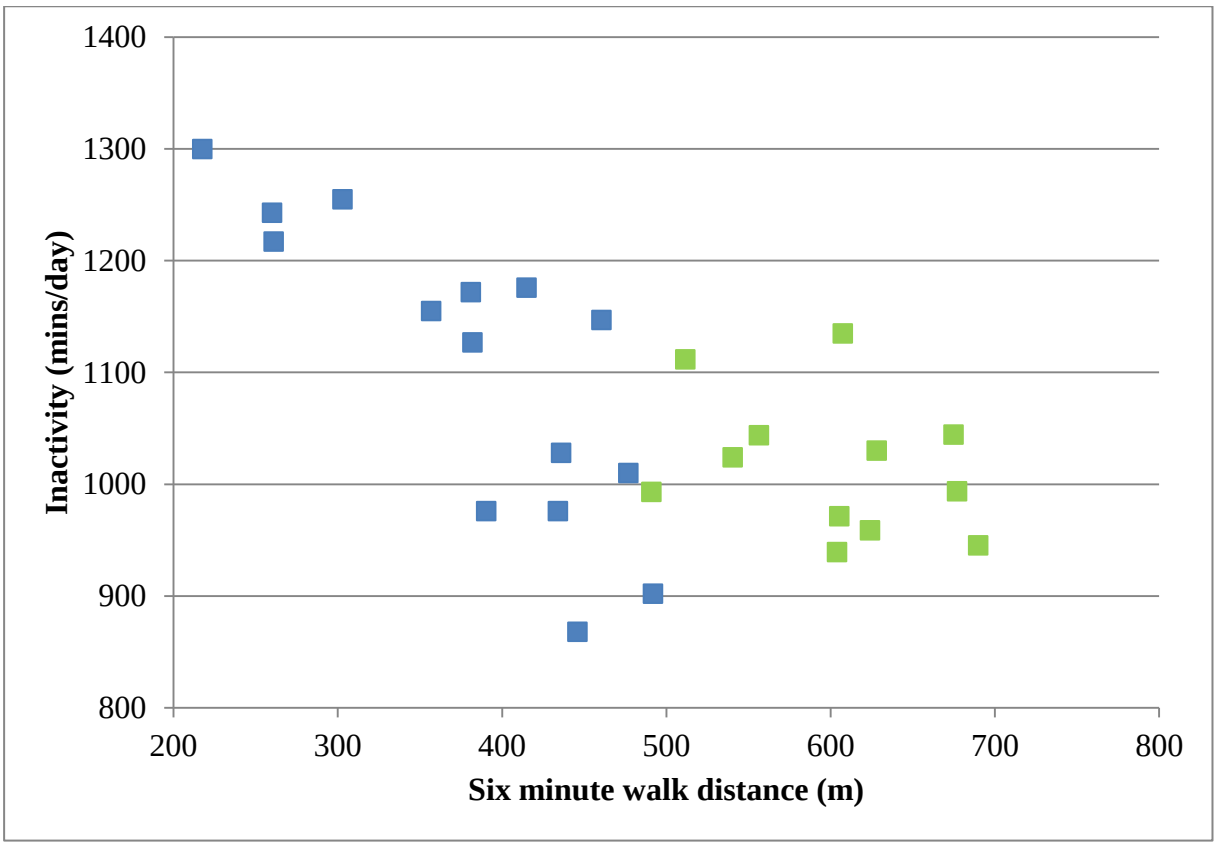

Figure 4-5 Inverse relationship between six minute walk distance and inactivity (minutes/day) measured by StepWatch™ activity monitoring in boys with DMD and control participants

The blue squares represent boys with DMD; green squares represent control participants.

4.4.2 Energy Intake

The physical characteristics and energy expenditure data of fourteen boys diagnosed with DMD are summarised in Table 4-3. All children attended regular school. Thirteen of the boys were receiving GCS treatment (prednisolone, n=12, 0.12- 0.65 mg/kg/day or deflazacort, n=1, 0.83mg/kg/day). All boys with DMD were able to ambulate independently although three boys used a motorised chair regularly. Similar anthropometric trends were observed in this cohort. The mean z-score for height indicated that the group was short for their age whilst the mean z-score for weight was within a healthy range (Box 2); the mean BMI z-score reflects the disparity between height and weight.

Table 4-3 Physical characteristics and energy expenditure in boys with DMD

Characteristic	DMD n=14
Age (years)	8.4 ± 1.9
Weight z-score	0.69 ± 1.21
Height z-score	-1.38 ± 1.25
BMI z-score	1.54 ± 0.89
Measured total energy expenditure (kJ/day)	7400 ± 1100
Estimated energy intake (kJ/day)	6900 ± 1100

Data presented at mean ± SD. BMI body mass index

Food diaries were completed by parents concurrently with measures of TEE for all fourteen boys. There was no difference between mean estimated energy intake and mean daily TEE (p = 0.196, 95% CI -306 to 1419 kJ). Using Bland Altman analysis, the bias between TEE and estimated energy intake was 556 ± 1627 kJ; 95% limits of agreement 3745 kJ to -2632 kJ (Figure 4-6). The correlation of the difference and the mean of the two measures was close to zero (r = 0.04) suggesting that the bias was consistent across a range of energy intakes values despite a wide variance.

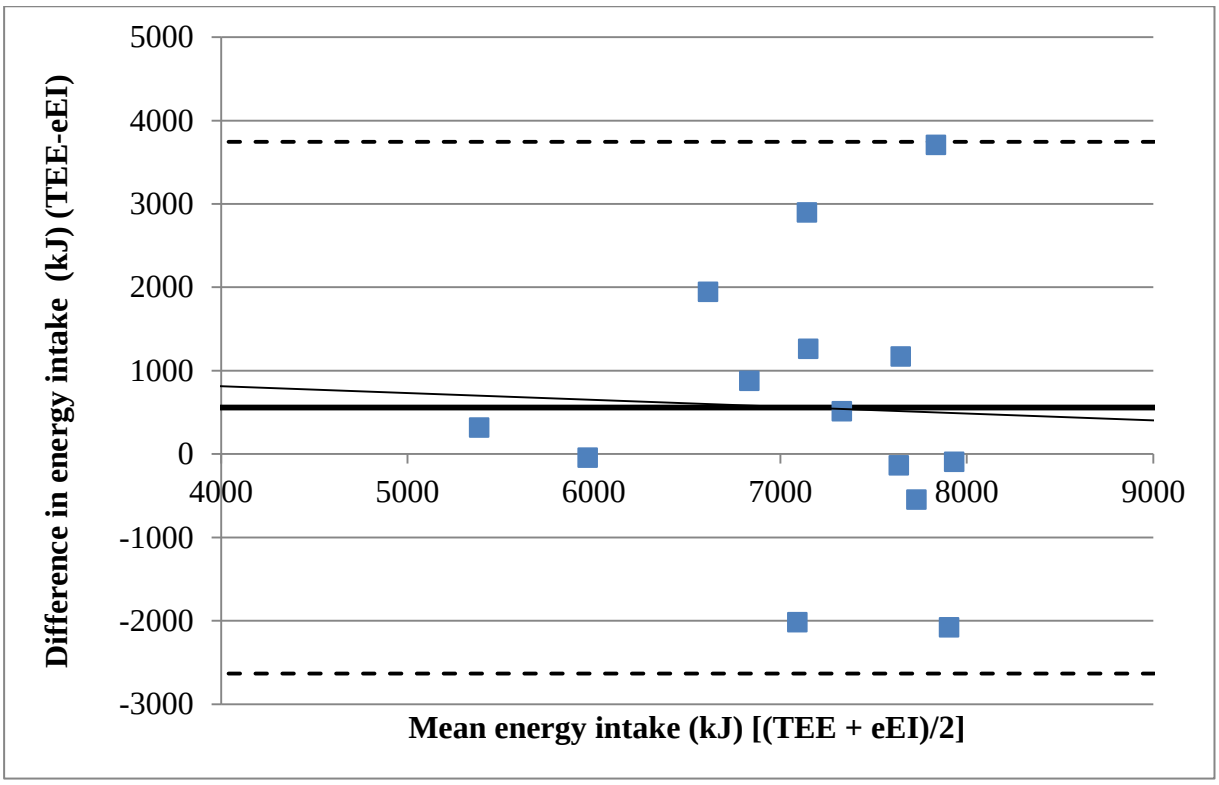

Figure 4-6 Bland Altman plot of the difference of estimated energy intake and total energy expenditure plotted against the mean of the two measures

eEI estimated energy intake; TEE total energy expenditure. The black line represents the mean bias between the two measures; dotted lines represent the 95% limits of agreement.

4.5 Discussion

The functional outcome measures investigated proved to be sensitive to identifying a decreased capacity for ambulation in boys with DMD when compared to healthy controls. StepWatch™ activity monitoring demonstrated that boys with DMD in a community setting, were less active, took fewer steps and spent less time at high activity. Interestingly, there was no difference between time spent at low and medium activity suggesting that the intensity of activity is an important consideration. Step activity monitoring has been investigated previously by two studies in DMD, where similar results were observed (256, 270). Specifically, when compared to a control cohort, boys with DMD had significantly more inactive minutes and took significantly less steps; however, a difference between time spent at medium activity was identified between boys with DMD and the control group in both studies. Longitudinal data from StepWatch™ data is currently lacking and would provide greater emphasis to these observed differences.

The StepWatch™ is a relatively new assessment tool, and as such literature on its use in paediatric populations is limited. The only evidence of reliability of the device exists in a Duchenne study (270, 271); test-retest correlation was high in this population (r=0.73-0.81). Validity of the StepWatch™ monitor was investigated in a sample of 6-20 year old healthy children (272). During a 10-min walk, the monitor had an agreement of almost 100% with observed steps and was moderately correlated with heart rate in a community setting (r=0.49). Control data obtained in this investigation (total steps/day 7239 ± 2621) was similar to values in the literature (7604 ± 2485 (273) and 7001 ± 580 (272)).

Compared to other physical activity measures, accelerometers such as the StepWatch™ monitor, offer a unique ability to predict energy expenditure as they are worn consistently for several days at a time. Two studies explored the potential of accelerometers in predicting

energy expenditure (274, 275). In both studies VO_2, which is the standard for determining energy expenditure during activity, was used as a surrogate measure of metabolic activity. Janz et al (274) evaluated the validity of an accelerometer for estimating energy expenditure in nine children and adolescents with cystic fibrosis. The mean validity correlation between calorie expenditure from the accelerometer and from VO_2 was 0.84 for treadmill walking and 0.97 for stair stepping. More recently, Norman (275) reported on the validity of the accelerometer in predicting absolute VO_2 in five subjects with spastic diplegia cerebral palsy. No significant differences were reported between the mean caloric expenditure estimated by accelerometers and by gas analysis unit VO_2 ($p=0.62$). Whilst these preliminary data require further investigation, using accelerometers as a surrogate measure for caloric expenditure may provide a reasonable means for estimating energy expenditure in boys with DMD, where little is known regarding energy metabolism. Concurrent measurement of TEE and community ambulation using StepWatch™ activity monitoring in boys with DMD would provide valuable data.

The second functional measure investigated, the 6MWT, also revealed significant differences between the DMD and control participants. On average, boys with DMD walked over 200 meters less than the control group in the six minute time frame. Since collection of these data, several papers have been published supporting the use 6MWT as an outcome measure in boys with DMD. Reliability of the 6MWT was assessed as high by two groups (253, 254). Also, longitudinal data demonstrates that the test is sensitive to functional deterioration in DMD over time, with distance walked over six minutes decreasing in a twelve month period by an average of 57 metres in boys 4-12 years old (255); and 26 metres in young males aged 4-17 years old (276). However, the changes over time in this parameter were quite variable, with younger boys showing improvements in the test over the 12 month period. To account for this limitation, Henricson et al. very recently explored the use of percentage predicted six minute

walk distance in boys with DMD (277). Using an age- and height-based equation from normative data, the group showed that younger boys with DMD, instead of improving in the 6MWT, were rather stable at 80% of that of their typically developing peers. The 6MWT is now used as the primary outcome measure in Phase 2 clinical trials investigating gene-related therapies for DMD (ataluren and exon skipping) (51, 56). In these clinical trials, a difference of 30m in distance walked is deemed clinically significant.

StepWatch™ activity monitoring has also been used alongside the 6MWT in Phase 2b trials of ataluren (PTC124®). Data from pre-treatment evaluations was used to explore the relationship between six minute walk distance and total steps/day (270). A moderate correlation (r=0.53) was revealed between the two outcomes. This relationship was weaker than that elucidated in this cohort (r=0.776). Strong, significant relationships were observed across all StepWatch™ parameters and six minute walk distance (Table 4-2), indicating that the 6MWT could be used as measure for absolute functional capacity, and also as a surrogate measure of community ambulation. This is the first study to show such strong relationships between these two functional outcomes.

In boys with DMD, it is often suggested that extra weight increases the burden on already weak muscles. Interestingly, there was no relationship between anthropometric measures and six minute walk distance or StepWatch™ variables in this cohort. This is in line with findings in Chapter 2, whereby BMI had little influence on age at LOA. These anthropometric variables most likely do not reflect body composition and one could assume a relationship would exist between functional outcome measures and lean or fat mass. Accordingly, McDonald et al. identified an inverse relationship between total steps/day and percentage body fat from DXA (r=-0.67); and in contrast to this study, a negative relationship between total steps/day and BMI (r=-0.52) (256). There was no relationship between age and

functional measures in the DMD cohort. This is most likely reflective of the heterogeneity in clinical decline in Duchenne. Conversely, the strong relationship between age and six minute walk distance in control participants is likely to be indicative of improvements associated with growth, development and maturation (277).

Accurate information on dietary intake is somewhat rare but extremely valuable. There are few valid methods available to assess intake. Stable isotopes, as used in this study, are considered to be the gold standard reference method for validation of measurements of energy intake. Due to the cost associated with this method and the complexities of analysing DLW, it remains impractical to use DLW routinely in a clinical setting (278). Under-reporting is the main bias that interferes with the food intake data obtained from food records, and in children this has been noted to be as high as 30% less than actual intake (278, 279). This study demonstrated that TEE was similar to estimated energy intake from parental-report food diaries in boys with DMD. Furthermore, consistent under or over reporting was not evident. Unlike previous work in children, there was no trend for under reporting to occur among the heaviest children and mis-reporting was not linked to weight status (280). Parents of boys with DMD may be more aware of their child's eating behaviours, as weight gain in boys with DMD is a particular parental concern (105). Although energy intake and energy expenditure were measured concurrently, they perhaps are not directly comparable because of the duration of data collection (three days vs. ten days). However, these findings are indicative that parents were reasonably accurate at reporting their child's intake over a short period of time. The use of parental reported diaries for the estimation of energy intake values in boys with DMD may prove advantageous for understanding energy requirements.

Data on energy intake of boys with DMD treated with GCS is sparse. It is speculated that boys consume excessive dietary energy as result of increased appetite when taking GCS.

Also, because of the burden of chronic illness on the child, there may be relaxed restriction of provision of energy dense foods by parents. On average, boys in this study were consuming 6,900 kJ/day. As this is by no means excessive, metabolism of dietary energy may indeed be altered in DMD. Despite these complexities, there is very little evidence about energy needs available to inform dietetic management. This is largely due to study design, in which resting energy expenditure instead of total energy expenditure has been explored (Chapter 1). This study is also the first to examine the validity of food diaries for the estimation of energy intake in boys with DMD. The use of simple parent-report tools such as those described here can inform dietetic practice and enable anticipatory guidance to be given to the family to try and prevent obesity by manipulation of energy balance using dietary intake.

The three day food diaries will be used to assess dietary changes across the course of a nutrient intervention (Chapter 5). An example of the diary is included in Appendix 20. Parents will be asked to record all food and drink provided to their child together with the amount consumed. Food diaries are appropriate tools to assess energy and macro and micro intake in children (264). The practical considerations for using food diaries are presented in Figure 4-7.

Burden	Researcher	Collection	L
		Analysis	H
	Participant		H
Sample Size	Individual		✓
	Group		✓
	Population		
Potential for Bias	Recall		✓
	Measurement		✓
Represents Usual Intakes	Individual level		?
	Group level		✓
Type of Data	Retrospective		
	Prospective		✓
Budget	$ (Low)		
	$$ (Medium)		✓
	$$$ (High)		

Figure 4-7 Practical considerations for using food diaries

L: low; H: high. Figure obtained from The Australasian Child and Adolescent Obesity Research Network (264).

4.6 Summary

This chapter presents data and literature to support the outcome measures used in the randomised control trial described next. Specifically, both StepWatch™ activity monitoring, and the 6MWT were sensitive to differences in ambulatory capacity in boys with DMD compared to control participants. Of interest, StepWatch™ parameters correlated strongly with six minute walk distance, indicating that the later could potentially be used as a surrogate measure for community ambulation. Furthermore, self-reported energy intake using food diaries was similar to TEE determined with DLW in boys with DMD. These food diaries will be used to assess changes in energy and protein intake over the course of the dietary intervention.

5 Nutriceuticals in Duchenne muscular dystrophy

A pilot randomised controlled trial of a multi-component nutritional supplement

The full trial protocol is provided on the accompanying CD.

5.1 Introduction

There are currently no curative therapies that halt or reverse muscle weakness in DMD, so care is designed on the principles of prevent and/or manage complications. Although the use of GCS as best practice in DMD has been previously highlighted; GCS only ameliorate the manifestations of DMD and slow the progression of muscle weakness and do not address its underlying cause (37). Their use is also associated with significant and debilitating side effects such as excessive weight gain, cushingoid features, osteopaenia, delayed puberty, hirsutism, glucose intolerance, cataracts and behavioural changes, which can be of sufficient concern to cause their withdrawal (35, 42, 45). Understandably, parents sometimes seek remedies in complementary medicines, often incurring great expense but without proven efficacy. In a survey conducted across four neuromuscular centres, 80% of parents reported "ever" using complementary and alternative medicines for their affected child (281). Nutritional interventions could provide a better, simple, acceptable and cost-effective strategy to maintain muscle function and mobility and enhance quality of life for males with DMD. As demonstrated in Chapter 1, a lack of consistent high-quality evidence exists to support nutriceutical use. This study aims to address this gap utilising a rigorous study design - a double blind randomised controlled cross-over trial to provide Oxford Centre for Evidence-based Medicine level 2 evidence of efficacy (1). It has a translational approach in that it takes interventions previously shown to be effective in the *mdx* mouse model into a human intervention study. Functional measures tested in Chapter 4 are used as outcomes because this is important at a practical level for families. Here, the results of a pilot study are presented, together with research plans for the multicentre trial which commenced in 2012.

5.2 Background

The supplement regimens used in this pilot study are indicated in Box 5. The components in this novel nutritional supplement have been chosen for investigation due to evidence of their potential benefit to muscle function in the *mdx* mouse model of DMD and they have been shown to be safe in humans. The most promising is creatine monohydrate (CrM), an amino acid derivative found in muscle which acts to store energy in the form of the high energy phosphate bond of phosphocreatine ready for immediate use in the ATP-ADP reaction (45). In healthy subjects, supplementation with CrM results in an increase in creatine and phosphocreatine concentrations in skeletal muscle; and this increase is greater for individuals with lower endogenous stores of both components (282). It has been demonstrated that subjects with neuromuscular conditions have lower creatine and phosphocreatine concentrations in skeletal muscle when compared to healthy controls (283). Because of this, CrM supplementation may have potential to enhance performance in people with neuromuscular disorders (118).

Standard Supplement Regimen
Protein shake, kids multivitamin, vitamin D, and fish oil
Enhanced Supplement Regimen
Protein shake, kids multivitamin, vitamin D, fish oil plus creatine monohydrate (CrM), glutamine and β-hydroxy-β-methylbutyrate (HMB)

Box 5 Supplement regimens used in the pilot study
Further details on supplements provided in Table 5-1.

Several studies have supported the use of CrM for increasing athletic performance and strength in healthy populations (119). Also, four previous RCTs, summarised in a Cochrane review (118), concluded that short- and medium-term CrM treatment is well tolerated and improves muscle strength in people with neuromuscular diseases. All bar one of these studies

were conducted in steroid-naïve males with DMD (122). In 138 participants treated with CrM, there was a significant increase in maximum voluntary contraction and lean body mass in the CrM group compared to placebo. CrM doses given to boys in these trials were 5g/day (120), 3g/day (121), 100 mg/kg/day (122) and 5g/day (123). Of interest was the additional effect on bone health. CrM treatment increased bone mineral density by 3% (121), and also reduced levels of ***N***-telopeptides, a marker of bone degradation (122). A CrM dose of 5g/day was used in this study as it has proven to be efficacious in the DMD population without any adverse effects. Although published data on CrM toxicity was not located, a risk assessment concluded that the evidence of safety is strong at intakes of up to 5g/day for chronic supplementation (284). Numerous review articles have also found that few adverse effects are associated with CrM supplementation (285-289).

The second nutriceutical chosen is glutamine, the most abundant amino acid in the body (290). Although glutamine has multiple functions, of interest is its role in protein synthesis. In animals, protein synthesis is correlated with intramuscular glutamine concentrations (291). In addition, provision of glutamine produces an anabolic effect in healthy humans (128). Glutamine may also be used for a fuel substrate. In both the liver and muscle tissue, glutamine is an effective amino acid for enhancing glycogen formation (292). Glutamine is converted to glucose in the tricarboxylic acid cycle (citric acid cycle) via conversion to glutamate and then α-ketoglutarate (Figure 5-1).

In boys with DMD, low levels of intramuscular glutamine have been documented and early supplementation studies concluded that glutamine may have a protein-sparing effect, decreasing estimated whole body protein degradation (130, 132, 293). These results led to the initiation of two clinical trials. Although the first of these trials was unable to demonstrate a significant effect of glutamine supplementation on manual and quantitative measurements of

muscle strength, a disease-modifying effect of glutamine in younger patients with dystrophies could not be excluded because the placebo group did not decline as predicted (120). More recently, Mok et al. documented a lack of functional benefit with glutamine versus a placebo in a group of boys with DMD (133). This study utilised a double blind, randomised crossover trial with two intervention periods: glutamine and placebo for four months each with a one month washout. The primary outcome measure used by the authors to assess functional ability was unusual – change in walking speed from a two minute walk test. Of the 30 boys recruited for the trial (aged 2 to 10 years), only five boys were being treated with GCS. Interestingly, a subgroup analysis revealed a significant effect of glutamine on functional measures in boys taking GCS, indicating a possible synergistic action.

The glutamine doses administered in these previous studies (0.6g/kg/day and 0.5g/kg/day, respectively) were safe and well tolerated, with no subjects withdrawing from the trials. In the pilot study described here, glutamine was dosed at 0.6g/kg of lean mass/day as measured using DXA. Glutamine is a non-essential amino acid that is mainly produced and stored in skeletal muscle (133). It is therefore reasonable to presume that the glutamine dose can be based on lean mass as opposed to body weight. The practical implications of this included reduced cost and improved palatability of the supplement. No reports of adverse events have been documented at doses of 0.6g/kg/day and 0.5g/kg/day in boys with DMD (120, 133). A thirteen week oral toxicity study of L-Glutamine in rats (294) estimated the 'no observed adverse effect level' at 1.25% of standard diet; equating to 0.83g/kg/day and 0.96g/kg/day for males and females, respectively. Similarly, a risk assessment of glutamine determined a conservative estimate of the observed safe level at 14g/day (295).

β-hydroxy-β-methylbutyrate (HMB), the third nutriceutical, is a metabolite of the amino acid leucine. Both leucine and its primary metabolite, α-ketoisocaproate, are known to be potent

anti-catabolic compounds (296). Other branched chain amino acids have been unable to achieve similar effects, suggesting that either α-ketoisocaproate or HMB are producing the anti-catabolic effects (297). From α-ketoisocaproate there are two main pathways of metabolism (Figure 5-1). The majority of α-ketoisocaproate will be converted to isovaleryl-CoA and eventually to acetyl-CoA; whilst approximately 5% will be converted to HMB (298). Based on this estimate, an individual would need to consume 60g of leucine to achieve a 3g dose of HMB (the common dose used in studies)(299). HMB can then be directed to cholesterol synthesis via β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) (300).

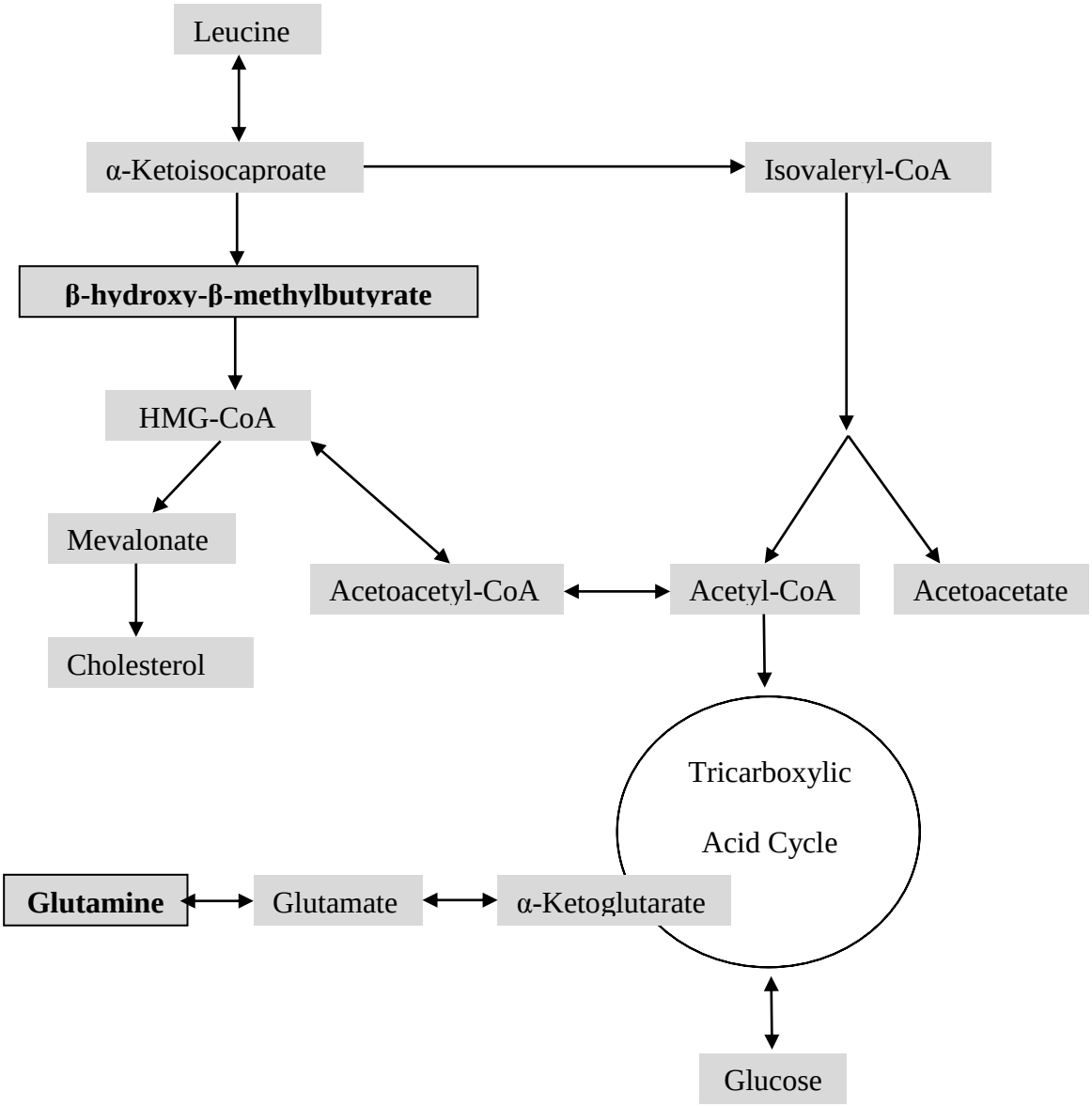

Figure 5-1 Pathways of metabolism for β-hydroxy-β-methylbutyrate and glutamine

Modified from Zanchi et al. and Stumvoll et al. (292, 300)

The proposed mechanisms of action of HMB are summarised by Wilson et al. (299). The first of these is related to stabilisation of the sarcolemma as described by the 'Cholesterol Synthesis Hypothesis'. This hypothesis purports that a damaged muscle is unable to produce sufficient cholesterol for various cell functions including sarcolemmal integrity (301). By increasing intramuscular HMB, the available substrate for conversion to cholesterol is also increased and this assists to stabilise the muscle cell wall. This mechanism of action is particularly pertinent given the aetiology of DMD where the sarcolemma is compromised due to the lack of dystrophin. It is also proposed that HMB can increase both performance of muscle and its rate of repair. This is through a combination of enhanced protein synthesis via the mTOR pathway (mammalian target of rapamysin pathway) and depression of protein degradation via the ubiquitin proteasome dependant pathway. These mechanisms are not yet fully understood. HMB has been shown to be effective for increasing fat-free mass in people with HIV (139), in cancer-related wasting (302), in the elderly (303), and in untrained males (304). Although HMB has not yet been tested in DMD, it was well tolerated in the above studies in humans and in studies using the *mdx* mouse (125).

The HMB dose used in this pilot study was 38mg/kg/day. The vast majority of studies investigating HMB use a dose of 3g/day, however all of these studies are in adults with body weights ranging from approximately 65kg to >100kg. Gallagher et al. determined that the 3g/day dose equates to 38mg/kg/day (304). Study participants randomly assigned to the 38mg/kg/day dose exhibited a significantly greater increase in fat free mass compared to participants assigned to 0g/day or 76mg/kg/day (~6g/day). Objective data indicate that 3g/day of HMB can be taken safely as an ergogenic aid for exercise (305). In addition, a 13 week dietary toxicity study of the calcium salt of HMB (CaHMB) in rats determined the 'no observed adverse effect level' at 5% CaHMB mixed with diet; 3.49g/kg/day and 4.16g/kg/day for males and females, respectively (306).

There is evidence to support the use of combined nutriceutical supplementation together with GCS treatment to increase the positive effect on muscle function. Using the *mdx* mouse, Payne et al. tested several compounds (CrM, conjugated linoleic acid, α-lipoic acid and HMB) in combination with GCS (prednisolone), which provided the most consistent evidence of efficacy than any single component alone; an increase in peak grip strength and decrease in grip strength fatigue was demonstrated (125). Similar complementary or synergistic effects have been reported between prednisone and CrM treatment *in vitro* (307). In combination, these data provide biological plausibility and preliminary evidence for the potential benefit of nutriceuticals as an 'add on' therapy to GCS in the treatment of DMD.

The synergy of the combined nutriceuticals (CrM, glutamine and HMB) may also increase the energy potential of the muscle. Essentially, a working muscle has four sources of energy: phosphocreatine which reacts with ADP to form ATP; glucose from glycogen stores in the muscle; glucose from glycogen stores in the liver; and aerobic metabolism via the mitochondria (308). CrM may increase the intramuscular concentration of creatine and phosphocreatine; glutamine may contribute to glycogen stores; and both glutamine and HMB may increase substrate available for energy production via metabolic pathways involving the tricarboxylic acid cycle (Figure 5-1).

There has been little investigation into the macronutrient and micronutrient requirements of boys with DMD. Dietary protein needs in this population are of obvious interest, but the use of high-protein supplements has not yet been investigated. Whey protein isolate was chosen for use because of its high protein quality score and its relatively high proportion of branched chain amino acids (26%, leucine, isoleucine and valine) (309). Moore et al. determined that the dose response to dietary protein ingestion was maximally stimulated at 20 g, and hence this dose (20g protein/day) was used in the study (310).

Since the advent of GCS therapy, the micronutrients that require immediate attention are vitamin D and calcium; however, few other micronutrients have been explored. As discussed in Chapter 3, low levels of serum 25(OH)D have been documented in our own studies and those of others (66). Based on the calculated vitamin D supplementation requirement of a patient using GCS, a dose of 2000 IU/day has been used as a component of this novel nutritional supplement.

In summary, these nutriceuticals were chosen for investigation because of their proven efficacy in increasing sports performance in healthy individuals, increasing lean body mass in both healthy individuals and those with suboptimal nutritional status, and because of the benefits observed in the *mdx* mouse model and early clinical trials in Duchenne populations. There is also evidence to suggest that nutriceuticals may work in a synergistic manner with GCS, the use of which are now best practice.

5.3 Aims and hypothesis

Our overarching aim was to keep these boys with DMD independently mobile for as long as possible. Specifically, we aimed to test the efficacy of a standard nutritional supplement in comparison to one enhanced with three nutriceuticals (CrM, glutamine, and HMB) in maintaining or improving community ambulation as measured by the StepWatch™ activity monitor in boys with DMD. Secondary objectives tested were the impact of the supplement regimens on functional ability as measured by the 6MWT, body composition (using DXA), and quality of life.

5.3.1 Hypothesis to be tested

That: 'A standard nutritional supplement enhanced with three nutriceuticals (CrM, glutamine and HMB) consumed for 20 weeks will maintain or reduce inactive time (minutes) as measured by the StepWatch™ activity monitor in boys with DMD when compared to a standard nutritional supplement'.

5.4 Methods

5.4.1 Study design

This was a single centre, double blind, randomised (1:1), controlled cross over trial conducted at The Royal Children's Hospital, Melbourne, Australia during 2011. The 50 week trial period incorporated an eight week lead in period and a two week washout between treatments. The study design is illustrated in Figure 5-2. By utilizing a cross-over design, the study was able to determine whether the standard nutritional supplement is effective alone and if an improved response is obtained with additional dietary supplementation of the active nutriceuticals CrM, glutamine and HMB.

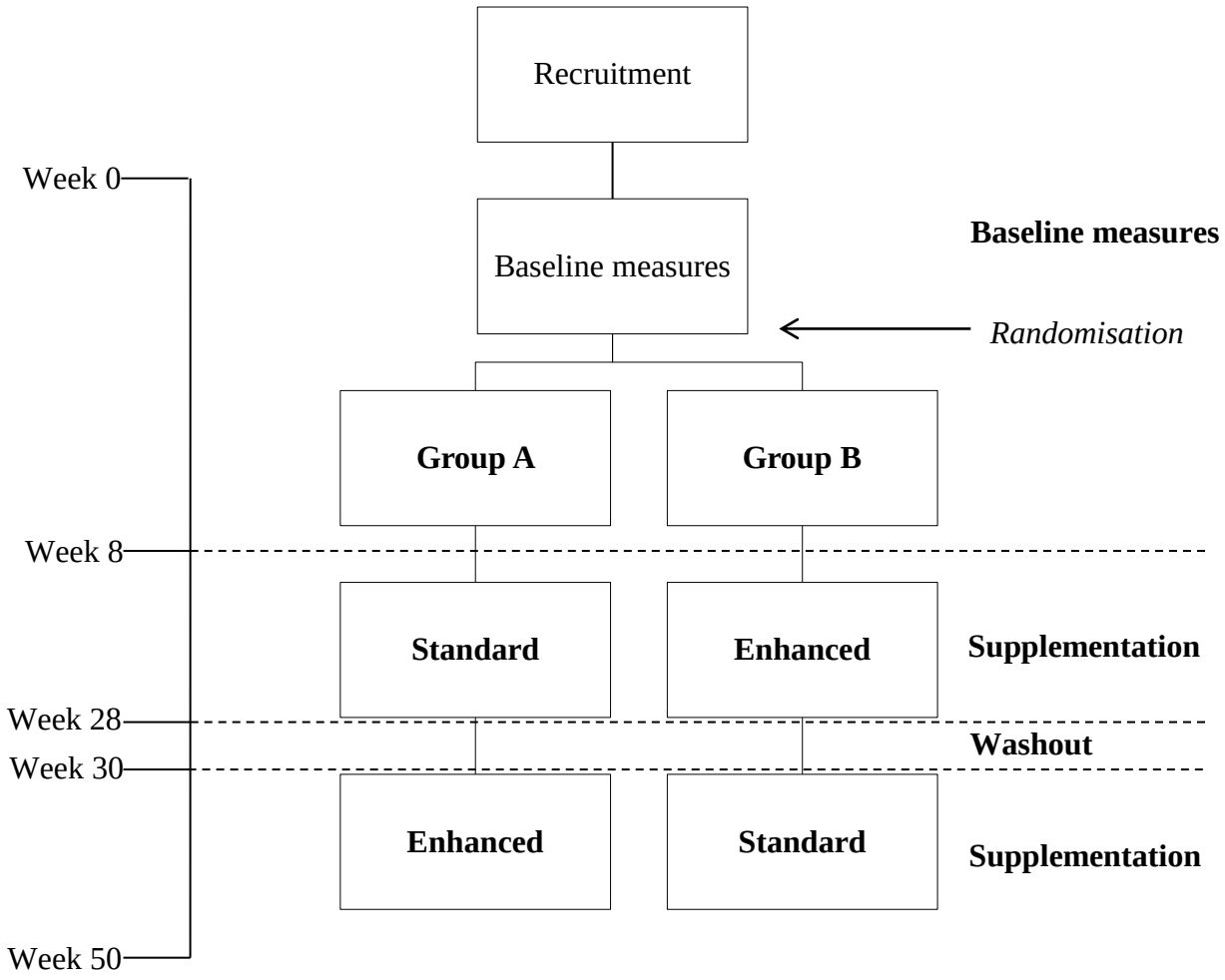

Figure 5-2 Study design

5.4.2 Participants

Participants were recruited through the neuromuscular clinic at The Royal Children's Hospital, Melbourne. Eligible participants were boys aged 5 to 13 years with a definite diagnosis of DMD and sufficiently ambulatory to walk ≥75 metres unassisted during a 6MWT. Exclusion criteria were: personal assistance required to aid walking or assistive devices (short leg braces, long leg braces) or use of a wheelchair/motorised scooter/similar mobility aid for >75 % of activity during the day; cow's milk protein allergy or lactose intolerance; history of kidney or liver disease; inability to attend the seven required hospital appointments within the 12 month period; inability to swallow tablets; parents wishing to continue using any complementary therapies (including other nutritional supplements); participation in other muscular dystrophy trials involving an active intervention; and use of medications which are contraindicated due to possible drug interactions. Eligible children were identified and their families approached initially by the neuromuscular clinic coordinator (Daniella Villano). The recruitment process together with the blinding process (Section 5.4.3) is summarised in Figure 5-3.

The trial protocol (included on attached CD) was approved by The Royal Children's Hospital and Monash University Human Research Ethics Committees (HREC# 30022 and 2010001692, respectively, Appendix 11 and Appendix 12). Informed consent was obtained from parents or guardians. The parent/guardian information sheet and consent from are provided in Appendix 13. A collaborative research group clinical trial agreement was prepared and executed by Monash University and The Royal Children's Hospital. The trial was registered with Australian New Zealand Clinical Trials Registry (#ACTRN12610000462088) and a clinical trial notification was submitted to the Therapeutic Goods Administration (Trial Number 2010/0561).

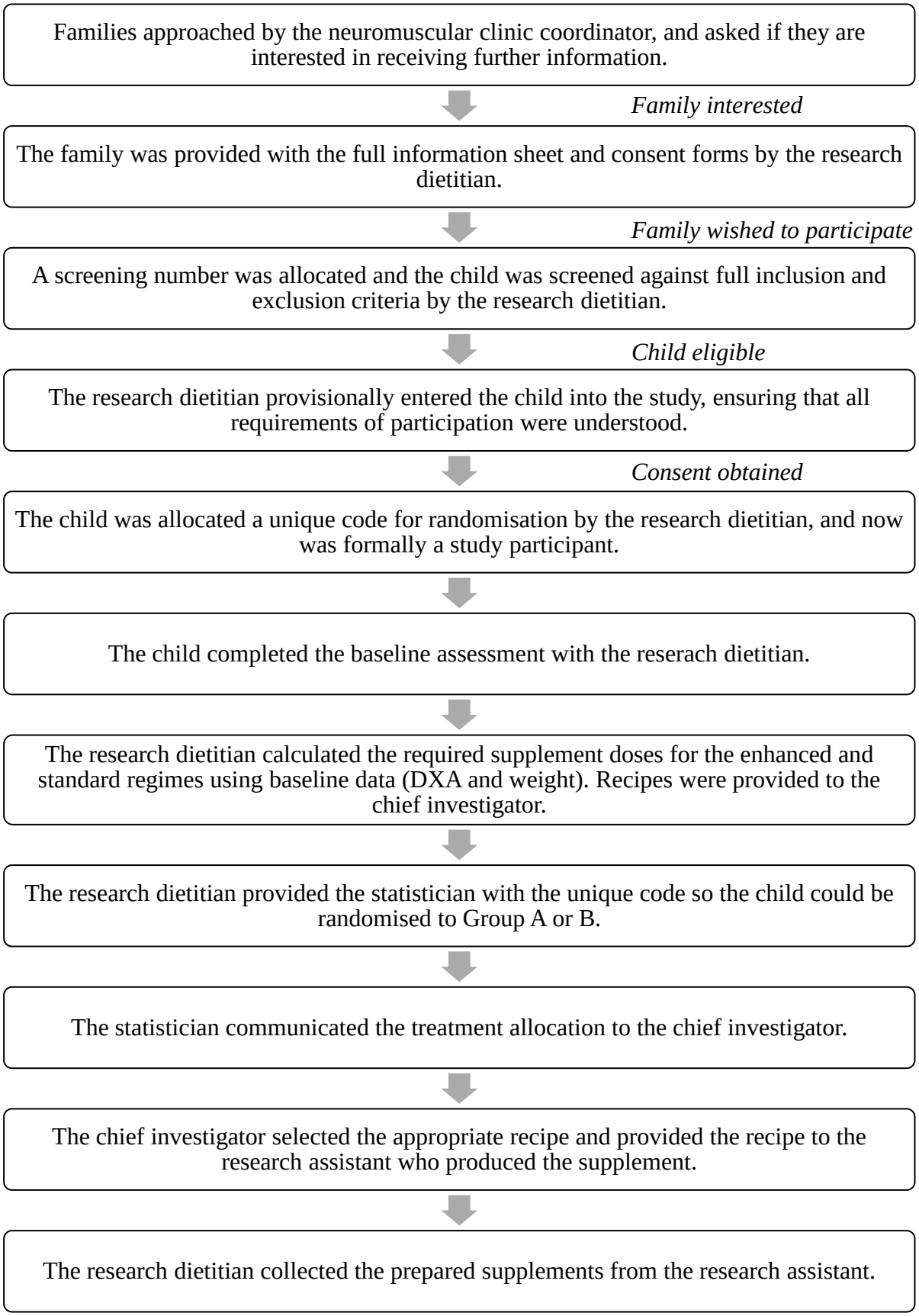

Figure 5-3 Flow diagram summarising the recruitment and blinding process

Neuromuscular clinic coordinator (Daniella Villano); research dietitian (ZD); statistician (Dr Ian Hughes); chief investigator (Helen Truby); research assistant (Deanna Pattieson).

5.4.3 Blinding

The blinding process is summarised in Figure 5-3. Although the research dietitian calculated the required formulation for each supplement regimen, there was no involvement with treatment allocation so this remained concealed. Study specific recipe forms were developed to assist with this process (Appendix 21). A research assistant (Deanna Pattieson), who had no contact with participants, prepared the shakes and HMB tablets in a formula room at Monash University in accordance with the allocated recipe.

Recipes were recalculated during the washout period using weight and DXA measures from week 28 by the study dietitian for the second supplementation phase (both standard and enhanced). These were provided to the chief investigator who selected the appropriate recipe and provided this to the research assistant for production of the supplements.

5.4.4 Supplements

The individual components of the supplement regimens are summarised in Table 5-1. Products were purchased at cost from Blackmores (Blackmores Australia, Warriewood, NSW) and Musashi (Nestlé Australia Ltd, Melbourne, VIC). Successful prototypes, acceptable in taste and indistinguishable from each other, for the standard and enhanced shake were developed prior to commencement of the trial. Splenda® (Johnson & Johnson Pacific Pty Ltd, Broadway, NSW) (sucralose) and maltodextrin were added to both shakes in order to mask the active ingredients, and so to reduce the caloric difference between the two shakes. The short half-life of the nutriceuticals requires a daily intake and the HMB was encapsulated due to taste. The estimated half-life of the nutriceuticals are: CrM 20 – 172 minutes (311); glutamine (containing dipeptides) < 3mins in healthy volunteers and < 10 minutes in intensive care patients (312); and HMB approximately 2 h and 3 h in pigs and sheep, respectively (304). Each supplement regimen was consumed for 20 weeks. The supplements (shakes and

tablets) were dispensed by the research dietitian (ZD) during study visits at week 8, 12, 28, 30, and 34. At week 20 and week 42 supplements were delivered to the participant's home. The glutamine dose was calculated on lean mass as measured by DXA at week 0 and week 28. Similarly, the HMB dose was based on body weight measurements at week 0 and week 28. All supplements were labelled and accounted for by the study dietitian (ZD). Figure 5-4 presents photographs of the shakes as they would be dispensed to study participants, and Figure 5-5 illustrates the different capsules used. Subject compliance was measured by unused shake sachets and capsules returned at weeks 12, 20, 28, 30, 34, 42 and 50.

5.4.5 Dietary prescription to support supplementation

All participants were provided with individually tailored dietary advice by the study dietitian (ZD) on how to incorporate the shake into their habitual diet in order to maintain an adequate energy intake. Food diaries completed at baseline were analysed using FoodWorks v 6.0 nutritional analysis software (Xyris Software, Brisbane, Australia, 2009) so that individual recommendations could be provided. Where possible, the shake was incorporated at a time when the child would usually consume milk. Alternatively, the shake was recommended as a meal replacement (breakfast) for boys who had difficulty with weight management prior to study involvement. Most of the advice provided to ensure energy balance was constructed around the theme of reducing calorie dense liquids (juice, soft drink, or cordial). Families were also educated to maintain their 'usual' intake of protein foods (especially diary and meat/meat alternatives) so that the protein received in the shake was additional to 'usual' intake. Dietary recommendations were reviewed at each visit in relation to weight gain and further guidance provided to ensure weight gain was appropriate for growth and development needs.

Table 5-1 Supplement regimens

	Standard Supplement (daily dose)	Enhanced Supplement (daily dose)	Route of Administration
Shake			
Whey protein isolate	22g (20g protein)	22g (20g protein)	Powder added to skim milk to make a shake.
Creatine monohydrate	0g	5g	Powder added to protein shake
Glutamine	0g	0.6g/kg fat free mass/day rounded up to nearest 0.5g	Powder added to protein shake
Placebo (maltodextrin)	16g	8g	Powder added to protein shake
Splenda®	5g	5g	Powder added to protein shake
Tablets			
Kids Multi Blackmores	5-6 years –1 capsules 7-12 years – 2 capsules	5-6 years –1 capsules 7-12 years – 2 capsules	Chewable capsule
Vitamin D3 Blackmores	2000 IU (2 capsules)	2000 IU (2 capsules)	Capsule
Kids Fruity Fishies™ Blackmores	1050mg Omega 3 triglycerides (2 capsules)	1050mg Omega 3 triglycerides (2 capsules)	Chewable capsule
HMB	0	38mg/kg/day rounded up to nearest 500mg (1-3 capsules)	Capsules
Placebo capsules (maltodextrin)	38mg/kg/day rounded up to nearest 500mg (1-3 capsules)	0	Capsules
Energy	**648 kJ**	**518 kJ**	
With skim milk	**1017kJ**	**887kJ**	

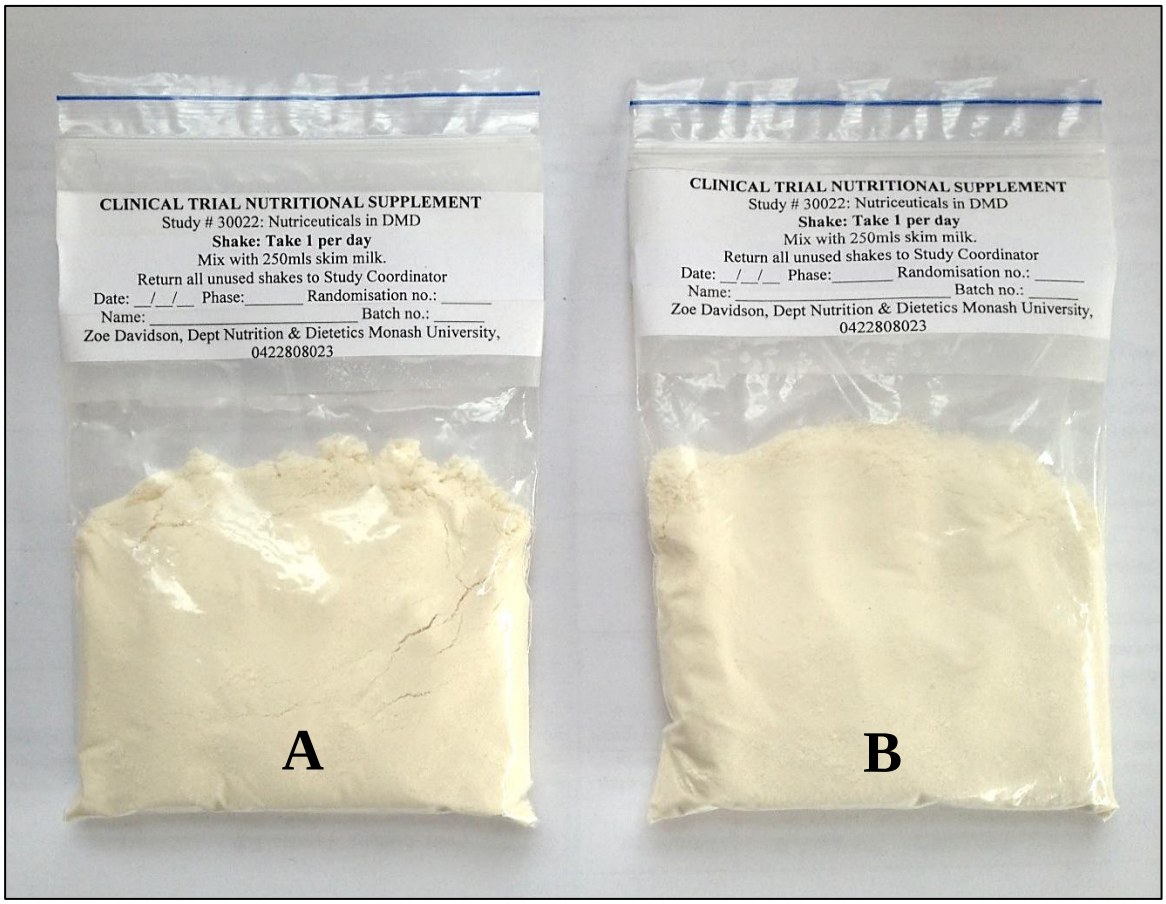

Figure 5-4 Protein shakes

Shake A is the standard shake; Shake B is the enhanced shake and contains CrM and glutamine.

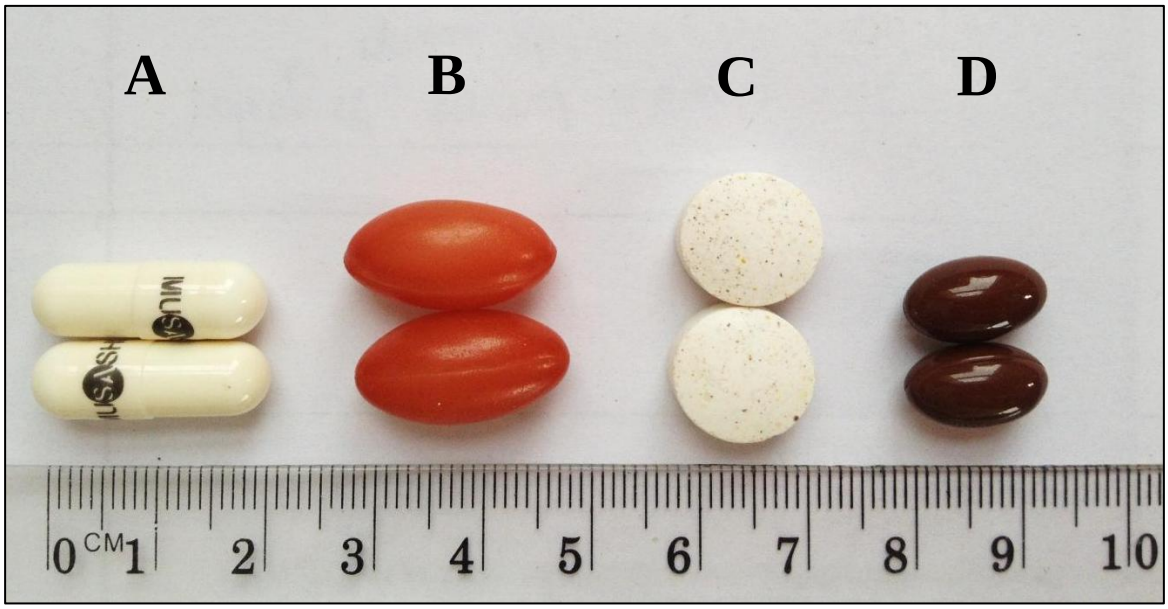

Figure 5-5 Capsules

Capsule A: HMB or placebo capsules (filled with maltodextrin); Capsule B: *Kids Fruity Fishies*™, Blackmores; Capsule C: *Kids Multi*, Blackmores; D: *Vitamin D3*, Blackmores.

5.4.6 Outcome measures

All study assessments occurred at the Royal Children's Hospital, Melbourne. Chapter 4 details the functional outcome methods used to assess change in this study. Inactive minutes/day as measured by StepWatch™ activity monitoring was the primary outcome measure in this supplement trial. Secondary outcome measures were the 6MWT; lean body mass as measured by DXA; serum 25(OH)D concentrations; the ACTIVLIM questionnaire (Appendix 22); and quality of life. DXA scans have been used by several authors to assess body composition in boys with DMD (66, 68, 88). The ACTIVLIM survey is a self-reported scale of activity limitations. The survey was recently developed and validated using the Rasch model and demonstrated a good sensitivity to change which is useful in a research setting (313-315). A score is obtained between zero and 36 with 36 being no activity limitations. The raw score is then converted to logits; a score of zero equates to
-6.869 logits, and a score of 36 equates to 6.571 logits.

Quality of life was measured using the age-appropriate general and neuromuscular Peds QL questionnaires, a well-validated tool for children with chronic disease (316). Parents responded on a scale of zero ('never') to four ('almost always'). The questionnaire was then reversed scored as follows: 0=100, 1=75, 2=50, 3=25, and 4=0. An average score across all questions was then generated with a score of 100 indicating the highest quality of life. An example of both the general and neuromuscular Peds QL for an 8-12 year old child is presented in Appendix 23. Parents completed all the surveys and questionnaires on behalf of their child.

The primary outcome, StepWatch™ activity monitoring, and secondary outcome measures were assessed at weeks 0, week 8, 28, 30, and week 50 (6MWT, Peds QL questionnaire, ACTIVLIM questionnaire, height and weight, blood test for analysis of 25(OH)D and

electrolytes and liver function tests, and a three day food record). The StepWatch™ monitor was worn for five continuous days. A total body DXA scan was conducted at week 0, week 28, and week 50. All assessments were conducted by trained health professionals (dietitian (ZD), physiotherapists, radiographers, and phlebotomists) blinded to group allocation. Safety assessments (biochemistry and medical examination) occurred at weeks 12 and 34. Medical examinations were conducted by paediatric neurologists.

5.4.7 Participant feedback

Feedback from participants regarding the supplement regimens was collected at the final study visit (week 50) using a brief questionnaire (Appendix 24). Participants completed the form with the assistance of their parent/guardian. Using a child-friendly Leichardt scale, the questionnaire asked participants to identify their perception of the taste, mouth-feel, and appearance of shakes; a response of '1' indicated 'yuk', '3' indicted 'ok', and '5' indicated 'yum'. Participants were also asked about the ease of taking the shakes and tablets daily; a response of '1' indicated very hard, '3' indicated 'ok', and '5' indicated 'very easy'. Finally, participants were asked if they could detect a difference between the two supplement regimens, and if so, which regimen they preferred. A general comments section was provided at the conclusion of the questionnaire.

5.4.8 Statistical analysis

Food diaries were analysed using FoodWorks v 6.0 nutritional analysis software (Xyris Software, Brisbane, Australia, 2009) based on Australian food composition tables from NUTTAB 2006 and AUSNUT 2007. Nutritional information for food and drink items not present in these databases were located on the Calorie King Australia website (265). All diaries were entered by a trained dietitian (Nicola Abel), who maintained a standard list of assumptions throughout the entry process. New foods were created to enable entry of the

protein shake, multivitamin, fish oil and vitamin D. Energy requirements were estimated using the Schofield weight only equation and a physical activity level of 1.4 (317). Our recent publication demonstrates that this equation is the most accurate and precise for predicting REE in boys with DMD (Appendix 2) (62). Micronutrient intake was assessed individually as the proportion of the recommended dietary intake or adequate intake achieved. Age appropriate nutrient reference values were utilised (318).

From collected anthropometry data, BMI (weight (kg)/height $(m)^2$) was calculated. Weight, height and BMI z-scores were determined using the CDC reference values for children (157). With a small sample size, it is difficult to assess normality, so data from the pilot study are presented as median (interquartile range (IQR)) to best demonstrate the spread of observations. The Freidman Test for repeated measures was used to explore the change in 25(OH)D and dietary intake over the study period. Statistical analysis was not performed on the remaining pilot data due to the small sample size and large variation in measures. Analysis would be inappropriate as the pilot study is not adequately powered to detect change. A detailed plan for statistical analysis in the multicentre, powered study is summarised in section 5.6. The statistical package used in this analysis was SPSS, Version 18.0 (SPSS Inc., Chicago, IL).

5.5 Results

5.5.1 Participants

Recruitment occurred between December 2010 and March 2011. Initial assessments commenced in January 2011, and the last assessment was conducted in February 2012. Participant flow is summarised in Figure 5-6. Families responded enthusiastically to recruitment. Of the twelve families provided with information, three declined to participate (unsuitable timing for family n=2, wished to continue current nutritional supplements n=1), and one boy did not meet the eligibility criteria because he was unable to swallow tablets. The remaining eight boys successfully completed the 50 week protocol, median age 8.2 (6.4, 9.7). As expected, five out of the eight participants had a deletion in the dystrophin gene. Other mutations included a duplication/triplication (n=1), a splice site mutation (n=1), and a point mutation (n=1). Seven boys were receiving GCS treatment (prednisolone, n=4, 0.45-0.70mg/kg/day; deflazacort, n=3, 0.53-0.73mg/kg/day). Baseline characteristics are summarised in Figure 5-6.

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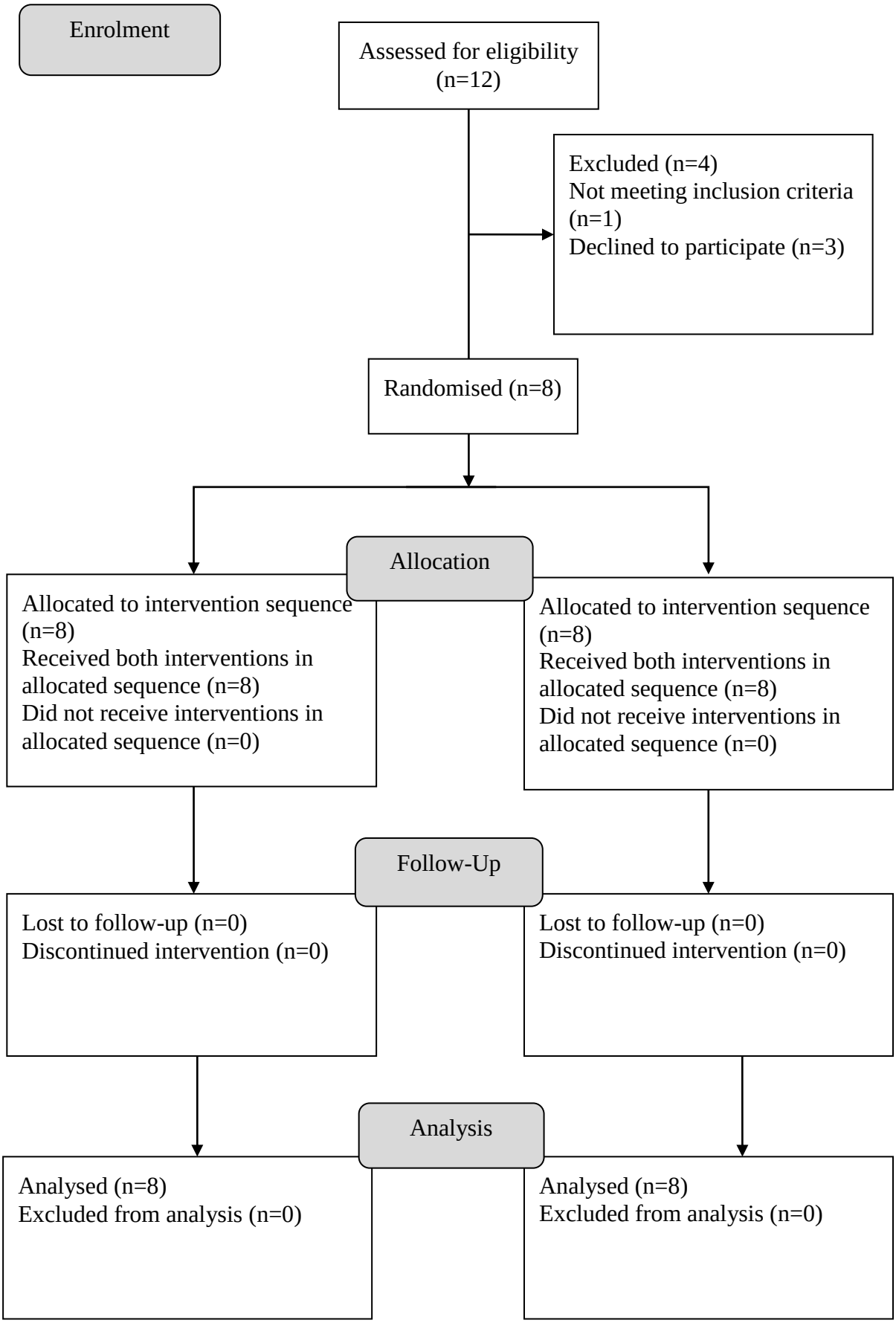


Figure 5-6 Participant flow

Table 5-2 Baseline characteristics of eight boys enrolled in the pilot study

Outcome	Median (IQR)
BMI z-score	1.95 (0.50, 2.42)
StepWatch™ Activity Monitoring	
Inactive minutes/day	1160 (985, 1226)
Total steps/day	4308 (3108, 6928)
Six Minute Walk Test	
Six minute walk distance (m)	403 (290, 457)
DXA	
Fat mass (%)	27.6 (17.2, 40.8)
Lean mass (%)	69.9 (57.2, 80.1)
Peds QL (general)	
Total scale score	51 (40, 70)
Peds QL (neuromuscular)	
Total scale score	72 (60, 89)
ACTIVLIM survey	
Total score	29 (25, 32)
Patient measure (logits)	2.013 (0.963, 3.007)

Peds QL (core and neuromuscular) scores can range between 0 (lowest) and 100 (highest); ACTIVLIM total scores can range between 0 (lowest) and 36 (highest); ACTIVLIM patient measures can range between -6.869 logits (lowest) and 6.571 logits (highest).

5.5.2 Primary and secondary outcomes

An observational analysis of these data from eight children is presented in Table 5-3. As indicated by change in BMI z-scores, weight and growth remained stable throughout both the standard and enhanced supplementation phases. This provides important evidence that the tailored dietary prescription can achieve energy balance. Body composition results from DXA scans are equivocal and typical of the natural history of DMD in this age group. The data from the StepWatch™ activity monitoring and the 6MWT are suggestive of **greater functional improvement during the enhanced supplement regimen**. When comparing the difference of the change between the two supplement regimens, on average, boys were less inactive following the enhanced regimen (-32 minutes) and took more steps per day (1251 steps). Similarly, boys walked 25 metres further in the 6MWT following the enhanced supplement regimen in comparison to the standard supplement regimen. There were minimal changes in quality of life over the course of the study. No intervention-related adverse events were recorded, and liver function and electrolytes remained stable over the 50 weeks.

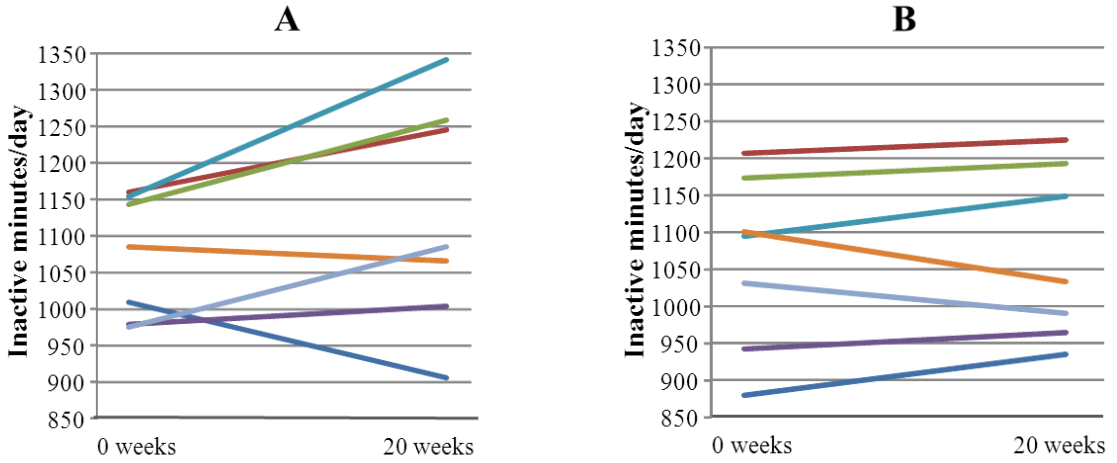
Individual responses in the functional outcome measures to the supplement regimens are illustrated in Figure 5-7. The variation in treatment response is evident in these graphs.

Table 5-3 Observational data comparing change in outcome measures during the eight week lead-in period, and the standard and enhanced supplement regimens

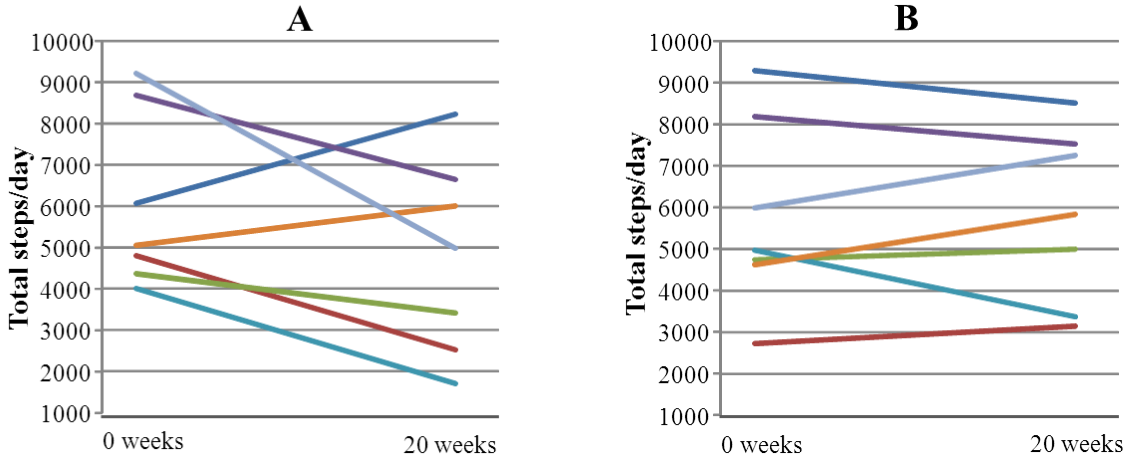
N=8	Week 0-8	Standard	Enhanced
BMI z-score change	-0.01 (-0.15, 0.08)	-0.02 (-0.05, 0.03)	0.05 (-0.13, 0.29)
StepWatch™ Activity Monitoring			
Change in inactive min/day	-3 (-36, 12)	86 (-19, 115)	20 (-41, 53)
Change in total steps/day	-306 (-467, 1482)	-2028 (-2301, 958)	263 (-773, 1202)
Six Minute Walk Test			
Change in Distance (m)	15.5 (-43, 46.5)	6 (-29, 18)	16 (-1, 55)
DXA [a]			
% fat change		0.9 (-0.2, 1.4)	2.0 (1.4, 2.8)
% lean change		-1.0 (-2.8, 0.0)	-2.0 (-3.0, -1.2)
Peds QL (general)			
Total scale score change	4 (-11, 9)	-1 (-7, 6)	2 (-1, 10)
Peds QL (neuromuscular)			
Total scale score change	-1 (-8, 6)	2 (-3, 7)	-3 (-6, 3)
ACTIVLIM survey			
Total score change	-3 (-5, 2)	-1 (-3, 2)	1 (-1, 3)
Change in patient measure (logits)	-0.084 (-1.213, 0.617)	0.027 (-0.401, 0.417)	0.166 (-0.158, 0.657)

Data presented as median (IQR); [a]DXA scans at week 0, 28, 50. Peds QL (core and neuromuscular) scores can range between 0 (lowest) and 100 (highest); ACTIVLIM total scores can range between 0 (lowest) and 36 (highest); ACTIVLIM patient measures can range between -6.869 logits (lowest) and 6.571 logits (highest).

StepWatch™ activity monitor – inactive time/day



StepWatch™ activity monitor – total steps/day



Six minute walk test – distance walked (m)

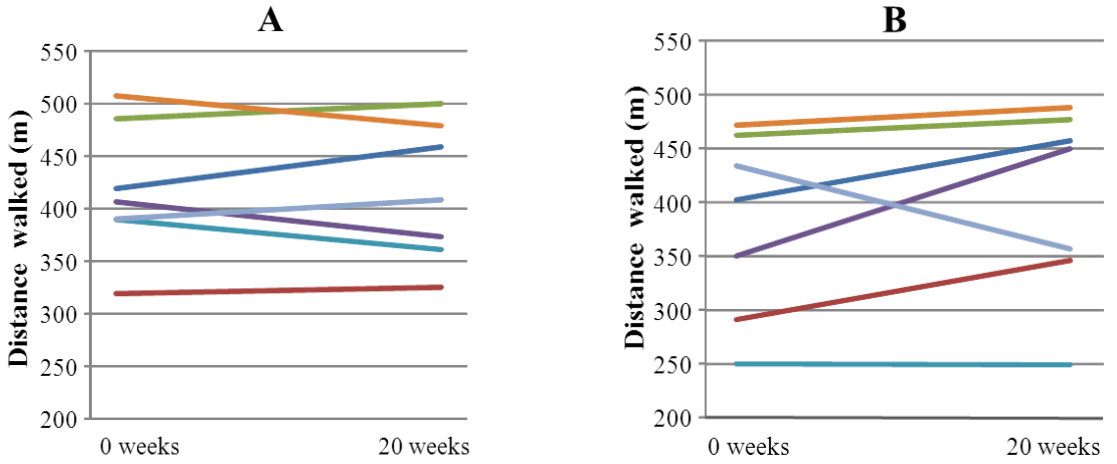


Figure 5-7 Individual responses in functional outcome measures

Diagrams A and B represent response to the standard and enhanced supplement regimens, respectively. The same colour is used for each participant in all graphs. Note: a decrease in inactive minutes/day measured by StepWatch™ activity monitoring is considered an improvement, whilst a decrease in the other two measures is considered a decline.

5.5.3 Response to vitamin D supplementation

Change in serum 25(OH)D concentrations across the 50 week intervention are summarised in Table 5-4. Supplementation commenced at week 8 with each participant receiving 2000IU/day. By week 28, median serum 25(OH)D had risen above 80 nmol/L, a level deemed optimal for bone health (319). This trend was significant ($p < 0.0005$, Friedman test). The dose of vitamin D chosen was thus effective in increasing serum 25(OH)D concentrations to an optimal level for improved bone health.

Table 5-4 Mean serum 25-hydroxyvitamin D concentrations at pre and post vitamin D supplementation

	Week 0	**Week 8**	**Week 28**	**Week 50**
25-hydroxyvitamin D (nmol/L)	48 (40, 69)	49 (45, 57)	85 (67, 95)	93 (77, 125)

Data presented at median (IQR)

5.5.4 Dietary intake

Energy intake as a proportion of estimated requirements varied between 76% and 83% over the course of the active intervention period; however there were no significant trends in energy intake data (Table 5-5). Changes in macronutrient and micronutrient intake are illustrated in Figure 5-8 and Figure 5-9, respectively. Following the introduction of the protein supplement, median proportion of energy contribution from protein increased from 19% to 23%; and the trend from week 0 to week 50 was significant (p=0.048). Similarly, when the multivitamin was introduced, significant trends in the median proportion of the recommended dietary intake or adequate intake achieved were evident for riboflavin (p=0.007), vitamin A (p=0.013), calcium (p=0.044), iodine (p=0.008), and vitamin E (p=0.008). An increasing trend for vitamin D intake was also observed (p=0.009) (data not shown).

Table 5-5 Energy intake over the course of the 50 week nutriceutical intervention

	Week 0	Week 8	Week 28	Week 30	Week 50
Energy intake (kJ)	6314 (6103, 7260)	5885 (5245, 6367)	6702 (6130, 8333)	7119 (6728, 7608)	6813 (6492, 8499)
Energy intake as a proportion of estimated energy requirements (%)	91 (68, 109)	76 (71, 86)	87 (62, 135)	81 (73, 120)	83 (77, 125)

Data presented as mean (IQR)

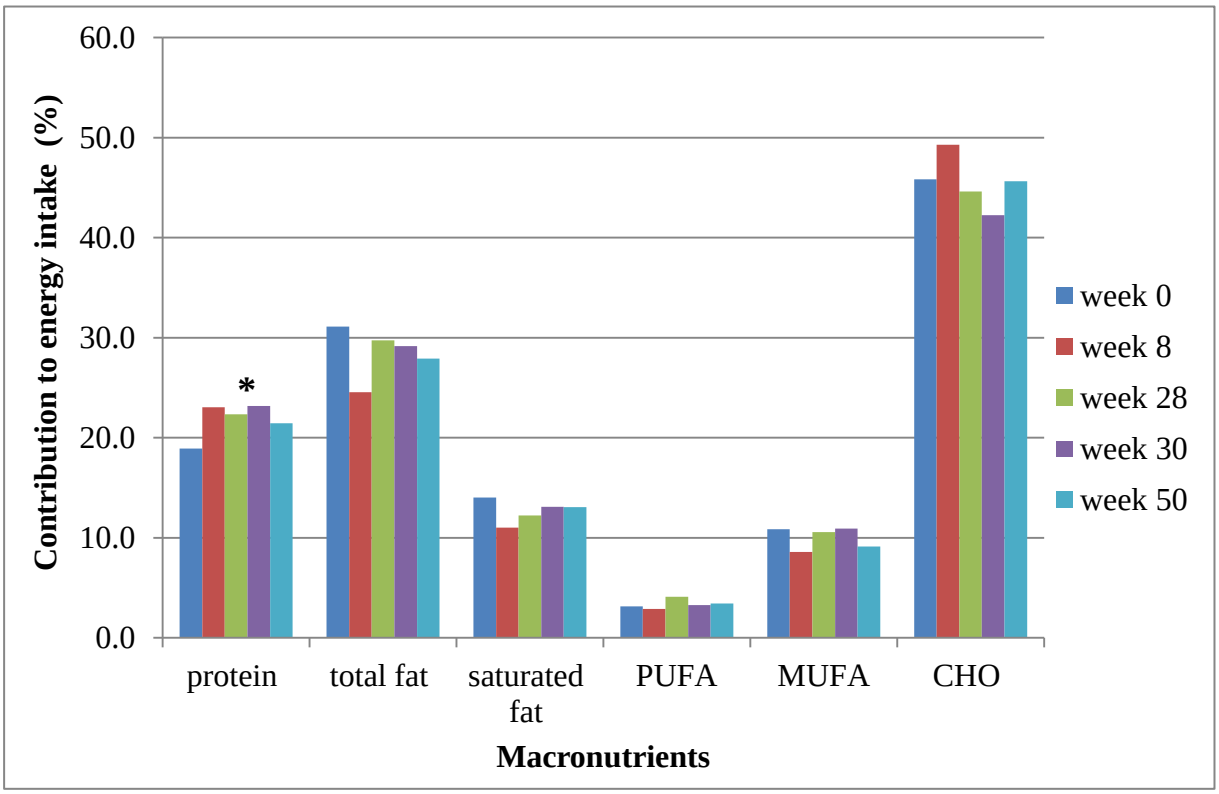


Figure 5-8 Median percentage contribution to energy intake by macronutrients across the 50 week nutriceutical intervention

PUFA polyunsaturated fatty acids; MUFA monounsaturated fatty acids; CHO carbohydrate; trend from week 0 to week 50 * $p<0.05$.

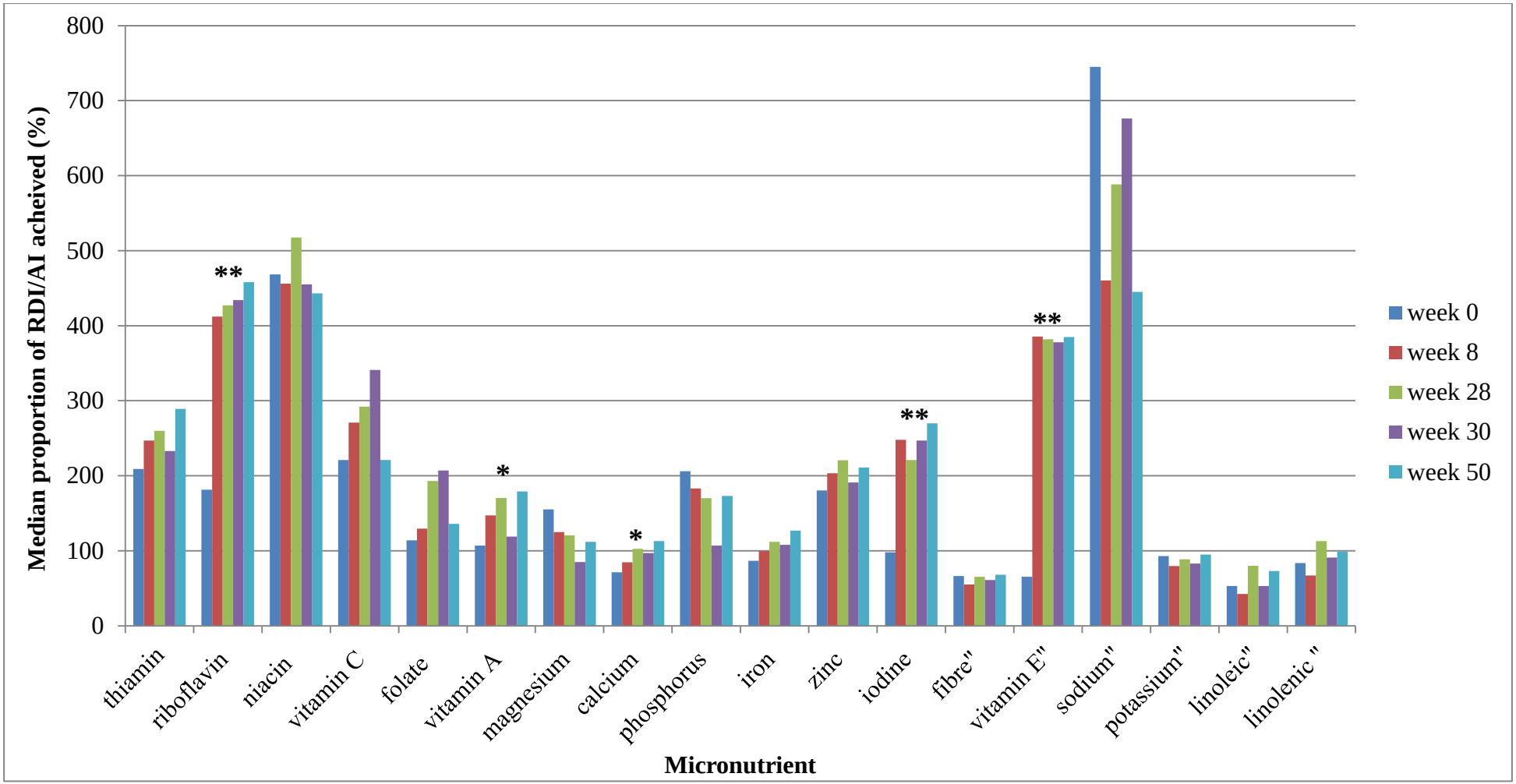


Figure 5-9 Median proportion of recommended dietary intake or adequate intake achieved across the 50 week nutriceutical intervention

" indicates nutrients with an adequate intake (AI); trend from week 0 to week 50 * $p<0.05$, ** $p<0.01$.

5.5.5 Participant feedback

Participant feedback is summarised in Table 5-6. The preference for the tablet regimen over the shakes was evident in both formal and informal feedback. Blinding was successful as participants did not know when they had received the active supplement. Six participants reported no detectable difference between the standard and enhanced regimen. Without revealing their treatment allocation, the two participants that reported a difference between the shakes indicated a preference for the standard shake.

Table 5-6 Participant feedback on supplement regimens

Supplement characteristics	Median (IQR)
Shake	
Taste	2 (1, 3)
Mouth feel	1.5 (1, 2)
Appearance	2 (1, 3)
Ease of taking daily	1.5 (1, 2)
Capsules	
Ease of taking daily	4.5 (3.3, 5)

A score of ‘1’ indicates dislike or difficulty; a score of ‘5’ indicates like or ease.

5.5.6 Compliance

Despite the low scores for the sensory characteristics, compliance for both the shake and capsule regimens was excellent. The calculated compliance and intake based on returned supplements is presented in Table 5-7. Compliance was observed to decrease in the second 20 week supplementation period.

Table 5-7 Compliance to supplement regimens

	Week 8 – Week 28	Week 30 – Week 50
Multivitamin		
% compliance	98 (92, 101)	93 (86, 96)
Vitamin D		
% compliance	100 (85, 101)	96 (91, 97)
Daily intake, vitamin D (IU)	1998 (1700, 2012)	1906 (1835, 1935)
Fruity Fishies		
% compliance	98 (84, 100)	94 (87, 98)
Daily intake, omega 3 triglycerides (mg)	1024 (879, 1048)	981 (908, 1034)
Shake		
% compliance	91 (80, 95)	85 (80, 89)
Daily intake, protein (g)	18.0 (15.9, 18.9)	17.0 (15.9, 17.9)
HMB/Placebo Capsules		
% compliance	98 (85, 101)	96 (89, 96)

Data presented at median (IQR)

5.6 Future research plans

These pilot data have led to the initiation of a multicentre trial involving a larger number of participants as required to fully test the hypothesis. A second recruitment site was opened via the neuromuscular clinic at MontroseAccess, Brisbane, Australia in October 2011. This clinic is staffed by visiting neurologists from Royal Children's Hospital and Mater Children's Hospital. Ethics approval was obtained from both organisations (# HREC/11/QRCH/2 and 1730, respectively, Appendix 25 and Appendix 26). Collaborative research group clinical trial agreements were prepared and executed by Monash University and Royal Children's Hospital, and Monash University and Mater Children's Hospital. The Therapeutic Goods Administration was notified of the additional trial site. The Brisbane site is funded by a grant from John T Reid Charitable Trust. A further 15 boys have been recruited (eight in Melbourne and seven in Brisbane). There have been no adverse events to date and no boys have dropped out of the expanded study. Additional funding has now been sought to support a third trial site based at The Children's Hospital at Westmead, Sydney, Australia.

The original protocol has been amended to incorporate a six month follow up assessment. Families will be invited back to participate and the following measures will be completed: step activity monitoring, 6MWT, DXA scan, height, weight, biochemistry (electrolytes, liver function tests, 25(OH)D), food diary, Peds QL (core and neuromuscular) questionnaires, and the ACTIVLIM survey. Information on continued supplement use will also be collected. Also, an additional eligibility criterion has been included for the multicentre trial. Only boys who are on a stable regimen of GCS will be eligible. This is to minimize the variation seen in the functional outcome measures.

5.6.1 Statistical analysis

Data analysis will involve tests of repeated measures to compare means within subjects. Parametric or non-parametric analysis will be used according to the distribution of the data. The primary outcome, change in inactive minutes/day as measured by StepWatch™, will be compared between the standard and enhanced supplements regimens. Multivariable regression analysis will be used to uncover influences that may be due to period (sequence), or treatment (GCS) effects and interactions. A method is described by Grizzle (320) to identify and account for a sequence effect. Similarly, Altman (321) discusses statistical analyses to identify period effects and treatment period interactions (sequence) effects. Analysis will incorporate these methods so to uncover influences that may not be attributed to treatment.

5.6.2 Sample size estimation based on pilot data results

G*Power software was used to calculate sample size (322). The study will report on a continuous response variable: change in inactive minutes/day. Data from the pilot study was used to inform power calculations, with an error probability (α) of 0.05. Specifically, the observed mean difference between the two supplement regimens was used together with the standard deviation of the change. The standard deviation of the change used in the calculation was 105 inactive minutes/day. With a sample size of 55, if the true difference in the mean response is 50 inactive minutes/day, we will be able to reject the null hypothesis that this response difference is zero with probability (power) of 0.97. The sample size calculation allows for 10% attrition, so that if only 50 participants complete the trial, the test will be able to detect a difference of 50 inactive minutes/day with a power of 95%. The study will be also adequately powered to detect the observed difference in total steps/day (Table 5-8).

The power for detecting observed differences in the secondary outcome measures was also calculated (Table 5-8). Additionally, the power to detect *desired* differences in the 6MWT, body composition (DXA), and quality of life (Peds QL questionnaires) was estimated.

Table 5-8 Estimated power for primary and secondary outcomes

Outcome	Change	Power at n = 55 (%)	Power at n = 50 (%)
Step Activity Monitoring			
Inactive minutes	50 mins	97%	95%
Total steps	1250 steps	97%	96%
Six Minute Walk Test			
	25m	85%	82%
	30m	94%	92%
DXA			
% Fat Mass	0.7%	88%	85%
	1.0%	99%	98%
% Lean Mass	0.5%	51%	47%
	1.0%	95%	93%
Peds QL			
Core	1.8 points	35%	33%
	5 points	97%	95%
Neuromuscular	2.8 points	89%	86%
	5 points	99%	99%
ACTIVLIM Survey			
Total Score	3 points	98%	96%
Patient measure	0.4 logits	95%	93%

Power calculations based on differences observed in the pilot study. Desired differences also calculated for the six minute walk test, body composition (DXA), and quality of life (Peds QL).

5.6.3 Timeline

Figure 5-10 details the projected study timeline by visit schedule for the multicentre study. The aim is for recruitment to be open across all sites by January 2013 for a twelve month period. Data management including resolving data queries and data cleaning will be performed on an ongoing basis throughout the trial period. Data analysis will commence when all participants have finished the 50 week protocol and will occur simultaneously with preparation of a manuscript for dissemination of results. It is estimated that analysis and manuscript preparation and dissemination will take 7 months.

	2013												2014												2015					
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J
Recrui-tment																														
Week 0 Visits																														
Week 8 Visits																														
Week 28 Visits																														
Week 30 Visits																														
Week 50 Visits																														
6 month post																														
Data manag-ement																														
Data analysis																														
Manusc-ript																														

Figure 5-10 Project timeline for nutriceuticals in DMD

5.7 Discussion

Internationally, this pilot study and the subsequent multicentre trial are the first to investigate the use of a multi-component nutritional supplement in boys with DMD. Although the pilot study was not powered appropriately to detect statistical differences, the trends in the data suggest that a nutriceutically enhanced regimen is well tolerated and may improve functional outcomes in boys with DMD making it worthwhile to conduct a fully powered randomised control trial. Inactive minutes/day were considerably lower following enhanced supplementation compared to standard supplementation (median (IQR), 20 (-41, 53) vs. 86 (-19, 115), respectively). Similarly, a median improvement of 16 (-1, 55) metres was observed in the 6MWT after enhanced supplementation. To provide a quantitative perspective on this response, longitudinal data from the 6MWT demonstrates that boys with DMD experience an average deterioration of between 26 and 57 metres in a year (255, 276). Similar data for the StepWatch™ is unknown. However, as StepWatch™ outcomes are highly correlated with the distance walked in a 6MWT (Chapter 4), one would expect a similar decline in these measures also. The multicentre trial has commenced to definitively test this hypothesis. The pilot study, however, provides proof of concept and evidence of feasibility of the trial protocol.

Feasibility was demonstrated by several outcomes from this pilot study. In the first instance, dietetic counselling provided to families achieved the desired dietary changes. Specifically, data from the food diaries demonstrated a significant increase in protein intake, in the context of stable energy intake. It is unclear at this stage which macronutrient was displaced by the increase in protein because there were no significant decreases in fat or carbohydrate; however, it is likely to be a combination of both. Positively, a significant increasing trend in calcium intake was also observed.

Despite some low scores regarding the shakes received from participant feedback, boys were exceptionally compliant to the supplement regimens. Compliance data is supported by the increase in 25(OH)D over the course of the intervention. Compliance declined in the second supplementation period, suggesting fatigue in consuming both the shakes and tablets daily. This time effect will be accounted for using multivariable regression analysis in the multicentre trial. Supplement fatigue could have influenced the participant feedback scores; the low scores received may have been a reflection of the monotony of the daily regimen as opposed to dislike of the supplement. Additional phone coaching will be provided to families in the multicentre trial for ongoing encouragement to assist compliance. Also, in further studies, consideration should be given to improving the sensory profile of the shake. Other supplementation trials in DMD cohorts have not provided information on compliance, a limitation of these studies (122, 123, 133); however, anecdotal evidence indicates that families living with DMD are particularly well motivated.

The blinding protocol was also successful in this pilot study. The study dietitian (ZD) and the patients were unaware of treatment allocations. Although two boys reported a difference between the regimens, they were unaware of which regimen they were receiving. These two boys had a higher lean body mass than the other children in the study, resulting in an increased dose of glutamine. Glutamine has a gritty texture which may have been detected at increasing doses. Future studies should consider introducing a maximal threshold for glutamine so that the gritty texture is avoided.

The dose of vitamin D (2000 IU) provided in this pilot study was appropriate to raise serum 25(OH)D above optimal concentrations. This supports the conclusions of Chapter 3, whereby it was proposed that 2000IU would be required to raise 25(OH)D levels in patients treated with GCS above 80nmol/L. However, the aetiology of decreased 25(OH)D levels in boys

with DMD remains unclear. A sequestering effect due to altered body composition is a possible explanation. Alternatively, as vitamin D supplementation is efficacious in increasing serum 25(OH)D concentrations, conversion in the liver of cholecalciferol to 25(OH)D may be disrupted (195). Serum transaminase levels are dramatically increased in boys with DMD due to the muscle breakdown (7). Whilst this is considered an innocuous phenomenon, little is known about the effect on liver function.

The mechanistic action of the nutriceutically enhanced supplement can only be hypothesised. Improvements in functional outcomes but not body composition were observed in this pilot study. Based on the ergogenic potential of the nutriceuticals, it could be postulated that the enhanced supplement may not slow the progression of the disease but rather improve the energy potential of the remaining muscle. The current study design is unable to separate the effects of individual supplements. Yet, to separate the individual elements would most likely be inappropriate because studies in the mouse model suggest a synergistic mechanism of multiple supplements with GCS therapy (125, 307). Using the mouse model to understand the mechanistic action and synergism of the multi-component regimen could assist in the development of an improved and more efficient supplement.

The study design used in the pilot study was scientifically robust - a double blinded, randomised, controlled, cross-over design. The advantages of the cross-over study design make it particularly suitable for use within the DMD population. The principal advantage of this design is efficiency; a smaller number of patients is required to achieve similar statistical power when compared to a parallel design (133, 323, 324). Given the heterogeneity in treatment response observed here, within subject comparisons enables the cross-over design to account for these variations (323, 324). Also, the design permits us to offer “active”

treatment to all those who enrol into the study rather than withholding treatment for six months in a control group.

An untreated control group was not incorporated into the design for a specific reason: to act as a true control all other nutritional therapies would need to be withdrawn for six months. Many families and caregivers provide their sons with a variety of nutritional supplements with and without proven efficacy. It would be unethical to withdraw these supplements for the period of the trial without provision of an alternative. Thus, standard care was provided through the use of a standard nutritional supplement. Multivitamins, fish oil and vitamin D are key ingredients for optimising nutrition within the general population. The literature on nutrition in DMD is limited such that even the use of a standard supplement has not been investigated. The design offers the advantage of consenting participants to explore the use of a standard supplement as well as the use of a standard supplement with CrM, glutamine and HMB.

Clinical sources of confounding within cross-over designs include: residual (carry over) effects; failure to return subjects to baseline state; non-uniform changes in patients over time; and time dependent response measures (323). Due to the nature of disease progression in DMD, the assumptions of returning subjects to baseline and uniform changes in patients cannot be met. However, it can be argued that boys with DMD are in a steady state of decline, and do not go through periods of instability as for example, a newly diagnosed child with epilepsy. To counterbalance these effects, the use of baseline measures is recommended (323). Accordingly, a two month lead in period was included in the study design as a control period. Similarly, a six month follow up in the multicentre trial will provide a post supplementation comparison.

Developing appropriate designs for investigating therapeutics in neuromuscular disorders is particularly challenging. These difficulties are discussed in depth by Conwit et al.(325). Most relevant to this study, is the need for innovative randomised controlled trial design with sufficient power that can be completed in a *timely* fashion. The large variation in individual response is evident in Figure 5-7. This heterogeneity is typical in Duchenne and difficult to control in trials, as demonstrated in the recent Phase 2b trial of ataluren (PTC124®), an orally administered drug that promotes ribosomal read-through of nonsense mutations. An improvement in the 6MWT of 29.7m was observed; however, the trial was halted as there was insufficient power to detect a statistical difference (p=0.058) (51). Associated power calculations based on pilot data here necessitate a third site to complete recruitment.

Boys with DMD are a neglected group with few resources being directed to improving their quality of life whilst curative research avenues such as gene therapies are explored. Nutriceuticals may provide a cost-effective alternative which could assist in preventing or delaying complications and enable boys with DMD to remain ambulatory for longer. Whilst it is acknowledged that this is not a cure, it could delay the need for the use of a wheelchair, preserving muscle strength and function required for activities of daily living. Such benefits would also assist to reduce the very high cost of muscular dystrophies. The annual cost of muscular dystrophy is $126,000 per person, and the value of lost well-being (disability and premature death) adds a further $1 billion (326).

The results of the multicentre study will translate into more structured guidelines regarding nutrition and the use of supplements for the Duchenne community, potentially saving families from great expense spent on unproven remedies. Recently published care guidelines for the management of boys with DMD were unable to make recommendations on the use of

supplements due to an absence of data (3). The panel involved in the review process identified nutritional supplements as an area in which additional research is required.

If shown to be of benefit in the final fully powered study, it is envisaged the enhanced nutritional supplement will be combined into a single milk beverage and be made available to families through the pharmaceutical benefits scheme. The enhanced supplement regimen also has potential to provide benefits to those with other common neuromuscular disorders such as BMD or spinal muscular atrophy. The functional muscle benefits that may be associated with the enhanced supplement would also be beneficial to an ageing population in which sarcopaenia is an emerging issue and preserving muscle function and strength a clear target area.

5.8 Summary

Nutriceuticals are a supportive intervention that may work synergistically with standard therapy to assist with walking ability in boys with DMD. This pilot study indicates that the 50 week intervention is feasible, with excellent compliance to the supplement regimens. The most exciting data is the improvement in functional outcome measures following the nutriceutically enhanced supplement. A multicentre trial has commenced and will provide conclusive evidence as to the benefits of nutriceuticals for boys with DMD.

6 Future Directions

The past ten years has seen major advances in treatment options for boys with DMD. Phase 2 trials of gene-related pharmaceutical therapies are looking very promising; and whilst the possibility of a 'cure' provides hope for many, the eradication of Duchenne is many years way. It is likely to be several years before current therapies under investigation can be used routinely in a clinical setting. The development of such high level pharmaceuticals requires rigorous testing and lengthy regulatory processes. Such therapies will have limited reach because they are targeted at specific mutations. Also, these pharmaceuticals will not 'cure' Duchenne. Instead, they will be able to attenuate disease progression so that a milder phenotype is present, similar to that of BMD. As health professionals we need to be cognisant of these issues because these children will still require ongoing, specialised, medical and supportive management, regardless of whether they are receiving these new therapies.

In order to provide the best care for boys with DMD, evidence based clinical guidelines that are standardised across centres would assist greatly. In 2009, detailed practice guidelines were released. This document was lacking depth in the area of nutritional management; and ideally, stand-alone guidelines for nutritional management should be developed. As outlined in Chapter 1, there is limited high quality evidence to guide practice. This thesis progresses the evidence in a move towards clinical guidelines, specifically in the areas of nutriceuticals, vitamin D and understanding growth patterns. This work also contributes to clinical understanding of DMD by identifying genotype-phenotype relationships. Despite this growing body of evidence, there are still many knowledge gaps that need to be addressed before nutritional guidelines are a reality.

Understanding energy metabolism in DMD is fundamental to provision of appropriate nutrition counselling, yet the relationship between energy expenditure and body composition is still very much unclear. Research by Ms Sarah Elliott in collaboration with this author and

Prof Helen Truby has seen significant advances in understanding energy metabolism, with the first measures of TEE being taken in boys with DMD. Ongoing recruitment and measurement of TEE in boys with DMD is occurring concurrently with the trial of nutriceuticals, and we hope to expand our sample size to be able to make meaningful conclusions. The multicentre trial investigating the use of nutriceuticals will also expand the evidence in this area. Several funding applications have been submitted, which if successful will enable us to initiate a third trial site to facilitate timely recruitment. The minor amends to the trial protocol have improved its rigour and the outcome of the nutriceutical study will be sufficiently conclusive as to the use of supplements for boys with DMD to inform practice.

The other concept that warrants further consideration is that of weight management in boys and young males with DMD. However, before this concept is fully explored, the effects of weight gain on mobility should be more thoroughly investigated. In the studies presented here, BMI was not associated with loss of mobility or walking function. Although it is frequently purported that excess weight increases the burden on already weak musculature; there is no evidence to support this statement. There also needs to more investigation into the effect of weight status on prognostic factors including cardiac and lung function. Similarly, the effect of excess weight on psychosocial factors should be explored. Once, and perhaps not before these questions are answered, we should focus our efforts on creating effective weight management solutions for these boys.

To fill these gaps in dietetic knowledge, and build the evidence base to such a point that clinical guidelines are a realistic notion, a prospective longitudinal study of growth is warranted. This study would measure energy expenditure (total and resting) and body composition (DXA) over several years. Basic anthropometric measures, as well as clinical outcomes, would also be collected including functional measures (6MWT), age at loss of

ambulation, genetic mutation, respiratory and cardiac function, and biochemical indices related to bone metabolism. Assessments could be incorporated into routine clinical visits to ease subject burden. Several centres would need to be involved, and for efficiency, the study could include all neuromuscular conditions. Collaboration between sites is key to achieving successful research outcomes in neuromuscular disorders. Small numbers of eligible participants drives collaborative research bringing people together to achieve necessary sample sizes. The longitudinal nature of such a study would make it unique, as only one study to date has prospectively documented growth, and this was before the introduction of GCS as standard treatment.

Obviously this type of study would require significant funding and effort. Here in lies the problem. Funding would be required from a large competitive grant scheme, such as a National Health and Medical Research Council project grant. Alternatively, some funding support could be obtained through patient advocacy groups. In both instances, it is likely that this study would be in competition with genetic therapies, making success in such funding rounds difficult. The issue of timely development of treatment options in DMD is so important and the writer does not begrudge these decisions. Unfortunately, development of supportive therapies, such as understanding growth and how best to manage it, will take time. However, supportive interventions will ultimately improve quality of life for boys living with DMD whilst treatment options are investigated. Awareness about the role of supportive interventions in neuromuscular conditions needs to be increased to enable these therapies to progress and improve.

The majority of work presented in this thesis would not have been possible, if it were not for some ‘enablers’: forward thinking physicians who value supportive therapies and interventions. Despite this support, nutrition and dietetics tends to be disregarded, and its

importance against medical management and physiotherapy is frequently overlooked. Boys with DMD are now living well into adulthood, studying at university, and having families. The full spectrum of supportive therapies should be emphasised and research and development in these areas needs to progress concurrently with genetic research to enable young men with DMD to reach their full potential. This includes nutrition and dietetics, but also nursing, occupational therapy, social work and psychology, and speech therapy. Allied health professionals need to collaborate to progress research, and to be acknowledged as a group of neuromuscular professionals. To do this, international interests groups need to be initiated within but also across disciplines.

This research does not stop with the completion of the thesis. The post-doctoral goals of the candidate are to see the nutriceuticals trial through to completion and to initiate a multicentre longitudinal study to monitor growth. The other ambition on conclusion of this thesis is to unify dietetic professionals working in neuromuscular disorders. In Australia, the time is right to move with these ideas. Recently, the Australasian Neuromuscular Network was established, and in line with this network, a virtual centre of research excellence was funded incorporating sites at Melbourne, Sydney and Perth. These collaborations have a united goal of improving clinical care of children with chronic neuromuscular disorders through well-designed collaborative multidisciplinary clinical research. This is an ideal platform to support the ongoing nutriceutical trial and also commencement of a longitudinal growth study.

It is anticipated that the culmination of this thesis and the proposed post-doctoral research will advance the nutritional evidence to such an extent that evidence based guidelines for nutritional management in DMD can be developed; and that high quality dietetic care is standard for all boys living with Duchenne.

7 References

1. Phillips B, Ball C, Sackett D, Badenoch D, Straus S, Haynes B, et al. Oxford Centre for Evidence-based Medicine Levels of Evidence [Web Page]. 1998 [cited 2012 January 10]; available from: http://www.cebm.net/index.aspx?o=1025.

2. Emery AE. Population frequencies of inherited neuromuscular diseases-a world survey. Neuromuscul Disord. 1991;1(1):19-29.

3. Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol. 2010;9(1):77-93.

4. Strober JB. Genetics of pediatric neuromuscular disease. Current Opinion in Pediatrics. 2000;12(6):549-53.

5. Blake DJ, Weir A, Newey SE, Davies KE. Function and genetics of dystrophin and dystrophin-related proteins in muscle. Physiol Rev. 2002;82(2):291-329.

6. Yiu EM, Kornberg AJ. Duchenne muscular dystrophy. Neurol India. 2008;56(3):236-47.

7. McMillan HJ, Campbell C, Mah JK, Canadian Paediatric Neuromuscular G. Duchenne muscular dystrophy: Canadian paediatric neuromuscular physicians survey. Can J Neurol Sci. 2010;37(2):195-205.

8. Bennett R, den Dunnen J, O'Brien K, Darras B, Kunkel L. Detection of mutations in the dystrophin gene via automated DHPLC screening and direct sequencing. BMC Genet. 2001;2(1):17.

9. Bushby KMD, Hill A, Steele JG. Failure of early diagnosis in symptomatic Duchenne muscular dystrophy. Lancet. 1999;353(9152):557-8.

10. Ciafaloni E, Fox DJ, Pandya S, Westfield CP, Puzhankara S, Romitti PA, et al. Delayed diagnosis in duchenne muscular dystrophy: data from the Muscular Dystrophy Surveillance, Tracking, and Research Network (MD STARnet). J Pediatr. 2009;155(3):380-5.

11. Pichavant C, Aartsma-Rus A, Clemens PR, Davies KE, Dickson G, Takeda Si, et al. Current status of pharmaceutical and genetic therapeutic approaches to treat DMD. Mol Ther. 2011;19(5):830-40.

12. Bartels B, Pangalila RF, Bergen MP, Cobben NAM, Stam HJ, Roebroeck ME. Upper limb function in adults with Duchenne muscular dystrophy. J Rehabil Med. 2011;43(9):770-5.

13. Roberto R, Fritz A, Hagar Y, Boice B, Skalsky A, Hwang H, et al. The natural history of cardiac and pulmonary function decline in patients with duchenne muscular dystrophy. Spine. 2011;36(15):1009-17.

14. Kinali M, Messina S, Mercuri E, Lehovsky J, Edge G, Manzur AY, et al. Management of scoliosis in Duchenne muscular dystrophy: a large 10-year retrospective study. Dev Med Child Neurol. 2006;48(6):513-8.

15. Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. Lancet Neurol. 2010;9(2):177-89.

16. Rahbek J, Werge B, Madsen A, Marquardt J, Steffensen BF, Jeppesen J. Adult life with Duchenne muscular dystrophy: Observations among an emerging and unforeseen patient population. Pediatr Rehabil. 2005;8(1):17-28.

17. Bach JR, Decicco A. Forty-eight years with duchenne muscular dystrophy. Am J Phys Med Rehabil. 2011;90(10):868-70.

18. Barohn RJ, Levine EJ, Olson JO, Mendell JR. Gastric hypomotility in Duchenne's muscular dystrophy. N Engl J Med. 1988;319(1):15-8.

19. Borrelli O, Salvia G, Mancini V, Santoro L, Tagliente F, Romeo EF, et al. Evolution of Gastric Electrical Features and Gastric Emptying in Children with Duchenne and Becker Muscular Dystrophy. Am J Gastroenterol. 2005;100(3):695-702.

20. Backhouse M, Harding L. Do Males Diagnosed with Duchenne Muscular Dystrophy (DMD) Unnecessarily Suffer Bowel and Bladder Problems? The Australian and New Zealand Continence Journal. 2006;12(3):66-73.

21. Bensen ES, Jaffe KM, Tarr PI. Acute gastric dilatation in Duchenne muscular dystrophy: a case report and review of the literature. Arch Phys Med Rehabil. 1996;77(5):512-4.

22. Leon SH, Schuffler MD, Kettler M, Rohrmann CA. Chronic intestinal pseudoobstruction as a complication of Duchenne's muscular dystrophy. Gastroenterology. 1986;90(2):455-9.

23. Jaffe KM, McDonald CM, Ingman E, Haas J. Symptoms of upper gastrointestinal dysfunction in Duchenne muscular dystrophy: case-control study. Arch Phys Med Rehabil. 1990;71(10):742-4.

24. Pane M, Vasta I, Messina S, Sorleti D, Aloysius A, Sciarra F, et al. Feeding problems and weight gain in Duchenne muscular dystrophy. Eur J Paediatr Neurol. 2006;10(5-6):231-6.

25. Ghafari J, Clark RE, Shofer FS, Berman PH. Dental and occlusal characteristics of children with neuromuscular disease. Am J Orthod Dentofacial Orthop. 1988;93(2):126-32.

26. Berardinelli A, Gorni K, Orcesi S, Tagliabue A, Uggetti C, Lanzi G. Dystrophinopathies: peculiar clinical and laboratory aspects. Funct Neurol. 2001;16 (Suppl):255-62.

27. Fabbro F, Marini A, Felisari G, Comi GP, D'Angelo MG, Turconi AC, et al. Language disturbances in a group of participants suffering from Duchenne muscular dystrophy: a pilot study. Percept Mot Skills. 2007;104(2):663-76.

28. Hinton VJ, De Vivo DC, Nereo NE, Goldstein E, Stern Y. Poor verbal working memory across intellectual level in boys with Duchenne dystrophy. Neurology. 2000;54(11):2127-32.

29. Cyrulnik SE, Fee RJ, Batchelder A, Kiefel J, Goldstein E, Hinton VJ. Cognitive and adaptive deficits in young children with Duchenne muscular dystrophy (DMD). J Int Neuropsychol Soc. 2008;14(5):853-61.

30. Donders J, Taneja C. Neurobehavioral characteristics of children with Duchenne muscular dystrophy. Child Neuropsychol. 2009;15(3):295-304.

31. Hendriksen JGM, Vles JSH. Neuropsychiatric disorders in males with duchenne muscular dystrophy: frequency rate of attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder, and obsessive-compulsive disorder. J Child Neurol. 2008;23(5):477-81.

32. Mochizuki H, Miyatake S, Suzuki M, Shigeyama T, Yatabe K, Ogata K, et al. Mental retardation and lifetime events of Duchenne muscular dystrophy in Japan. Intern Med. 2008;47(13):1207-10.

33. Abbs S, Tuffery-Giraud S, Bakker E, Ferlini A, Sejersen T, Mueller CR. Best practice guidelines on molecular diagnostics in Duchenne/Becker muscular dystrophies. Neuromuscul Disord. 2010;20(6):422-7.

34. Birnkrant DJ, Bushby KMD, Amin RS, Bach JR, Benditt JO, Eagle M, et al. The respiratory management of patients with duchenne muscular dystrophy: a DMD care considerations working group specialty article. Pediatr Pulmonol. 2010;45(8):739-48.

35. Bianchi ML, Biggar D, Bushby K, Rogol AD, Rutter MM, Tseng B. Endocrine aspects of Duchenne muscular dystrophy. Neuromuscul Disord. 2011;21(4):298-303.

36. Quinlivan R, Shaw N, Bushby K. 170th ENMC International Workshop: bone protection for corticosteroid treated Duchenne muscular dystrophy. 27-29 November 2009, Naarden, The Netherlands. Neuromuscul Disord. 2010;20(11):761-9.

37. Manzur AY, Kuntzer T, Pike M, Swan A. Glucocorticoid corticosteroids for Duchenne muscular dystrophy. Cochrane Database Syst Rev. 2008: (1) CD003725.

38. Moxley RT, 3rd, Ashwal S, Pandya S, Connolly A, Florence J, Mathews K, et al. Practice Parameter: Corticosteroid treatment of Duchenne dystrophy: Report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology. 2005;64:13-20.

39. Biggar WD, Harris VA, Eliasoph L, Alman B. Long-term benefits of deflazacort treatment for boys with Duchenne muscular dystrophy in their second decade. Neuromuscul Disord. 2006;16:249-55.

40. Lynch GS. Therapies for improving muscle function in neuromuscular disorders. Exerc Sport Sci Rev. 2001;29(4):141-8.

41. Yilmaz O, Karaduman A, Topaloglu H. Prednisolone therapy in Duchenne muscular dystrophy prolongs ambulation and prevents scoliosis. Eur J Neurol. 2004;11(8):541-4.

42. Balaban B, Matthews DJ, Clayton GH, Carry T. Corticosteroid treatment and functional improvement in Duchenne muscular dystrophy: long-term effect. Am J Phys Med Rehabil. 2005;84(11):843-50.

43. Daftary AS, Crisanti M, Kalra M, Wong B, Amin R. Effect of Long-term Steroids on Cough Efficiency and Respiratory Muscle Strength in Patients With Duchenne Muscular Dystrophy. Pediatrics. 2007;119(2):320-4.

44. Pradhan S, Ghosh D, Srivastava NK, Kumar A, Mittal B, Pandey CM, et al. Prednisolone in Duchenne muscular dystrophy with imminent loss of ambulation. J Neurol. 2006;253(10):1309-16.

45. Strober JB. Therapeutics in Duchenne Muscular Dystrophy. NeuroRx. 2006;3(2):225-34.

46. Sorva RA, Turpeinen MT. Asthma, glucocorticoids and growth. Ann Med. 1994;26(4):309-14.

47. Angelini C. The role of corticosteroids in muscular dystrophy: A critical appraisal. Muscle Nerve. 2007;36(4):424-35.

48. Matthews DJ, James KA, Miller LA, Pandya S, Campbell KA, Ciafaloni E, et al. Use of corticosteroids in a population-based cohort of boys with duchenne and becker muscular dystrophy. J Child Neurol. 2010;25(11):1319-24.

49. Moxley RT, 3rd, Pandya S, Ciafaloni E, Fox DJ, Campbell K. Change in natural history of Duchenne muscular dystrophy with long-term corticosteroid treatment: implications for management. J Child Neurol. 2010;25(9):1116-29.

50. Kilmer DD. The role of exercise in neuromuscular disease. Phys Med Rehabil Clin N Am. 1998;9(1):115-25.

51. PTC Therapeutics. Pivotal data presented at the world muscle society congress suggest ataluren slows the loss of walking ability in patients with nonsense mutation duchenne/becker muscular dystrophy [Web Page]. 2010 [cited 2012 January 19]; available from: http://ptct.client.shareholder.com/releasedetail.cfm?ReleaseID=518941.

52. PTC Therapeutics. Ataluren Update [Web Page]. 2011 [cited 2012 January 19]; available from: http://www.muscular-dystrophy.org/assets/0003/1015/2011-11-29_ataluren_update_d.pdf.

53. Aartsma-Rus A, Fokkema I, Verschuuren J, Ginjaar I, van Deutekom J, van Ommen G-J, et al. Theoretic applicability of antisense-mediated exon skipping for Duchenne muscular dystrophy mutations. Hum Mutat. 2009;30(3):293-9.

54. Cirak S, Arechavala-Gomeza V, Guglieri M, Feng L, Torelli S, Anthony K, et al. Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study. Lancet. 2011;378(9791):595-605.

55. Goemans NM, Tulinius M, van den Akker JT, Burm BE, Ekhart PF, Heuvelmans N, et al. Systemic Administration of PRO051 in Duchenne's Muscular Dystrophy. N Engl J Med. 2011;364(16):1513-22.

56. Prosensa. GSK and Prosensa announce start of Phase III study of investigational Duchenne Muscular Dystrophy medication [Web Page]. 2011 [cited 2012 January 18]; available from: http://www.prosensa.eu/press-release/gsk-and-prosensa-announce-start-phase-iii-study-investigational-dmd-medication.

57. Mendell JR, Campbell K, Rodino-Klapac L, Sahenk Z, Shilling C, Lewis S, et al. Dystrophin immunity in Duchenne's muscular dystrophy. N Engl J Med. 2010;363(15):1429-37.

58. Bodamer OAF, Hoffmann GF, Visser GH, Janecke A, Linderkamp O, Leonard JV, et al. Assessment of energy expenditure in metabolic disorders. Eur J Pediatr. 1997;156(1):S24-S8.

59. Okada K, Manabe S, Sakamoto S, Ohnaka M, Niiyama Y. Protein and energy metabolism in patients with progressive muscular dystrophy. J Nutr Sci Vitaminol. 1992;38(2):141-54.

60. Gonzalez-Bermejo J, Lofaso F, Falaize L, Lejaille M, Raphael JC, Similowski T, et al. Resting energy expenditure in Duchenne patients using home mechanical ventilation. Eur Respir J. 2005;25(4):682-7.

61. Shimizu-Fujiwara M, Komaki H, Nakagawa E, Mori-Yoshimura M, Oya Y, Fujisaki T, et al. Decreased resting energy expenditure in patients with Duchenne muscular dystrophy. Brain Dev. 2011;34(3):206-12.

62. Elliott SA, Davidson ZE, Davies PSW, Truby H. Predicting resting energy expenditure in boys with Duchenne muscular dystrophy. Eur J Paediatr Neurol 2012;(Epub ahead of print).

63. Department of Health. Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. London: The Stationery Office; 1991.

64. Almond S, Allott L, Hall K. Feeding Children with Neurodisabilities. In: Shaw V, Lawson M, editors. Clinical Paediatric Dietetics. third ed. Carlton, Victoria: Blackwell Publishing; 2007. p. 566-87.

65. Canalis E, Bilezikian JP, Angeli A, Giustina A, Canalis E, Bilezikian JP, et al. Perspectives on glucocorticoid-induced osteoporosis. Bone. 2004;34(4):593-8.

66. Söderpalm AC, Magnusson P, Ahlander AC, Karlsson J, Kroksmark AK, Tulinius M, et al. Low bone mineral density and decreased bone turnover in Duchenne muscular dystrophy. Neuromuscul Disord. 2007;17(11-12):919-28.

67. Aparicio LF, Jurkovic M, DeLullo J, Aparicio LF, Jurkovic M, DeLullo J. Decreased bone density in ambulatory patients with duchenne muscular dystrophy. J Pediatr Orthop. 2002;22(2):179-81.

68. Douvillez B, Braillon P, Hodgkinson I, Berard C. Pain, ostropenia and body composition of 22 patients with Duchenne muscular dystrophy: a descriptive study. Ann Readapt Med Phys. 2005;48:616-22.

69. Larson CM, Henderson RC. Bone mineral density and fractures in boys with Duchenne muscular dystrophy. J Pediatr Orthop. 2000;20(1):71-4.

70. Vestergaard P, Glerup H, Steffensen BF, Rejnmark L, Rahbek J, Moseklide L. Fracture risk in patients with muscular dystrophy and spinal muscular atrophy. J Rehabil Med. 2001;33(4):150-5.

71. Hankard R, Gottrand F, Turck D, Carpentier A, Romon M, Farriaux JP. Resting energy expenditure and energy substrate utilization in children with Duchenne muscular dystrophy. Pediatr Res. 1996;40(1):29-33.

72. Satomura S, Yokota I, Tatara K, Naito E, Ito M, Kuroda Y. Paradoxical weight loss with extra energy expenditure at brown adipose tissue in adolescent patients with Duchenne muscular dystrophy. Metabolism. 2001;50(10):1181-5.

73. Zanardi MC, Tagliabue A, Orcesi S, Berardinelli A, Uggetti C, Pichiecchio A. Body composition and energy expenditure in Duchenne muscular dystrophy. Eur J Clin Nutr. 2003;57(2):273-8.

74. Hogan SE. Body composition and resting energy expenditure of individuals with Duchenne and Becker muscular dystrophy. Can J Diet Pract Res. 2008;69(4):208-12.

75. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M, Drug, et al. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics. 2008;122(2):398-417.

76. Bianchi ML, Mazzanti A, Galbiati E, Saraifoger S, Dubini A, Cornelio F, et al. Bone mineral density and bone metabolism in Duchenne muscular dystrophy. Osteoporos Int. 2003;14(9):761-7.

77. Bianchi ML, Morandi L, Andreucci E, Vai S, Frasunkiewicz J, Cottafava R. Low bone density and bone metabolism alterations in Duchenne muscular dystrophy: response to calcium and vitamin D treatment. Osteoporos Int. 2011;22(2):529-39.

78. Eiholzer U, Boltshauser E, Frey D, Molinari L, Zachmann M. Short stature: a common feature in Duchenne muscular dystrophy. Eur J Pediatr. 1988;147(6):602-5.

79. Nagel BHP, Mortier W, Elmlinger M, Wollmann HA, Schmitt K, Ranke MB. Short stature in Duchenne muscular dystrophy: a study of 34 patients. Acta Paediatr. 1999;88(1):62-5.

80. Willig TN, Carlier L, Legrand M, Riviere H, Navarro J. Nutritional assessment in Duchenne muscular dystrophy. Dev Med Child Neurol. 1993;35(12):1074-82.

81. McDonald CM, Abresch RT, Carter GT, Fowler WM, Jr., Johnson ER, Kilmer DD, et al. Profiles of neuromuscular diseases. Duchenne muscular dystrophy. Am J Phys Med Rehabil. 1995;74(5):S70-92.

82. Stewart L, Mckaig N, Dunlop C, Daly H, Almond S. Guidelines on dietetic assessment and monitoring of children with special needs and faltering growth. Birmingham: British Dietetic Association; 2006.

83. Martigne L, Salleron J, Mayer M, Cuisset J-M, Carpentier A, Neve V, et al. Natural evolution of weight status in Duchenne muscular dystrophy: a retrospective audit. Br J Nutr. 2011;105(10):1486-91.

84. Leroy-Willig A, Willig TN, Henry-Feugeas MC, Frouin V, Marinier E, Boulier A, et al. Body composition determined with MR in patients with Duchenne muscular dystrophy, spinal muscular atrophy, and normal subjects. Magn Reson Imaging. 1997;15(7):737-44.

85. Pichiecchio A, Uggetti C, Egitto MG, Berardinelli A, Orcesi S, Gorni KOT, et al. Quantitative MR evaluation of body composition in patients with Duchenne muscular dystrophy. Eur Radiol. 2002;12(11):2704-9.

86. Mok E, Beghin L, Gachon P, Daubrosse C, Fontan J-E, Cuisset J-M, et al. Estimating body composition in children with Duchenne muscular dystrophy: comparison of bioelectrical impedance analysis and skinfold-thickness measurement. Am J Clin Nutr. 2006;83(1):65-9.

87. Mok E, Letellier G, Cuisset J-M, Denjean A, Gottrand F, Hankard R. Assessing change in body composition in children with Duchenne muscular dystrophy: anthropometry and bioelectrical impedance analysis versus dual-energy X-ray absorptiometry. Clin Nutr. 2010;29(5):633-8.

88. Palmieri GM, Bertorini TE, Griffin JW, Igarashi M, Karas JG. Assessment of whole body composition with dual energy x-ray absorptiometry in Duchenne muscular dystrophy: correlation of lean body mass with muscle function. Muscle Nerve. 1996;19(6):777-9.

89. Skalsky AJ, Han JJ, Abresch RT, Shin CS, McDonald CM. Assessment of regional body composition with dual-energy X-ray absorptiometry in Duchenne muscular dystrophy: correlation of regional lean mass and quantitative strength. Muscle Nerve. 2009;39(5):647-51.

90. McDonald CM, Carter GT, Abresch RT, Widman L, Styne DM, Warden N, et al. Body composition and water compartment measurements in boys with Duchenne muscular dystrophy. Am J Phys Med Rehabil. 2005;84(7):483-91.

91. Griggs RC, Forbes G, Moxley RT, Herr BE. The assessment of muscle mass in progressive neuromuscular disease. Neurology. 1983;33(2):158-65.

92. Edmonds CJ, Smith T, Griffiths RD, Mackenzie J, Edwards RH. Total body potassium and water, and exchangeable sodium in muscular dystrophy. Clin Sci. 1985;68(4):379-85.

93. Huang Y, Majumdar S, Genant HK, Chan WP, Sharma KR, Yu P, et al. Quantitative MR relaxometry study of muscle composition and function in duchenne muscular dystrophy. J Magn Reson Imaging. 1994;4(1):59-64.

94. Gong QY, Phoenix J, Kemp GJ, Garcia-Finana M, Frostick SP, Brodie DA, et al. Estimation of body composition in muscular dystrophy by MRI and stereology. J Magn Reson Imaging. 2000;12(3):467-75.

95. Franciotta D, Zanardi MC, Albertotti L, Orcesi S, Berardinelli A, Pichiecchio A, et al. Measurement of skeletal muscle mass in Duchenne muscular dystrophy: use of 24-h creatinine excretion. Acta Diabetologia. 2003;40:S290-2.

96. Pessolano FA, Suarez AA, Monteiro SG, Mesa L, Dubrovsky A, Roncoroni AJ, et al. Nutritional assessment of patients with neuromuscular diseases. Am J Phys Med Rehabil. 2003;82(3):182-5.

97. Griffiths RD, Edwards RH. A new chart for weight control in Duchenne muscular distrophy. Arch Dis Child. 1988;63(10):1256-8.

98. DeSilva S, Drachman DB, Mellits D, Kuncl RW. Prednisone treatment in Duchenne muscular dystrophy. Long-term benefit. Arch Neurol. 1987;44(8):818-22.

99. Bonifati MD, Ruzza G, Bonometto P, Berardinelli A, Gorni K, Orcesi S, et al. A multicenter, double-blind, randomized trial of deflazacort versus prednisone in Duchenne muscular dystrophy. Muscle Nerve. 2000;23(9):1344-7.

100. Iannaccone ST, Owens H, Scott J, Teitell B. Postoperative malnutrition in Duchenne muscular dystrophy. J Child Neurol. 2003;18(1):17-20.

101. Willig TN, Paulus J, Lacau Saint Guily J, Beon C, Navarro J. Swallowing problems in neuromuscular disorders. Arch Phys Med Rehabil. 1994;75(11):1175-81.

102. Truby H, Baic S, Delooy A, Fox KR, Livingstone MBE, Logan CM, et al. Randomised controlled trial of four commercial weight loss programmes in the UK: initial findings from the BBC "diet trials". Br Med J. 2006;332(7553):1309-11.

103. McDonald C. Physical Activity, Health Impairments, and Disability in Neuromuscular Disease. Am J Phys Med Rehabil. 2002;81(11):S108-20.

104. Edwards RH, Round JM, Jackson MJ, Griffiths RD, Lilburn MF. Weight reduction in boys with muscular dystrophy. Dev Med Child Neurol. 1984;26(3):382-90.

105. Arikian A, Boutelle K, Peterson CB, Dalton J, Day JW, Crow SJ. Targeting parents for the treatment of pediatric obesity in boys with Duchenne muscular dystrophy: a case series. Eat Weight Disord. 2010;15(3):161-5.

106. Casteels K, Fieuws S, van Helvoirt M, Verpoorten C, Goemans N, Coudyzer W, et al. Metformin therapy to reduce weight gain and visceral adiposity in children and adolescents with neurogenic or myogenic motor deficit. Pediatr Diabetes. 2010;11(1):61-9.

107. Carter GT, Yudkowsky MP, Han JJ, McCrory MA. Topiramate for weight reduction in duchenne muscular dystrophy. Muscle Nerve. 2005;31(6):788-9.

108. Martigne L, Seguy D, Pellegrini N, Orlikowski D, Cuisset JM, Carpentier A, et al. Efficacy and tolerance of gastrostomy feeding in Duchenne muscular dystrophy. Clin Nutr. 2010;29(1):60-4.

109. Diamanti A, Panetta F, Tentolini A. Efficacy and safety of gastrostomy feeding in Duchenne muscular dystrophy. Clin Nutr. 2011;30(2):263;.

110. Goldstein M, Meyer S, Freund HR. Effects of overfeeding in children with muscle dystrophies. JPEN J Parenter Enteral Nutr. 1989;13(6):603-7.

111. Hilton T, Orr RD, Perkin RM, Ashwal S. End of life care in Duchenne muscular dystrophy. Pediatr Neurol. 1993;9(3):165-77.

112. Bushby K, Bourke J, Bullock R, Eagle M, Gibson M, Quinby J. The multidisciplinary management of Duchenne muscular dystrophy. Current Paediatrics. 2005;15:292-300.

113. Birnkrant DJ, Ferguson RD, Martin JE, Gordon GJ. Noninvasive Ventilation During Gastrostomy Tube Placement in Patients with Severe Duchenne Muscular Dystrophy: Case Reports and Review of the Literature. Pediatr Pulmonol. 2006;41(2):188-93.

114. Parker AE, Robb SA, Chambers J, Davidson AC, Evans K, O'Dowd J, et al. Analysis of an adult Duchenne muscular dystrophy population. Q J Med. 2005;98:729-36.

115. Heyman MB, Harmatz P, Acree M, Wilson L, Moskowitz JT, Ferrando S, et al. Economic and psychologic costs for maternal caregivers of gastrostomy-dependent children. J Pediatr. 2004;145(4):511-6.

116. Cano NJM, Roth H, Court-Fortune I, Cynober L, Gerard-Boncompain M, Cuvelier A, et al. Nutritional depletion in patients on long-term oxygen therapy and/or home mechanical ventilation. Eur Resp J. 2002;20(1):30-7.

117. Leung DG, Germain-Lee EL, Denger BE, Wagner KR. Report on the Second Endocrine Aspects of Duchenne Muscular Dystrophy Conference December 1–2, 2010, Baltimore, Maryland, USA. Neuromuscul Disord. 2011;21(8):594-601.

118. Kley RA, Vorgerd M, Tarnopolsky MA. Creatine for treating muscle disorders Cochrane Database Syst Rev. 2007;(1):CD004760.

119. Grande BM, Graves BS. Creatine Supplementation: Forms, Function, and Effects. Strength Cond J. 2005;27(1):62.

120. Escolar D, Buyse G, Henrickson E, Leshner R, Group atC. CINRG randomized controlled trial of creatine and glutamine in Duchenne muscular dystrophy. Ann Neurol. 2005;58(1):151-5.

121. Louis M, Lebacq J, Poortmans JR, Belpaire-Dethiou M-C, Devogelaer J-P, Van Hecke P, et al. Beneficial effects of creatine supplementation in dystrophic patients. Muscle Nerve. 2003;27(5):604-10.

122. Tarnopolsky MA, Mahoney DJ, Vajsar J, Rodriguez C, Doherty TJ, Roy BD, et al. Creatine monohydrate enhances strength and body composition in Duchenne muscular dystrophy. Neurology. 2004;62(10):1771-7.

123. Walter MC, Lochmuller H, Reilich P, Klopstock T, Huber R, Hartard M, et al. Creatine monohydrate in muscular dystrophies: A double-blind, placebo-controlled clinical study. Neurology. 2000;54(9):1848-50.

124. Banerjee B, Sharma U, Balasubramanian K, Kalaivani M, Kalra V, Jagannathan NR. Effect of creatine monohydrate in improving cellular energetics and muscle strength in ambulatory Duchenne muscular dystrophy patients: a randomized, placebo-controlled 31P MRS study. Magn Reson Imaging. 2010;28(5):698-707.

125. Payne ET, Yasuda N, Bourgeois JM, Devries MC, Rodriguez MC, Yousuf J, et al. Nutritional therapy improves function and complements corticosteroid intervention in mdx mice. Muscle Nerve. 2006;33(1):66-77.

126. Vandenburgh H, Shansky J, Benesch-Lee F, Skelly K, Spinazzola JM, Saponjian Y, et al. Automated drug screening with contractile muscle tissue engineered from dystrophic myoblasts. FASEB J. 2009;23(10):3325-34.

127. Spurney CF, Rocha CT, Henricson E, Florence J, Mayhew J, Gorni K, et al. CINRG pilot trial of coenzyme Q10 in steroid-treated Duchenne muscular dystrophy. Muscle Nerve. 2011;44(2):174-8.

128. Hankard RG, Haymond MW, Darmaun D. Effect of glutamine on leucine metabolism in humans. Am J Physiol. 1996;271(4):748-54.

129. Darmaun D, Welch S, Rini A, Sager BK, Altomare A, Haymond MW. Phenylbutyrate-induced glutamine depletion in humans: effect on leucine metabolism. Am J Physiol. 1998;274(5):801-7.

130. Hankard R, Hammond D, Haymond M, Darmaun D. Oral glutamine slows down whole body protein breakdown in Duchenne muscular dystropy. Pediatr Res. 1998;43(2):222-6.

131. Hankard R, Mauras N, Hammond D, Hammond M, Darmaun D. Is glutamine a `conditionally essential' amino acid in Duchenne muscular dystrophy? Clin Nutr. 1999;18(6):365-9.

132. Mok E, Violante C, Daubrosse C, Gottrand F, Regal O, Fontan J-E, et al. Oral glutamine and amino acid supplementation inhibit whole-body protein degradation in children with Duchenne muscular dystrophy. Am J Clin Nutr. 2006;83(4):823-8.

133. Mok E, Letellier G, Cuisset J-M, Denjean A, Gottrand F, Alberti C, et al. Lack of functional benefit with glutamine versus placebo in Duchenne muscular dystrophy: a randomized crossover trial. PLoS ONE. 2009;4(5):e5448.

134. Chaubourt E, Fossier P, Baux G, Leprince C, Israel M, de la Porte S. Nitric oxide and L-Arginine cause an accumulation of Utrophine at the Sarcolemma: a possible

compensation for dystrophin loss in Duchenne muscular dystrophy. Neurobiol Dis. 1999;6:499-507.

135. Voisin V, Sebrie C, Matecki S, Yu H, Gillet B, Ramonatxo M, et al. L-arginine improves dystrophic phenotype in mdx mice. Neurobiol Dis. 2005;20:123-30.

136. Buetler TM, Renard M, Offord EA, Schneider H, Ruegg UT, Buetler TM, et al. Green tea extract decreases muscle necrosis in mdx mice and protects against reactive oxygen species. Am J Clin Nutr. 2002;75(4):749-53.

137. Dorchies OM, Wagner S, Vuadens O, Waldhauser K, Buetler TM, Kucera P, et al. Green tea extract and its major polyphenol (-)-epigallocatechin gallate improve muscle function in a mouse model for Duchenne muscular dystrophy. Am J Physiol Cell Physiol. 2006;290(2):616-25.

138. Lynch GS, Schertzer JD, Ryall JG. Therapeutic approaches for muscle wasting disorders. Pharmacol Ther. 2007;113:461-87.

139. Clark RH, Feleke G, Din M, Yasmin T, Singh G, Khan FA, et al. Nutritional treatment for acquired immunodeficiency virus-associated wasting using β-hydroxy-β-methylbutyrate, glutamine, and arginine: a randomized, double-blind, placebo-controlled study. JPEN J Parenter Enteral Nutr. 2000;24(3):133-9.

140. Eagle M, Baudouin SV, Chandler C, Giddings DR, Bullock R, Bushby K, et al. Survival in Duchenne muscular dystrophy: improvements in life expectancy since 1967 and the impact of home nocturnal ventilation. Neuromuscul Disord. 2002;12(10):926-9.

141. Aitkens S, Kilmer DD, Wright NC, McCrory MA. Metabolic syndrome in neuromuscular disease. Arch Phys Med Rehabil. 2005;86(5):1030-6.

142. Kilmer DD, Zhao HH. Obesity, physical activity, and the metabolic syndrome in adult neuromuscular disease. Phys Med Rehabil Clin N Am. 2005;16(4):1053-62.

143. McCrory MA, Wright NC, Kilmer DD. Nutritional aspects of neuromuscular diseases. Phys Med Rehabil Clin N Am. 1998;9(1):127-43.

144. Fenichel GM, Brooke MH, Griggs RC, Mendell JR, Miller JP, III RTM, et al. Clinical investigation in Duchenne muscular dystrophy: Penicillamine and vitamin E. Muscle Nerve. 1988;11(11):1164-8.

145. Monaco AP, Neve RL, Colletti-Feener C, Bertelson CJ, Kurnit DM, Kunkel LM. Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene. Nature. 1986;323(6089):646-50.

146. National Institutes of Health. Talking Glossary of Genetic Term [Web Page]. 2011 [cited 2012 February 12]; available from: http://www.genome.gov/Glossary/.

147. Pegoraro E, Hoffman EP, Piva L, Gavassini BF, Cagnin S, Ermani M, et al. SPP1 genotype is a determinant of disease severity in Duchenne muscular dystrophy. Neurology. 2011;76(3):219-26.

148. Koenig M, Beggs AH, Moyer M, Scherpf S, Heindrich K, Bettecken T, et al. The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. Am J Hum Genet. 1989;45(4):498-506.

149. Love DR, Flint TJ, Marsden RF, Bloomfield JF, Daniels RJ, Forrest SM, et al. Characterization of deletions in the dystrophin gene giving mild phenotypes. Am J Med Genet. 1990;37(1):136-42.

150. Leiden University Medical Center. Leiden Muscular Dystrophy pages: Dystrophin Isoforms [Web Page]. 2006 [cited 2012 12 February]; available from: http://www.dmd.nl/isoforms.html#proms.

151. Tokarz SA, Duncan NM, Rash SM, Sadeghi A, Dewan AK, Pillers D-AM. Redefinition of Dystrophin Isoform Distribution in Mouse Tissue by RT-PCR Implies Role in Nonmuscle Manifestations of Duchenne Muscular Dystrophy. Mol Genet Metab. 1998;65(4):272-81.

152. Taylor PJ, Betts GA, Maroulis S, Gilissen C, Pedersen RL, Mowat DR, et al. Dystrophin gene mutation location and the risk of cognitive impairment in Duchenne muscular dystrophy. PLoS ONE. 2010;5(1):e8803.

153. Rapaport D, Passos-Bueno MR, Brandao L, Love D, Vainzof M, Zatz M. Apparent association of mental retardation and specific patterns of deletions screened with probes cf56a and cf23a in Duchenne muscular dystrophy. Am J Med Genet. 1991;39(4):437-41.

154. Bushby KMD, Appleton R, Anderson LVB, Welch JL, Kelly P, Gardner-Medwin D. Deletion status and intellectual impairment in Duchenne muscular dystrophy. Dev Med Child Neurol. 1995;37(3):260-9.

155. Kaspar RW, Allen HD, Ray WC, Alvarez CE, Kissel JT, Pestronk A, et al. Analysis of dystrophin deletion mutations predicts age of cardiomyopathy onset in becker muscular dystrophy. Circ Cardiovasc Genet. 2009;2(6):544-51.

156. Desguerre I, Christov C, Mayer M, Zeller R, Becane HM, Bastuji-Garin S, et al. Clinical heterogeneity of duchenne muscular dystrophy (DMD): definition of sub-phenotypes and predictive criteria by long-term follow-up. PLoS ONE. 2009;4(2):e4347.

157. Kuczmarski R, Odgen C, Grummer-Strawn L, Flegal K, Guo S, Wei R, et al. CDC Growth Charts: United States. Hyattsville, Maryland: National Center for Health Statistics; 2000.

158. Laerd Statistics. Testing for Normality Using SPSS [Web Page]. 2012 [cited 2012 3 March]; available from: https://statistics.laerd.com/spss-tutorials/testing-for-normality-using-spss-statistics.php.

159. National Health and Medical Research Council. Clinical Practice Guidelines for the Management of Overweight and Obesity in Children and Adolescents. Canberra: Commonwealth of Australia; 2003.

160. Tuffery-Giraud S, Beroud C, Leturcq F, Yaou RB, Hamroun D, Michel-Calemard L, et al. Genotype-phenotype analysis in 2,405 patients with a dystrophinopathy using the UMD-DMD database: a model of nationwide knowledgebase. Hum Mutat. 2009;30(6):934-45.

161. Pallant J. SPSS Survival Manual. 4th ed. Crows Nest, NSW: Allen & Unwin; 2011.

162. Grossman JM, Gordon R, Ranganath VK, Deal C, Caplan L, Chen W, et al. American College of Rheumatology 2010 recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis. Arthritis Care Res. 2010;62(11):1515-26.

163. Bone and Tooth Society, National Osteoporosis Society, Royal College of Physicians. Glucocorticoid-induced osteoporosis: guidelines for prevention and treatment. London: Royal College of Physicians of London; 2002.

164. Shaker JL, Lukert BP. Osteoporosis Associated with Excess Glucocorticoids. Endocrinol Metab Clin North Am. 2005;34(2):341-56.

165. Van Staa TP, Leufkens HGM, Abenhaim L, Zhang B, Cooper C. Use of Oral Corticosteroids and Risk of Fractures. J Bone Miner Res. 2000;20(8):1486-93.

166. Todorova A, Todorov T, Georgieva B, Lukova M, Guergueltcheva V, Kremensky I, et al. MLPA analysis/complete sequencing of the DMD gene in a group of Bulgarian Duchenne/Becker muscular dystrophy patients. Neuromuscul Disord. 2008;18(8):667-70.

167. Liechti-Gallati S, Koenig M, Kunkel LM, Frey D, Boltshauser E, Schneider V, et al. Molecular deletion patterns in Duchenne and Becker type muscular dystrophy. Hum Genet. 1989;81(4):343-8.

168. Brabec P, Vondracek P, Klimes D, Baumeister S, Lochmuller H, Pavlik T, et al. Characterization of the DMD/BMD patient population in Czech Republic and Slovakia using an innovative registry approach. Neuromuscul Disord. 2009;19(4):250-4.

169. Takeshima Y, Yagi M, Okizuka Y, Awano H, Zhang Z, Yamauchi Y, et al. Mutation spectrum of the dystrophin gene in 442 Duchenne/Becker muscular dystrophy cases from one Japanese referral center. J Hum Genet. 2010;55(6):379-88.

170. Basak J, Dasgupta UB, Mukherjee SC, Das SK, Senapati AK, Banerjee TK. Deletional mutations of dystrophin gene and carrier detection in eastern India. Indian J Pediatr. 2009;76(10):1007-12.

171. Swaminathan B, Shubha GN, Shubha D, Murthy AR, Kiran Kumar HB, Shylashree S, et al. Duchenne muscular dystrophy: a clinical, histopathological and genetic study at a neurology tertiary care center in Southern India. Neurol India. 2009;57(6):734-8.

172. Cunniff C, Andrews J, Meaney FJ, Mathews KD, Matthews D, Ciafaloni E, et al. Mutation analysis in a population-based cohort of boys with Duchenne or Becker muscular dystrophy. J Child Neurol. 2009;24(4):425-30.

173. Biggar WD, Gingras M, Fehlings DL, Harris VA, Steele CA. Deflazacort treatment of Duchenne muscular dystrophy. J Pediatr. 2001;138(1):45-50.

174. Brewer C, Holloway S, Zawalnyski P, Schinzel A, FitzPatrick D. A chromosomal deletion map of human malformations. Am J Hum Genet. 1998;63(4):1153-9.

175. Hahn AF, Bolton CF, White CM, Brown WF, Tuuha SE, Tan CC, et al. Genotype/Phenotype Correlations in X-Linked Dominant Charcot-Marie-Tooth Disease. Ann N Y Acad Sci. 1999;883(1):366-82.

176. Hara Y, Balci-Hayta B, Yoshida-Moriguchi T, Kanagawa M, Beltrán-Valero de Bernabé D, Gündeşli H, et al. A Dystroglycan Mutation Associated with Limb-Girdle Muscular Dystrophy. N Engl J Med. 2011;364(10):939-46.

177. Johansen HK, Nir M, Koch C, Schwartz M, Høiby N. Severity of cystic fibrosis in patients homozygous and heterozygous for ΔF508 mutation. Lancet. 1991;337(8742):631-4.

178. Maffiuletti NA, Jubeau M, Munzinger U, Bizzini M, Agosti F, De Col A, et al. Differences in quadriceps muscle strength and fatigue between lean and obese subjects. Eur J Appl Physiol. 2007;101(1):51-9.

179. Maffiuletti NA, Jubeau M, Agosti F, De Col A, Sartorio A. Quadriceps muscle function characteristics in severely obese and nonobese adolescents. Eur J Appl Physiol. 2008;103(4):481-4.

180. Stenholm S, Sallinen J, Koster A, Rantanen T, Sainio P, Heliovaara M, et al. Association between obesity history and hand grip strength in older adults-exploring the roles of inflammation and insulin resistance as mediating factors. J Gerontol A Biol Sci Med Sci. 2011;66(3):341-8.

181. Hulens M, Vansant G, Claessens AL, Lysens R, Muls E. Predictors of 6-minute walk test results in lean, obese and morbidly obese women. Scand J Med Sci Sports. 2003;13(2):98-105.

182. Daly RM, Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Sikaris KA, et al. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: A national, population-based study. Clin Endocrinol. 2011 (Epub ahead of print).

183. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2011;96(7):1911-30.

184. Zittermann A. Vitamin D and disease prevention with special reference to cardiovascular disease. Prog Biophys Mol Biol. 2006;92(1):39-48.

185. Holick MF. Vitamin D deficiency in 2010: Health benefits of vitamin D and sunlight: a D-bate. Nat Rev Endocrinol. 2011;7(2):73-5.

186. Holick MF. Vitamin D Status: Measurement, Interpretation, and Clinical Application. Ann Epidemiol. 2009;19(2):73-8.

187. Holick MF. Optimal Vitamin D Status for the Prevention and Treatment of Osteoporosis. Drugs Aging. 2007;24(12):1017-29.

188. van der Mei IAF, Ponsonby A, Engelsen O, Pasco JA, McGrath JJ, Eyles DW, et al. The high prevalence of vitamin D insufficiency across Australian populations is only partly explained by season and latitude. Environ Health Perspect. 2007;115(8):1132-9.

189. Farrell CJ, Martin S, McWhinney B, Straub I, Williams P, Herrmann M. State-of-the-art vitamin D assays: a comparison of automated immunoassays with liquid chromatography-tandem mass spectrometry methods. Clin Chem. 2012;58(3):531-42.

190. Kimlin M, Harrison S, Nowak M, Moore M, Brodie A, Lang C. Does a high UV environment ensure adequate Vitamin D status? J Photochem Photobiol B. 2007;89(2–3):139-47.

191. Vu LH, Whiteman DC, van der Pols JC, Kimlin MG, Neale RE. Serum Vitamin D Levels in Office Workers in a Subtropical Climate. Photochem Photobiol. 2011;87(3):714-20.

192. Vu LH, van der Pols JC, Whiteman DC, Kimlin MG, Neale RE. Knowledge and Attitudes about Vitamin D and Impact on Sun Protection Practices among Urban Office Workers in Brisbane, Australia. Cancer Epidemiol Biomarkers Prev. 2010;19(7):1784-9.

193. Mazziotti G, Angeli A, Bilezikian JP, Canalis E, Giustina A. Glucocorticoid-induced osteoporosis: an update. Trends Endocrinol Metab. 2006;17(4):144-9.

194. Canalis E, Mazziotti G, Giustina A, Bilezikian JP. Glucocorticoid-induced osteoporosis: pathophysiology and therapy. Osteoporos Int. 2007;18(10):1319-28.

195. Borradale D, Kimlin M. Vitamin D in health and disease: an insight into traditional functions and new roles for the 'sunshine vitamin'. Nutr Res Rev. 2009;22(02):118-36.

196. Amin S, LaValley MP, Simms RW, Felson DT. The role of vitamin D in corticosteroid-induced osteoporosis: a meta-analytic approach. Arthritis Rheum. 1999;42(8):1740-51.

197. Homik J, Suarez-Almazor ME, Shea B, Cranney A, Wells G, Tugwell P. Calcium and vitamin D for corticosteroid-induced osteoporosis. Cochrane Database Syst Rev. 2000;(2):CD000952.

198. Kanis JA, Stevenson M, McCloskey EV, Davis S, Lloyd-Jones M. Glucocorticoid-induced osteoporosis: a systematic review and cost-utility analysis. Health Technol Assess. 2007;11(7):1-256.

199. Avioli LV, Birge SJ, Lee S. Effects of Prednisone on Vitamin D Metabolism in Man. J Clin Endocrinol Metab. 1968;28(9):1341-6.

200. Hahn TJ, Halstead LR, Haddad JG, Jr. Serum 25-hydroxyvitamin D concentrations in patients receiving chronic corticosteroid therapy. J Lab Clin Med. 1977;90(2):399-404.

201. Hahn TJ, Halstead LR, Teitelbaum SL, Hahn BH. Altered mineral metabolism in glucocorticoid-induced osteopenia. Effect of 25-hydroxyvitamin D administration. J Clin Invest. 1979;64(2):655-65.

202. Slovik DM, Neer RM, Ohman JL, Lowell FC, Clark MB, Segre GV, et al. Parathyroid hormone and 25-hydroxyvitamin D levels in glucocorticoid-treated patients. Clin Endocrinol. 1980;12(3):243-8.

203. Klein RG, Arnaud SB, Gallagher JC, Deluca HF, Riggs BL. Intestinal calcium absorption in exogenous hypercortisonism. Role of 25-hydroxyvitamin D and corticosteroid dose. J Clin Invest. 1977;60(1):253-9.

204. Seeman E, Kumar R, Hunder GG, Scott M, Heath H, 3rd, Riggs BL. Production, degradation, and circulating levels of 1,25-dihydroxyvitamin D in health and in chronic glucocorticoid excess. J Clin Invest. 1980;66(4):664-9.

205. Holick MF. Vitamin D Deficiency. N Engl J Med. 2007;357(3):266-81.

206. Heaney RP, Heaney RP. The Vitamin D requirement in health and disease. J Steroid Biochem Mol Biol. 2005;97(1-2):13-9.

207. American Dietetic Association. Evidence Analysis Manual: Steps in the ADA Evidence Analysis Process. Chicago, IL: Scientific Affairs and Research, American Dietetic Association; 2008.

208. National Health and Medical Research Council. A Guide to the Development, Implementation and Evaluation of Clinical Practice Guidelines. Canberra, ACT: National Health and Medical Research Council; 1998.

209. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. Introduction to Meta-Analysis. West Sussex, UK: John Wiley & Sons Ltd; 2009.

210. Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale, NJ: Lawrence Earlbaum Associates; 1988.

211. Bradburn MJ, Deeks JJ, Altman DG. metan: an alternative meta-analysis command. Stata J. 1999;44:4-15.

212. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. Br Med J. 2003;327(7414):557-60.

213. Skversky AL, Kumar J, Abramowitz MK, Kaskel FJ, Melamed ML. Association of Glucocorticoid Use and Low 25-Hydroxyvitamin D Levels: Results from the National Health and Nutrition Examination Survey (NHANES): 2001–2006. J Clin Endocrinol Metab. 2011; 96(12):3838-45.

214. Dykman TR, Haralson KM, Gluck OS, Murphy WA, Teitelbaum SL, Hahn TJ, et al. Effect of oral 1,25-dihydroxyvitamin D and calcium on glucocorticoid-induced osteopenia in patients with rheumatic diseases. Arthritis Rheum. 1984;27(12):1336-43.

215. Colette C, Monnier L, Pares Herbute N, Blotman F, Mirouze J. Calcium absorption in corticoid treated subjects effects of a single oral dose of calcitriol. Horm Metab Res. 1987;19(7):335-8.

216. Abitbol V, Roux C, Chaussade S, Guillemant S, Kolta S, Dougados M, et al. Metabolic bone assessment in patients with inflammatory bowel disease. Gastroenterology. 1995;108(2):417-22.

217. Adler RA, Funkhouser HL, Petkov VI, Berger MM, Adler RA, Funkhouser HL, et al. Glucocorticoid-induced osteoporosis in patients with sarcoidosis. Am J Med Sci. 2003;325(1):1-6.

218. Aloia JF, Roginsky M, Ellis K, Shukla K, Cohn S. Skeletal metabolism and body composition in Cushing's syndrome. J Clin Endocrinol Metab. 1974;39(6):981-5.

219. Als OS, Riis B, Christiansen C. Serum concentration of vitamin D metabolites in rheumatoid arthritis. Clin Rheumatol. 1987;6(2):238-43.

220. Bernstein CN, Seeger LL, Sayre JW, Anton PA, Artinian L, Shanahan F. Decreased bone density in inflammatory bowel disease is related to corticosteroid use and not disease diagnosis. J Bone Miner Res. 1995;10(2):250-6.

221. Bijlsma JW, Raymakers JA, Mosch C, Hoekstra A, Derksen RH, Baart de la Faille H, et al. Effect of oral calcium and vitamin D on glucocorticoid-induced osteopenia. Clin Exp Rheumatol. 1988;6(2):113-9.

222. Bikle DD, Halloran B, Fong L, Steinbach L, Shellito J. Elevated 1,25-dihydroxyvitamin D levels in patients with chronic obstructive pulmonary disease treated with prednisone. J Clin Endocrinol Metab. 1993;76(2):456-61.

223. Bonadonna S, Burattin A, Nuzzo M, Bugari G, Rosei EA, Valle D, et al. Chronic glucocorticoid treatment alters spontaneous pulsatile parathyroid hormone secretory dynamics in human subjects. Eur J Endocrinol. 2005;152(2):199-205.

224. Braun JJ, Birkenhager-Frenkel DH, Rietveld AH, Juttmann JR, Visser TJ, Birkenhager JC. Influence of 1 alpha-(OH)D3 administration on bone and bone mineral metabolism in patients on chronic glucocorticoid treatment; a double blind controlled study. Clin Endocrinol. 1983;19(2):265-73.

225. D'Angelo A, Fabris A, Sartori L, Malvasi L, Travaglia P, Gambari PF, et al. Mineral metabolism and bone mineral content in rheumatoid arthritis. Effect of corticosteroids. Clin Exp Rheumatol. 1985;3(2):143-6.

226. Driscoll RH, Jr., Meredith SC, Sitrin M, Rosenberg IH. Vitamin D deficiency and bone disease in patients with Crohn's disease. Gastroenterology. 1982;83(6):1252-8.

227. Grecu EO, Simmons R, Baylink DJ, Haloran BP, Spencer ME. Effects of medroxyprogesterone acetate on some parameters of calcium metabolism in patients with glucocorticoid-induced osteoporosis. Bone Miner. 1991;13(2):153-61.

228. Lund B, Storm TL, Melsen F, Mosekilde L, Andersen RB, Egmose C, et al. Bone mineral loss, bone histomorphometry and vitamin D metabolism in patients with rheumatoid arthritis on long-term glucocorticoid treatment. Clin Rheumatol. 1985;4(2):143-9.

229. Newman ED, Matzko CK, Olenginski TP, Perruquet JL, Harrington TM, Maloney-Saxon G, et al. Glucocorticoid-Induced Osteoporosis Program (GIOP): a novel, comprehensive, and highly successful care program with improved outcomes at 1 year. Osteoporos Int. 2006;17(9):1428-34.

230. Reid IR, Schooler BA, Stewart AW. Prevention of glucocorticoid-induced osteoporosis. J Bone Miner Res. 1990;5(6):619-23.

231. Rickers H, Deding A, Christiansen C, Rodbro P, Naestoft J. Corticosteroid-induced osteopenia and vitamin D metabolism. Effect of vitamin D2, calcium phosphate and sodium fluoride administration. Clin Endocrinol. 1982;16(4):409-15.

232. Toloza SM, Cole DE, Gladman DD, Ibanez D, Urowitz MB, Toloza SMA, et al. Vitamin D insufficiency in a large female SLE cohort. Lupus. 2010;19(1):13-9.

233. Worth H, Stammen D, Keck E. Therapy of steroid-induced bone loss in adult asthmatics with calcium, vitamin D, and a diphosphonate. Am J Respir Crit Care Med. 1994;150(2):394-7.

234. Zerwekh JE, Emkey RD, Harris ED, Jr. Low-dose prednisone therapy in rheumatoid arthritis: effect on vitamin D metabolism. Arthritis Rheum. 1984;27(9):1050-2.

235. Zhou C, Assem M, Tay JC, Watkins PB, Blumberg B, Schuetz EG, et al. Steroid and xenobiotic receptor and vitamin D receptor crosstalk mediates CYP24 expression and drug-induced osteomalacia. J Clin Invest. 2006;116(6):1703-12.

236. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72(3):690-3.

237. Elliott M, Farrah R, Binkley N, Carnes M, Gudmundsson A. Management of glucocorticoid-induced osteoporosis in male veterans. Ann Pharmacother. 2000;34(12):1380-4.

238. Feldstein A, Elmer P, Nichols G, Herson M. Practice patterns in patients at risk for glucocorticoid-induced osteoporosis. Osteoporos Int. 2005;16(12):2168-74.

239. Cruse LM, Valeriano J, Vasey FB, Carter JD. Prevalence of Evaluation and Treatment of Glucocorticoid-Induced Osteoporosis in Men. J Clin Rheumatol. 2006;12(5):221-5.

240. Saag KG, Gehlbach SH, Curtis JR, Youket TE, Worley K, Lange JL. Trends in prevention of glucocorticoid-induced osteoporosis. J Rheumatol. 2006;33(8):1651-7.

241. Adler R. Glucocorticoid-Induced Osteoporosis: Management Update. Curr Osteoporos Rep. 2010;8(1):10-4.

242. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington DC: The National Academics Press; 2011.

243. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr. 2003;77(1):204-10.

244. Hathcock JN, Shao A, Vieth R, Heaney R. Risk assessment for vitamin D. Am J Clin Nutr. 2007;85(1):6-18.

245. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA. 2010;303(18):1815-22.

246. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, et al. Effect of Vitamin D on falls: a meta-analysis. JAMA. 2004;291(16):1999-2006.

247. Visser M, Deeg DJ, Lips P, Longitudinal Aging Study A, Visser M, Deeg DJH, et al. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. J Clin Endocrinol Metab. 2003;88(12):5766-72.

248. Ward KA, Das G, Berry JL, Roberts SA, Rawer R, Adams JE, et al. Vitamin D Status and Muscle Function in Post-Menarchal Adolescent Girls. J Clin Endocrinol Metab. 2009;94(2):559-63.

249. Sullivan SS, Cobb JL, Rosen CJ, Holick MF, Chen TC, Kimlin MG, et al. Assessment of sun exposure in adolescent girls using activity diaries. Nutr Res. 2003;23(5):631-44.

250. Chodick G, Kleinerman R, Linet M, Fears T, Kwok R, Kimlin M, et al. Agreement Between Diary Records of Time Spent Outdoors and Personal Ultraviolet Radiation Dose Measurements. Photochem Photobiol. 2008;84(3):713.

251. Moher D, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF. Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Lancet. 1999;354(9193):1896-900.

252. Schlesselman JJ. Risk of endometrial cancer in relation to use of combined oral contraceptives. A practitioner's guide to meta-analysis. Hum Reprod. 1997;12(9):1851-63.

253. McDonald CM, Henricson EK, Han JJ, Abresch RT, Nicorici A, Elfring GL, et al. The 6-minute walk test as a new outcome measure in Duchenne muscular dystrophy. Muscle Nerve. 2010;41(4):500-10.

254. Mayhew J, Florence J, Mayhew TP, Henrickson E, Leshner R, McCarter RJ, et al. Reliable surrogate outcome measures in multicentre clinical trials of Duchenne muscular dystrophy. Muscle Nerve. 2007;35:36-42.

255. McDonald CM, Henricson EK, Han JJ, Abresch RT, Nicorici A, Atkinson L, et al. The 6-minute walk test in Duchenne/Becker muscular dystrophy: longitudinal observations. Muscle Nerve. 2010;42(6):966-74.

256. McDonald CM, Widman LM, Walsh DD, Walsh SA, Abresch RT. Use of step activity monitoring for continuous physical activity assessment in boys with Duchenne muscular dystrophy. Arch Phys Med Rehabil. 2005;86(4):802-8.

257. Livingstone M, Prentice A, Coward W, Strain J, Black A, Davies P, et al. Validation of estimates of energy intake by weighed dietary record and diet history in children and adolescents. Am J Clin Nutr. 1992;56(1):29-35.

258. Burrows TL, Martin RJ, Collins CE. A Systematic Review of the Validity of Dietary Assessment Methods in Children when Compared with the Method of Doubly Labeled Water. J Am Diet Assoc. 2010;110(10):1501-10.

259. Hill RJ, Davies PSW. The validity of self-reported energy intake as determined using the doubly labelled water technique. Br J Nutr. 2001;85(4):415-30.

260. Australasian Child and Adolescent Obesity Research Network. Dietary intake assessment - Weighed food records [Web Page]. 2010 [cited 2012 March 22]; available from: http://www.acaorn.org.au/streams/nutrition/assessment-methods/weighed-food-records.php.

261. Trabulsi J, Schoeller DA. Evaluation of dietary assessment instruments against doubly labeled water, a biomarker of habitual energy intake. Am J Physiol Endocrinol Metab. 2001;281(5):891-9.

262. American Thoracic Society (ATS). ATS Statement: Guidelines for the Six-Minute Walk Test. Am J Respir Crit Care Med. 2002;166:111–7.

263. Persson LA, Carlgren G. Measuring Children's Diets: Evaluation of Dietary Assessment Techniques in Infancy and Childhood. Int J Epidemiol. 1984;12(4):506-16.

264. Australasian Child and Adolescent Obesity Research Network. Dietary intake assessment - Food diary [Web Page]. 2010 [cited 2012 March 22]; available from: http://www.acaorn.org.au/streams/nutrition/assessment-methods/food-diary.php.

265. CalorieKing Australia. Nutritional Information [Web Page]. 2010 [cited 2012 March 30]; Available from: http://www.calorieking.com.au/foods/.

266. Schoeller DA, Webb P. Five-day comparison of the doubly labeled water method with respiratory gas exchange. Am J Clin Nutr. 1984;40:153-8.

267. Trabulsi J, Troiano RP, Subar AF, Charbaugh C. Precision of the doubly labelled water method in a large-scale application: evaluation of a streamlined-dosing protocol in the Observing Protein and Energy Nutrition (OPEN) study. Eur J Clin Nutr. 2003;57(11):1370-7.

268. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;328(8502):307-10.

269. Black AE. Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. Int J Obes Relat Metab Disord. 2000;24(9):1119-30.

270. McDonald C, Coleman K, Henricson E, Abresch RT, Eagle M, Florence J, et al. Assessment of StepWatch™ activity monitoring in phase 2b study of ataluren (PTC124™) in nmDMD/BMD. Neuromuscul Disord. 2009;19(8):602.

271. De Vries SI, Van Hirtum HWJEM, Bakker I, Hopman-Rock M, Hirasing RA, Van Mechelen W. Validity and Reproducibility of Motion Sensors in Youth: A Systematic Update. Med Sci Sports Exerc. 2009;41(4):818-27.

272. McDonald CM, Widman L, Abresch RT, Walsh SA, Walsh DD. Utility of a step activity monitor for the measurement of daily ambulatory activity in children. Arch Phys Med Rehabil. 2005;86(4):793-801.

273. Song KM, Bjornson KF, Cappello T, Coleman K. Use of the StepWatch Activity Monitor for Characterization of Normal Activity Levels of Children. J Pediatr Orthop. 2006;26(2):245-9.

274. Janz KF, Cassady SL, Barr RN, Kelly JM. Monitoring exercise in children and adolescents with cystic fibrosis: validation of the CSA accelerometer. Cardiopulm Phys Ther J. 1995;6(2):3-8.

275. Norman JF. Accelerometry as an estimate of energy expenditure in healthy children and children with cerebral palsy during self-paced ambulation. IJAHSP. 2006;4(1):1-5.

276. Mazzone E, Vasco G, Sormani MP, Torrente Y, Berardinelli A, Messina S, et al. Functional changes in Duchenne muscular dystrophy: a 12-month longitudinal cohort study. Neurology. 2011;77(3):250-6.

277. Henricson E, Abresch R, Han JJ, Nicorici A, Goude Keller E, Elfring G, et al. Percent-Predicted 6-Minute Walk Distance in Duchenne Muscular Dystrophy to Account for Maturational Influences. PLoS Curr. 2012;4:RRN1297.

278. Burrows TL, Martin RJ, Collins CE. A Systematic Review of the Validity of Dietary Assessment MEthods in Children when Compared with the Method of Doubly Labeled Water. Journal of the American Dietetic Association. 2010;110(10):1501-10.

279. Araujo MC, Yokoo EM, Pereira RA. Validation and Calibration of a Semiquantitative Food Frequency Questionnaire Designed for Adolescents. J Am Diet Assoc. 2010;110(8):1170-7.

280. Fisher JO, Johnson RK, Lindquist C, Birch LL, Goran MI. Influence of Body Composition on the Accuracy of Reported Energy Intake in Children. Obesity. 2000;8(8):597-603.

281. Nabukera SK, Romitti PA, Campbell KA, Meaney FJ, Caspers KM, Mathews KD, et al. Use of Complementary and Alternative Medicine by Males With Duchenne or Becker Muscular Dystrophy. J Child Neurol. 2011.

282. Harris RC, Soderlund K, Hultman E. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. Clin Sci. 1992;83(3):367-74.

283. Tarnopolsky MA, Parise G. Direct measurement of high-energy phosphate compounds in patients with neuromuscular disease. Muscle Nerve. 1999;22(9):1228-33.

284. Shao A, Hathcock JN, Shao A, Hathcock JN. Risk assessment for creatine monohydrate. Regul Toxicol Pharmacol. 2006;45(3):242-51.

285. Bizzarini E, De Angelis L. Is the use of oral creatine supplementation safe? J Sports Med Phys Fitness. 2004;44(4):411-6.

286. Lattavo A, Kopperud A, Rogers PD, Lattavo A, Kopperud A, Rogers PD. Creatine and other supplements. Pediatr Clin North Am.54(4):735-60.

287. Persky AM, Rawson ES, Persky AM, Rawson ES. Safety of creatine supplementation. Subcell Biochem. 2007;46:275-89.

288. Poortmans JR, Francaux M. Adverse effects of creatine supplementation: fact or fiction? Sports Med. 2000;30(3):155-70.

289. Terjung RL, Clarkson P, Eichner ER, Greenhaff PL, Hespel PJ, Israel RG, et al. American College of Sports Medicine roundtable. The physiological and health effects of oral creatine supplementation. Med Sci Sports Exerc. 2000;32(3):706-17.

290. Smith RJ. Glutamine Metabolism and Its Physiologic Importance. JPEN J Parenter Enteral Nutr. 1990;14(4):40S-4S.

291. Jepson MM, Bates PC, Broadbent P, Pell JM, Millward DJ. Relationship between glutamine concentration and protein synthesis in rat skeletal muscle. Am J Physiol Endocrinol Metab. 1988;255(2):166-72.

292. Stumvoll M, Perriello G, Meyer C, Gerich J. Role of glutamine in human carbohydrate metabolism in kidney and other tissues. Kidney Int. 1999;55(3):778-92.

293. Rennie MJ, MacLennan PA, Hundal HS, Weryk B, Smith K, Taylor PM, et al. Skeletal muscle glutamine transport, intramuscular glutamine concentration, and muscle-protein turnover. Metabolism. 1989;38(8):47-51.

294. Tsubuku S, Hatayama K, Mawatari K, Smriga M, Kimura T, Tsubuku S, et al. Thirteen-week oral toxicity study of L-glutamine in rats. Int J Toxicol. 2004;23(2):107-12.

295. Shao A, Hathcock JN, Shao A, Hathcock JN. Risk assessment for the amino acids taurine, L-glutamine and L-arginine. Regul Toxicol Pharmacol. 2008;50(3):376-99.

296. Zanchi NE, Nicastro H, Lancha AH, Jr. Potential antiproteolytic effects of L-leucine: observations of in vitro and in vivo studies. Nutr Metab. 2008;5:20.

297. Holecek M, Muthny T, Kovarik M, Sispera L. Effect of beta-hydroxy-beta-methylbutyrate (HMB) on protein metabolism in whole body and in selected tissues. Food Chem Toxicol. 2009;47(1):255-9.

298. Van Koevering M, Nissen S. Oxidation of leucine and alpha-ketoisocaproate to beta-hydroxy-beta-methylbutyrate in vivo. Am J Physiol Endocrinol Metab. 1992;262(1):27-31.

299. Wilson GJ, Wilson JM, Manninen AH. Effects of beta-hydroxy-beta-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex, and training experience: A review. Nutr Metab. 2008;5:1.

300. Zanchi NE, Gerlinger-Romero F, Guimaraes-Ferreira L, de Siqueira Filho MA, Felitti V, Lira FS, et al. HMB supplementation: clinical and athletic performance-related effects and mechanisms of action. Amino Acids. 2011;40(4):1015-25.

301. Nissen SL, Abumrad NN. Nutritional role of the leucine metabolite β-hydroxy β-methylbutyrate (HMB). J Nutr Biochem. 1997;8(6):300-11.

302. May PE, Barber A, D'Olimpio JT, Hourihane A, Abumrad NN, May PE, et al. Reversal of cancer-related wasting using oral supplementation with a combination of beta-hydroxy-beta-methylbutyrate, arginine, and glutamine. Am J Surg. 2002;183(4):471-9.

303. Baier S, Johannsen D, Abumrad N, Rathmacher JA, Nissen S, Flakoll P, et al. Year-long changes in protein metabolism in elderly men and women supplemented with a nutrition cocktail of beta-hydroxy-beta-methylbutyrate (HMB), L-arginine, and L-lysine. JPEN J Parenter Enteral Nutr. 2009;33(1):71-82.

304. Gallagher PM, Carrithers JA, Godard MP, Schulze KE, Trappe SW. Beta-hydroxy-beta-methylbutyrate ingestion, Part I: effects on strength and fat free mass. Med Sci Sports Exerc. 2000;32(12):2109-15.

305. Nissen S, Sharp RL, Panton L, Vukovich M, Trappe S, Fuller JC, Jr. beta-hydroxy-beta-methylbutyrate (HMB) supplementation in humans is safe and may decrease cardiovascular risk factors. J Nutr. 2000;130(8):1937-45.

306. Baxter JH, Carlos JL, Thurmond J, Rehani RN, Bultman J, Frost D. Dietary toxicity of calcium beta-hydroxy-beta-methyl butyrate (CaHMB). Food Chem Toxicol. 2005;43(12):1731-41.

307. Vandenburgh H, Shansky J, Benesch-Lee F, Skelly K, Spinazzola JM, Saponjian Y, et al. Automated drug screening with contractile muscle tissue engineered from dystrophic myoblasts. FASEB Journal. 2009;23(10):3325-34.

308. Campbell MK, Farrell SO. Biochemistry. 5th ed. Belmont, CA: Thomson Brooks/Cole; 2006.

309. Ha E, Zemel MB. Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people. J Nutr Biochem. 2003;14(5):251-8.

310. Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, et al. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. Am J Clin Nutr. 2009;89(1):161-8.

311. Persky AM, Brazeau GA. Clinical pharmacology of the dietary supplement creatine monohydrate. Pharmacol Rev. 2001;53(2):161-76.

312. Wernerman J. Clinical Use of Glutamine Supplementation. J Nutr. 2008;138(10):2040S.

313. Vandervelde L, Dispa D, Van den Bergh PY, Thonnard JL. A comparison between self-reported and observed activity limitations in adults with neuromuscular disorders. Arch Phys Med Rehabil. 2008;89:1720-3.

314. Vandervelde L, Van den Bergh PY, Goemans N, thonnard JL. ACTIVLIM: A Rasch-built measure of activity limitations in children and adults with neuromuscular disorders. Neuromuscul Disord. 2007;17:459-69.

315. Vandervelde L, Van den Bergh PY, Goemans N, Thonnard JL, Vandervelde L, Van den Bergh PYK, et al. Activity limitations in patients with neuromuscular disorders: a responsiveness study of the ACTIVLIM questionnaire. Neuromuscul Disord. 2009;19(2):99-103.

316. Varni J, Burwinkle T, Seid M, Skarr D. The PedsQL 4.0 as a pediatric population health measure: feasibility, reliability, and validity. Ambul Pediatr. 2003;3:329-41.

317. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr. 1985;39:5-41.

318. National Health and Medical Research Council. Nutrient Reference Values for Australia and New Zealand. Canberra: Commonwealth of Australia; 2006.

319. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr. 2006;84(1):18-28.

320. Grizzle JE. The Two-Period Change-Over Design and Its Use in Clinical Trials. Biometrics. 1965;21(2):467-80.

321. Altman DG. Practical Statistics for Medical Research. London: Chapman and Hall; 1991.

322. Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods. 2007;39(2):175-91.

323. Woods JR, Williams JG, Tavel M. The two-period crossover design in medical research. Ann Intern Med. 1989;110(7):560-6.

324. Richens A. Proof of efficacy trials: cross-over versus parallel-group. Epilepsy Res. 2001;45(1-3):43-7.

325. Conwit RA, Bhanushali MJ, Porter JD, Kaufmann P, Gutmann L. Adding more muscle and nerve to clinical trials. Muscle Nerve. 2011;44(5):695-702.

326. Access Economics Report. The cost of muscular dystrophy [Web Page]. 2007 [cited 2012 February 28]; available from: http://www.accesseconomics.com.au/publicationsreports/.

8 Appendices

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Appendix 1

Davidson ZE, Truby H. A review of nutrition in Duchenne muscular dystrophy. J Hum Nutr Diet. 2009;22(5):383-93.

Appendix 2

Elliott SA, Davidson ZE, Davies PSW, Truby H. Predicting resting energy expenditure in boys with Duchenne muscular dystrophy. Eur J Paediatr Neurol. 2012;(Epub ahead of print).

Appendix 3

The Royal Children's Hospital, Melbourne

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RCH HUMAN RESEARCH ETHICS COMMITTEE APPROVAL

HREC REF. No:	**29075 B**
PROJECT TITLE:	Energy requirements and body composition in children with neuromuscular disease, a cross sectional study.
DOCUMENTS APPROVED:	Recruitment Letter v1 dated 7 Sep 09
PRINCIPAL INVESTIGATOR:	H Truby
DATE OF MODIFICATION APPROVAL:	**28 September 2009**
DURATION:	23 months
DATE OF APPROV[redacted]	**[redacted] 2011**
SIGNED:	....[redacted] 30 / 9 / 2009

APPROVED SUBJECT TO THE FOLLOWING CONDITIONS:

ALL PROJECTS

1. Any proposed change in protocol or any approved documents or the addition of any documents (including flyers, brochures, advertising material etc) and the reasons for that change or addition, together with an indication of ethical implications (if any), must be submitted to the Human Research Ethics Committee for Approval prior to implementation.
2. The Principal Investigator must notify the Secretary of the Human Research Ethics Committee of:
   - Any serious adverse effects of the study on participants and steps taken to deal with them.
   - Any unforeseen events (e.g. protocol violations).
   - Investigators withdrawing from or joining the project.
3. A progress report must be submitted annually and at the conclusion of the project, with special emphasis on ethical matters.
4. All research information collected whilst individual participants are children must be kept until the individual turns 25 (i.e. 7 years after their 18th birthday).

Please note that it is the investigators responsibility to ensure that the RCH HREC Approval remains current for the entire duration of the project. Investigators undertaking projects without current HREC approval risk their indemnity, funding and publication rights.

DRUG/DEVICE TRIALS

5. The investigator(s) must report all **internal* SAEs (occurring in RCH participants)** to the sponsor and the RCH HREC within 24-72 hours of occurrence.
6. The investigators must ensure that all externally sponsored Clinical Drug Studies have insurance coverage that is current for the duration of the study.

Appendix 4

Queensland Government

CHILDREN'S HEALTH SERVICES DISTRICT
ETHICS COMMITTEE

Professor John Pearn (Chair) 3365 5323
Mrs Amanda Smith (Co-ordinator) 3636 9167

Queensland **Health**

Level 3, RCH Foundation Building
Royal Children's Hospital
Herston QLD 4029 Australia
Telephone (07) 3636 9167
Facsimile (07) 3365 5455

10th August 2009

Professor Helen Truby
Children's Nutrition Research Centre
Royal Children's Hospital
Herston QLD 4029

Dear Professor Truby,

HREC Reference number: HREC/09/QRCH/86
Project title: Investigating the relationship between genotype and phenotype , a retrospective analysis.

Thank you for submitting the above project for ethical and scientific review.

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) *National Statement on Ethical Conduct in Human Research (2007), NHMRC and Universities Australia Australian Code for the Responsible Conduct of Research (2007)* and the *CPMP/ICH Note for Guidance on Good Clinical Practice*. Attached is the HREC Composition with specialty and affiliation with the Hospital (Attachment I).

I am pleased to advise that the Human Research Ethics Committee has granted approval of this research project. The documents reviewed and approved include:

Document	*Version*	*Date*
Covering Letter		
Application: Low Risk Application		

The HREC wishes you every success in your research.

With kind regards,

[redacted]

Professor John Pearn
Chair
Children's Health Services District Ethics Committee

Cc: Ethics Committee Files
Members of the Ethics Committee

Appendix 5

Size of exons in the Dystrophin gene used in the analysis for Chapter 2

Exon Number	Exon size (base pairs)	Exon Number	Exon size (base pairs)	Exon Number	Exon size (base pairs)
1	275	27	183	54	155
2	62	28	135	55	190
3	93	29	150	56	173
4	78	30	162	57	157
5	93	31	111	58	121
6	173	32	174	59	269
7	119	33	156	60	147
8	182	34	171	61	79
9	129	35	180	62	61
10	189	36	129	63	62
11	182	37	171	64	75
12	151	38	123	65	202
13	120	39	138	66	86
14	102	40	153	67	158
15	108	41	183	68	167
16	180	42	195	69	112
17	176	43	173	70	137
18	124	44	149	71	39
19	88	45	176	72	66
20	242	46	148	73	66
21	181	47	150	74	159
22	146	48	186	75	244
23	213	49	102	76	124
24	114	50	109	77	93
25	156	51	233	78	32
26	171	52	118	79	2703

Appendix 6

Sets of brothers identified in the review of medical records and their genitive profile.

	State	Mutation	Exons involve in mutations
1	VIC	nonsense	8
2	VIC	deletion	51-52
3	VIC	deletion	47-50
4	VIC	duplication	52
5	VIC	deletion	30-47
6	VIC	deletion	56
7	QLD	deletion	50
8	QLD	nonsense	58
9	QLD	point	22
10	QLD	nonsense	39
11	QLD	duplication	14-17
12	QLD	deletion	51-53
13	QLD	deletion	3-43
14	QLD	unknown	

Appendix 7

Davidson ZE, Walker KZ, Truby H. Do Glucocorticosteroids Alter Vitamin D Status? A Systematic Review with Meta-Analyses of Observational Studies. J Clin Endocrinol Metab. 2012;97(3):738-44.

Appendix 8

The Royal Children's Hospital, Melbourne

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PARENT/GUARDIAN INFORMATION STATEMENT AND CONSENT FORM

HREC Project Number: **29075**

Research Project Title: **Investigating nutritional markers in children with neuromuscular disease: DMD participant**

Thank you for taking the time to read this Information Statement. This Information Statement and Consent Form are 7 pages long. Please make sure you have all the pages.

Your child is invited to participate in a research project that is explained below.

What is an Information Statement?
These pages tell you about the research project. It explains to you clearly and openly all the steps and procedures of the project. The information is to help you to decide whether or not you would like your child to take part in the research.

Please read this Information Statement carefully. You can ask us questions about anything in it. You may want to talk about the project with your family, friends or health care worker.

Participation in this research project is voluntary. If you don't want your child to take part, you don't have to. You can withdraw your child from the project at any time without explanation and this will not affect their access to the best available treatment options and care from The Royal Children's Hospital.

Once you have understood what the project is about, if you would like your child to take part please sign the consent form at the end of this information statement. You will be given a copy of this information and consent form to keep.

1. What is the research project about?

We already know that the body compartments, such as muscle and fat change in boys with neuromuscular disease, in particular Duchenne muscular dystrophy (DMD). Because the changes seen in DMD are not seen in other children, managing this from a nutritional point of view can be difficult. Boys with DMD can move between being over and underweight, but we are unsure of the best way to manage this.

We want to find out more about nutrition in boys with DMD living in Victoria. There is very little known about nutrition in DMD and there have been no studies that look at nutrition of boys with DMD living in Australia. We want to look at body composition (muscle and fat), energy requirements and Vitamin D levels in boys with DMD compared to healthy boys of similar age.

It is hoped that this information will lead to a better understanding of the impact DMD has nutritional status. The findings from this study will help inform doctors and dietitians of the best dietary management options in this group.

This is a pilot project. We estimate 20 – 40 boys with DMD living in Victoria who are attending the neuromuscular clinic at The Royal Children's Hospital will participate in this research project. We are also going to be looking at vitamin D levels in some of the brothers and sisters of the boys with DMD. The researchers have done the same project in Brisbane, Queensland.

A word on energy requirements:

The amount of energy your child takes in each day is decided by how much they eat and drink. The amount of energy they need and use up depends on the amount of activity they undertake each day.

We usually estimate energy requirements using standard equations. However, for children with NMD we are not aware of exactly how much energy is needed due to changes in their body composition. Our study is trying to investigate these needs.

A word on Vitamin D:

To date, results of the same study in boys living in Queensland have shown that many of the boys have low vitamin D measurements. Vitamin D is known as the "sunshine vitamin" as we usually get what we need from sun exposure. Although steroid therapy can affect vitamin D levels, the levels we are seeing are very low. We therefore wish to determine if there is an environmental effect. That is, are the boys getting enough sun exposure? We can see if environment does have an effect by comparing the vitamin D levels measured in boys with DMD to children living in the same area. Your other children may be asked to participate in the research to help has work this out.

2. Who are the researchers?

- Professor Helen Truby is a children's dietitian who works at Monash University.
- Dr Andrew Kornberg is a special children's doctor at The Royal Children's Hospital.
- Dr Monique Ryan is a special children's doctor at The Royal Children's Hospital.
- Ms Zoe Davidson is a children's dietitian. This project is part of her PhD program which she is completing through The University of Queensland.

3. Who is funding this research project?

The study is partly funded by the Children's Nutrition Research Centre, The University of Queensland and The Duchenne Foundation.

4. Why is my child being asked to be in this research project?

We are asking you child to take part in this project because he has been diagnosed with DMD.

5. What do we need to do to be in this research project?

Appointment at The Royal Children's Hospital

Your child needs to attend a one-off out-patient appointment at The Royal Children's Hospital. The appointment will take 2-3 hours and will usually be in the morning. It is very important that **your child has nothing to eat or drink (except water) before coming** to the appointment because a fasting blood sample is required. You will be given a voucher to buy breakfast for your child. Your child can continue with all medications and treatment during project involvement. The schedule for the appointment is outlined below.

1) Your child will have a fasting blood sample collected by a professional experienced in taking blood from children. They will need to collect about 10 mls (2 teaspoons) of blood from your son.
2) Your child will have breakfast.
3) Height, weight and waist and hip circumference will be measured.
4) A special machine that measures 'bioelectrical impedance' will be used to tell us how much water you child has in his body. A small harmless electrical current runs through the body for a few seconds. It is completely painless.

5) We will measure energy expenditure with doubly labelled water (DLW). DLW is water that has been 'labelled' with two naturally occurring and stable isotopes; deuterium and oxygen. By giving water with extra deuterium and oxygen, it boosts the levels which are already present in the body. These isotopes are non-toxic and the water is not dangerous or harmful to your child in anyway. DLW has been used on pre-term babies, infants, children, and adolescents. Your child will need to give a spot urine sample (10 mls) and then drink a small glass of the DLW water which is tasteless. The DLW stays in your child's body for 10 days. We will collect another urine sample (10 mls) after 5 hours.
6) Your child will have a dual energy x-ray absorptiometry (DXA) scan. This scan is painless, and will provide another measure of body composition (fat and muscle in the body), as well as information about your child's bone mineral density – a measure of bone health.
7) You will be provided with instructions to collect a urine sample over the next 10 days. Only one sample needs to be collected for each day. The urine samples will be picked up by the research team or a courier.

Other information we will collect includes:

- yours son's genotype (if this has been analysed)
- medical history
- physiotherapist assessments
- medications that your son is taking
- any nutritional supplements that your son is taking
- pubertal status

Parental Questionnaire
We would also like to know what parents see as the main nutritional concerns for their children. We would like to ask you a few questions whilst you are at The Royal Children's Hospital. This will take no more than 5 minutes.

At Home

Food Diary
You will need to fill out a food diary for 3 days recording everything that your son eats and drinks. Completion of the food diary will take approximately 10 minutes per day. We will collect this with the urine samples.

Food Frequency Questionnaire
This is a 5 minute questionnaire that asks you about types of food your son eats. We will collect this with the urine samples.

Physical Activity Diary
You will need to complete a physical activity diary for 4 days recording your sons' physical activity. The 4 day activity diary needs to be completed on days when you are collecting urine samples. We will collect this with the urine samples.

Sun Exposure Questionnaire
We would like to know how much sun your son is exposed to. To do this, we would like you to complete a 5 minute questionnaire. We will collect this with the urine samples.

Storage of blood
When your son has his blood test, we would like to keep some of the blood and store it at the Queensland University of Technology (QUT). This will allow us to explore if there are any special genes linked to low vitamin D levels in the future without doing further bloods tests. The blood sample will be stored at QUT for up to 15 years and will only be used to look at genetic factors associated with the production of vitamin D.

On the consent form you can consent to storage of blood. By doing this you allow us to look at the blood in the future for the purposes stated above and will we not ask for further consent from you. We will not release the blood for any other research purposes without your consent. If you do not wish for your son's blood to be stored for future use, you can consent to the blood test and we will simply discard blood after testing.

In The future

Collection of repeat vitamin D levels
If it is found that your son has a low vitamin D level, a repeat blood test should be done in the future as part of routine clinical monitoring. If this occurs, we wish to collect the results from this test and ask you if you have been using any vitamin supplements.

6. What are my child's alternatives to taking part in this project?

Your child doesn't have to take part if you don't want him to. If you and your child decide to participate, you are still free to withdraw at any time without giving a reason. If your son does not take part or withdraws it will not affect the standard of care he receives or any future relationship with The Royal Children's Hospital.

7. What are the possible benefits for my child?

Your son may not get any direct benefit from taking part. We will give you the results of all the tests we are doing. This may help to inform your son's nutritional management.

8. What are the benefits for other people in the future?

Currently there is no information collected routinely about the nutritional status of boys in Victoria with neuromuscular disease. By participating in this study you and your child can contribute to improving our understanding of body composition change in DMD and how this affects energy requirements. The information gathered in this study will help guide future research in this area as well as helping health professionals provide the best dietary advice for boys with DMD.

9. What are the possible risks, side-effects and/or discomforts?

The research involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2,000 micro Sieverts each year. The effective dose from this study is about 40 micro Sieverts. At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. The risk is believed to be minimal. For comparison, the radiation is equivalent to 1/3rd of a simple chest x-ray or about seven days worth of natural background radiation.

Some children may be nervous and anxious about the blood test and it may cause some physical discomfort. To help overcome this staff highly experienced in taking blood from children will be taking the blood sample and anaesthetic cream can be used where requested. Some children may experience bruising following a blood test. A bandaid can be provided for this.

There may be additional unforeseen or unknown risks that are not known to us. If new information about risks becomes known during the project you will be informed of this.

10. What are the possible inconveniences?

Possible inconveniences may include time off school and work to attend the appointment at the hospital. Also, there is a small amount of time required for questionnaire completion whilst at home.

11. What will be done to make sure my child's information is confidential?

Any information we collect from you and your child will remain confidential. We will use your child's information only for this research project. Only the researchers involved with this project and The Royal Children's Hospital Ethics Committee will have access to this information. We can disclose the information only with your permission, except as required by law. You have the right to access and request correction of your information in accordance with the Freedom of Information Act 1982 (Vic).

Blood and urine samples will be stored in an identifiable format. They will be stored in fridges in locked rooms. Urine will be stored at The University of Queensland and Monash University and blood at Monash University and QUT. Urine samples will be destroyed immediately after testing. Where consent is given, blood samples will be stored for up to 15 years. Only researchers involved with this project and approved scientists performing the required testing will have access to the samples. The University of Queensland will be said to "own" the samples.

Information will be re-identifiable. This means we will remove names from the information and use a special code number. Only the research team can match names to code numbers, if it is necessary to do so.

Paper questionnaires which will be stored in a locked filing cabinet at Monash University or The University of Queensland. Information will also be stored on a password-protected database through The University of Queensland. Only members of the research team and the Ethics Committee can have access to this information.

Information will be kept until the youngest participant turns 25 years old. After this time it will be destroyed. A copy of the results of the individual assessments will be placed in your child's medical record which is kept until your child turns 25 years.

Results will be published professional journals and presented at conferences. Names will never be used in any publications, reports or presentations.

12. Will we be informed of the results when the research project is finished?

We will send you a summary of the overall project results. We will also send your sons' test results. Results from future research with blood samples will not be sent.

If you would like more information about the project or if you need to speak to a member of the research team in an emergency please contact:

Name: Zoe Davidson

Contact telephone: [redacted]

Or you may email: [redacted]

If you have any concerns about the project or the way it is being conducted, and would like to speak to someone independent of the project, please contact:

Head of Department
Ethics and Research Department
Human Research Ethics Committee
The Royal Children's Hospital
Telephone: (03) 9345 5044

The Royal Children's Hospital, Melbourne

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CONSENT FORM FOR PARENT/GUARDIAN TO GIVE INFORMED CONSENT FOR THEIR CHILD TO TAKE PART IN A RESEARCH PROJECT

HREC Project Number:

Research Project Title: **Investigating nutritional markers in children with neuromuscular disease**

I (Parent/Guardian name) _____

of (child's name) _____

voluntarily consent for my child to take part in the above research project

- I believe I understand the purpose, extent and possible effects of my child's involvement in this project.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I understand that this project has been approved by The Royal Children's Hospital Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007).
- I understand I will receive a copy of this Parent/Guardian Information Statement and Consent Form.

Parent/Guardian Signature _____ **Date** _____

Print name of witness to parent/guardian's signature

_____

Witness Signature _____ **Date** _____

I have explained the project to the parent/guardian who has signed above, and believe that they understand the purpose, extent and possible effects of their child's involvement in this project.

Researcher Signature _____ **Date** _____

Note: All parties signing the Consent Form must date their own signature.

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PARENT/GUARDIAN CONSENT FORM FOR RESEARCH INVOLVING BLOOD OR TISSUE OR GENETIC SAMPLES

HREC Project Number:

Research Project Title: **Investigating nutritional markers in children with neuromuscular disease**

I (Parent/Guardian name) _____

of (child's name) _____

voluntarily consent to (tick applicable boxes below)

☐ The taking of a blood/tissue sample for use in this research project only.

☐ The taking of a blood/tissue sample for use in **future ethically approved** research projects. I understand that **I WILL NOT** be contacted when my child's blood/tissue sample is used in future research and by consenting now, I consent to all future uses of my child's blood/tissue sample.

Parent/Guardian Signature _____ **Date** _____

Print name of witness to parent/guardian's signature

_____

Witness Signature _____ **Date** _____

I have explained the project to the parent/guardian who has signed above, and believe that they understand the purpose, extent and possible effects of their child's involvement in this project.

Researcher Signature _____ **Date** _____

Note: All parties signing the Consent Form must date their own signature.

Appendix 9

The Royal Children's Hospital, Melbourne

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PARENT/GUARDIAN INFORMATION STATEMENT AND CONSENT FORM

HREC Project Number: **29075**

Research Project Title: **Investigating nutritional markers in children with neuromuscular disease: Siblings**

Thank you for taking the time to read this Information Statement. This Information Statement and Consent Form are 6 pages long. Please make sure you have all the pages.

Your child is invited to participate in a research project that is explained below.

What is an Information Statement?
These pages tell you about the research project. It explains to you clearly and openly all the steps and procedures of the project. The information is to help you to decide whether or not you would like your child to take part in the research.

Please read this Information Statement carefully. You can ask us questions about anything in it. You may want to talk about the project with your family, friends or health care worker.

Participation in this research project is voluntary. If you don't want your child to take part, you don't have to. You can withdraw your child from the project at any time without explanation and this will not affect their access to the best available treatment options and care from The Royal Children's Hospital.

Once you have understood what the project is about, if you would like your child to take part please sign the consent form at the end of this information statement. You will be given a copy of this information and consent form to keep.

---

13. What is the research project about?
We already know that the body compartments, such as muscle and fat change in boys with neuromuscular disease, in particular Duchenne muscular dystrophy (DMD). Because the changes seen in DMD are not seen in other children, managing this from a nutritional point of view can be difficult. Boys with DMD can move between being over and underweight, but we are unsure of the best way to manage this.

We want to find out more about nutrition in boys with DMD living in Victoria. There is very little known about nutrition in DMD and there have been no studies that look at nutrition of boys with DMD living in Australia. We want to look at body composition (muscle and fat), energy requirements and Vitamin D levels in boys with DMD compared to healthy boys of similar age.

It is hoped that this information will lead to a better understanding of the impact that DMD has on nutritional status. The findings from this study will help inform doctors and dietitians of the best dietary management options in this group.

Your child has been invited to participate in this study as a healthy 'control'. This means his/her

measurements will be compared to the measurements of young boys who have DMD. Control data is used in research to look at differences between groups. This would help us to understand the differences in nutritional status between children who have neuromuscular disease and those who do not.

Your son with DMD has participated or will be participating in another arm of this study. To date, results of this part of the study from boys in Queensland have shown that many of the boys have low vitamin D measurements. Vitamin D is known as the "sunshine vitamin" as we usually get what we need from sun exposure. Although steroid therapy can affect vitamin D levels, the levels we are seeing are very low. We therefore wish to determine if there is an environmental effect. That is, are the boys getting enough sun exposure? We can see if environment does have an effect by comparing the vitamin D levels measured in boys with DMD to those of children living in the same area. Unfortunately, we do not have any information about vitamin D levels of children living in Victoria. We wish to invite a sibling of your son to participate in this study as a healthy 'control' for vitamin D measurements. This would help us to understand if there is an environmental effect on vitamin D levels in boys living in Queensland. Vitamin D is measured in a single blood test. We would also like to compare the amount of sun exposure that both you son with DMD and your other children receive.

This is a pilot project. We estimate 40-80 siblings of boys with DMD living in Victoria will participate in this research project. The researchers have done the same project in Brisbane, Queensland.

14. Who are the researchers?

- Professor Helen Truby is a children's dietitian who works at Monash University.
- Dr Andrew Kornberg is a special children's doctor at The Royal Children's Hospital.
- Dr Monique Ryan is a special children's doctor at The Royal Children's Hospital.
- Ms Zoe Davidson is a children's dietitian. This project is part of her PhD program which she is completing through The University of Queensland.

15. Who is funding this research project?

The study is being partially funded by the Children's Nutrition Research Centre, The University of Queensland and The Duchenne Foundation.

16. Why is my child being asked to be in this research project?

We are asking you child to take part in this project because he/she has a brother who has been diagnosed with DMD.

17. What do we need to do to be in this research project?

<u>Appointment at The Royal Children's Hospital</u>

Your child will need to come to Royal Children's Hospital for a blood test to measure vitamin D. They will need to collect about 10 mls (2 teaspoons) of blood from your child. **It is very important that your child has nothing to eat or drink (except water) before coming to the appointment.** The total time spent at the hospital should be less than an hour. The test itself will only take 10 minutes but sometimes there is a short wait at pathology.

Sun Exposure Questionnaire
We would like to know how much sun your child is exposed to. To do this, we would like you to complete a 5 minute questionnaire. We will arrange to collect the survey from you in person or by mail.

Storage of blood
When your child has their blood test, we would like to keep some of the blood and store it at the

Queensland University of Technology (QUT). This will allow us to explore if there are any special genes linked to low vitamin D levels in the future without doing further bloods tests. The blood sample will be stored at QUT for up to 15 years and will only be used to look at genetic factors associated with the production of vitamin D.

On the consent form you can consent to storage of blood. By doing this you allow us to look at the blood in the future for the purposes stated above and will we not ask for further consent from you. We will not release the blood for any other research purposes without your consent. If you do not wish for your son's blood to be stored for future use, you can consent to the blood test and we will simply discard blood after testing.

18. What are my child's alternatives to taking part in this project?
Your child doesn't have to take part if you don't want him/her to. If you and your child decide to participate, you are still free to withdraw at any time without giving a reason. If your child does not take part or withdraws it will not affect the standard of care he/she receives or any future relationship with The Royal Children's Hospital.

19. What are the possible benefits for my child?
Your child may not get any direct benefit from taking part. We will give you the results of all the tests we are doing and you will find out your child's Vitamin D level.

20. What are the benefits for other people in the future?
Currently there is no information collected routinely about the nutritional status of boys in Victoria with neuromuscular disease. By participating in this study you and your child can contribute to improving our understanding of body composition change in DMD and how this affects energy requirements. The information gathered in this study will help guide future research in this area as well as helping health professionals provide the best dietary advice for boys with DMD.

21. What are the possible risks, side-effects and/or discomforts?
The risks of taking part in the study are very minimal. Some children may be nervous and anxious about the blood test and it may cause some physical discomfort. To help overcome this staff highly experienced in taking blood from children will be taking the blood sample and anaesthetic cream can be used where requested. Some children may experience bruising following a blood test. A bandaid can be provided for this.

There may be additional unforeseen or unknown risks that are not known to us. If new information about risks becomes known during the project you will be informed of this.

22. What are the possible inconveniences?
Possible inconveniences may include time off school and work to attend the appointment at the hospital. Also, there is a small amount of time required for questionnaire completion whilst at home.

23. What will be done to make sure my child's information is confidential?
Any information we collect from you and your child will remain confidential. We will use your child's information only for this research project. Only the researchers involved with this project and The Royal Children's Hospital Ethics Committee will have access to this information. We can disclose the information only with your permission, except as required by law. You have the right to access and request correction of your information in accordance with the Freedom of Information Act 1982 (Vic).

Blood and urine samples will be stored in an identifiable format. They will be stored in fridges in locked rooms. Urine will be stored at The University of Queensland and Monash University and blood at Monash University and QUT. Urine samples will be destroyed immediately after testing. Where consent is given, blood samples will be stored for up to 15 years. Only researchers involved with this project and approved scientists performing the required testing will have access to the samples. The University of Queensland will be said to "own" the samples.

Information will be re-identifiable. This means we will remove names from the information and use a special code number. Only the research team can match names to code numbers, if it is necessary to do so.

Paper questionnaires which will be stored in a locked filing cabinet at Monash University or The University of Queensland. Information will also be stored on a password-protected database through The University of Queensland. Only members of the research team and the Ethics Committee can

have access to this information.

Information will be kept until the youngest participant turns 25 years old. After this time it will be destroyed. A copy of the results of the individual assessments will be placed in your child's medical record which is kept until your child turns 25 years.

Results will be published professional journals and presented at conferences. Names will never be used in any publications, reports or presentations.

24. Will we be informed of the results when the research project is finished?
We will send you a summary of the overall project results. We will also send your sons' test results. Results from future research with blood samples will not be sent.

If you would like more information about the project or if you need to speak to a member of the research team in an emergency please contact:

Name: Zoe Davidson

Contact telephone: [redacted]

Or you may email:[redacted]

If you have any concerns about the project or the way it is being conducted, and would like to speak to someone independent of the project, please contact:

Head of Department
Ethics and Research Department
Human Research Ethics Committee
The Royal Children's Hospital
Telephone: (03) 9345 5044

The Royal Children's Hospital, Melbourne

Flemington Road, Parkville
Victoria, Australia, 3052

Telephone (03) 9345 5522
ISD (+613) 9345 5522
Facsimile (03) 9345 5789
Web www.rch.org.au

CONSENT FORM FOR PARENT/GUARDIAN TO GIVE INFORMED CONSENT FOR THEIR CHILD TO TAKE PART IN A RESEARCH PROJECT

HREC Project Number:

Research Project Title: **Investigating nutritional markers in children with neuromuscular disease**

I (Parent/Guardian name) _____

of (child's name) _____

voluntarily consent for my child to take part in the above research project

- I believe I understand the purpose, extent and possible effects of my child's involvement in this project.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I understand that this project has been approved by The Royal Children's Hospital Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007).
- I understand I will receive a copy of this Parent/Guardian Information Statement and Consent Form.

Parent/Guardian Signature _____ **Date** _____

Print name of witness to parent/guardian's signature

_____

Witness Signature _____ **Date** _____

I have explained the project to the parent/guardian who has signed above, and believe that they understand the purpose, extent and possible effects of their child's involvement in this project.

Researcher Signature _____ **Date** _____

Note: All parties signing the Consent Form must date their own signature.

The Royal Children's Hospital, Melbourne

Flemington Road, Parkville
Victoria, Australia, 3052

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PARENT/GUARDIAN CONSENT FORM FOR RESEARCH INVOLVING BLOOD OR TISSUE OR GENETIC SAMPLES

HREC Project Number:

Research Project Title: **Investigating nutritional markers in children with neuromuscular disease**

I (Parent/Guardian name) _____

of (child's name) _____

voluntarily consent to (tick applicable boxes below)

☐ The taking of a blood/tissue sample for use in this research project only.

☐ The taking of a blood/tissue sample for use in **future ethically approved** research projects. I understand that **I WILL NOT** be contacted when my child's blood/tissue sample is used in future research and by consenting now, I consent to all future uses of my child's blood/tissue sample.

Parent/Guardian Signature _____ **Date** _____

Print name of witness to parent/guardian's signature

_____

Witness Signature _____ **Date** _____

I have explained the project to the parent/guardian who has signed above, and believe that they understand the purpose, extent and possible effects of their child's involvement in this project.

Researcher Signature _____ **Date** _____

Note: All parties signing the Consent Form must date their own signature.

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SUN EXPOSURE QUESTIONNAIRE

SECTION 1: ABOUT YOUR SON

1. What is the age of your son in years?

_____

2. Is your son able to walk? Yes/No (please circle)

3. Does your son attend school 5 days each week? Yes/No (Please circle)

If no, how many times does your son attend school each week?

_____

4. Is your son currently taking steroid medication? Yes/No (please circle

If yes, how much does your son take daily?

_____

SECTION 2: YOUR SKIN AND THE SUN

The following questions ask about your child's skin and how it reacts to the sun.

5. How does your child's skin react when they sit in the sun in your current area of residence, in the middle of the day, for the first time in summer, without sunscreen? *(please tick one box)*

- ☐ Never burn
- ☐ Burn after more than 2 hours sun exposure
- ☐ Burn after 1 –2 hours
- ☐ Burn after ½ - 1 hour
- ☐ Burn within half an hour

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SUN EXPOSURE QUESTIONNAIRE (SIBLING)

SECTION 1: ABOUT YOUR CHILD

1. What is the age of your child in years?

_____

2. Does your child attend school 5 days each week? Yes/No (Please circle)

If no, how many times does your child attend school each week?

_____

SECTION 2: YOUR SKIN AND THE SUN

The following questions ask about your child's skin and how it reacts to the sun.

5. How does your child's skin react when they sit in the sun in your current area of residence, in the middle of the day, for the first time in summer, without sunscreen? *(please tick one box)*

- ☐ Never burn
- ☐ Burn after more than 2 hours sun exposure
- ☐ Burn after 1 –2 hours
- ☐ Burn after ½ - 1 hour
- ☐ Burn within half an hour

6. At the end of summer or after a two-week holiday in the sun, what kind of a tan would your child have? *(please tick one box)*

- ☐ A dark tan
- ☐ A medium tan
- ☐ Light tan
- ☐ Practically no tan

6. At the end of summer or after a two-week holiday in the sun, what kind of a tan would your child have? *(please tick one box)*

- ☐ A dark tan
- ☐ A medium tan
- ☐ Light tan
- ☐ Practically no tan

7. How does your child's skin react when they go out in the sun in your current area of residence, for one hour in the middle of the day, for the first time in summer, without sunscreen? *(please tick one box)*

- ☐ Burn then peel
- ☐ Burn then tan
- ☐ Tan only

SECTION 3: TIME SPENT IN THE SUN

The following questions ask about your child's indoor and outdoor activities and your child's sun exposure.

8. Thinking about the PAST MONTH, we would like to know the TIMES OF DAY as well as the USUAL LENGTH OF TIME that your child would spend OUTSIDE between sunrise and sunset on:

8a. A typical **MONDAY** *(Tick one response for EACH time period)*

	Length of time spent outside				
	Never	<15 minutes	15-30 minutes	30-45 minutes	45-60 minutes
Morning (am)					
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐
7-8	☐	☐	☐	☐	☐
8-9	☐	☐	☐	☐	☐
9-10	☐	☐	☐	☐	☐
10-11	☐	☐	☐	☐	☐
11-12	☐	☐	☐	☐	☐
Afternoon (pm)					
12-1	☐	☐	☐	☐	☐
1-2	☐	☐	☐	☐	☐
2-3	☐	☐	☐	☐	☐
3-4	☐	☐	☐	☐	☐
4-5	☐	☐	☐	☐	☐
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐

8b. A typical **TUESDAY** *(Tick one response for EACH time period)*

	Length of time spent outside				
	Never	<15 minutes	15-30 minutes	30-45 minutes	45-60 minutes
Morning (am)					
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐
7-8	☐	☐	☐	☐	☐
8-9	☐	☐	☐	☐	☐
9-10	☐	☐	☐	☐	☐
10-11	☐	☐	☐	☐	☐
11-12	☐	☐	☐	☐	☐
Afternoon (pm)					
12-1	☐	☐	☐	☐	☐
1-2	☐	☐	☐	☐	☐
2-3	☐	☐	☐	☐	☐
3-4	☐	☐	☐	☐	☐
4-5	☐	☐	☐	☐	☐
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐

8c. A typical **WEDNESDAY** *(Tick one response for EACH time period)*

	Length of time spent outside				
	Never	<15 minutes	15-30 minutes	30-45 minutes	45-60 minutes
Morning (am)					
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐
7-8	☐	☐	☐	☐	☐
8-9	☐	☐	☐	☐	☐
9-10	☐	☐	☐	☐	☐
10-11	☐	☐	☐	☐	☐
11-12	☐	☐	☐	☐	☐
Afternoon (pm)					
12-1	☐	☐	☐	☐	☐
1-2	☐	☐	☐	☐	☐
2-3	☐	☐	☐	☐	☐
3-4	☐	☐	☐	☐	☐
4-5	☐	☐	☐	☐	☐
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐

8d. A typical **THURSDAY** *(Tick one response for EACH time period)*

	Length of time spent outside				
	Never	<15 minutes	15-30 minutes	30-45 minutes	45-60 minutes
Morning (am)					
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐
7-8	☐	☐	☐	☐	☐
8-9	☐	☐	☐	☐	☐
9-10	☐	☐	☐	☐	☐
10-11	☐	☐	☐	☐	☐
11-12	☐	☐	☐	☐	☐
Afternoon (pm)					
12-1	☐	☐	☐	☐	☐
1-2	☐	☐	☐	☐	☐
2-3	☐	☐	☐	☐	☐
3-4	☐	☐	☐	☐	☐
4-5	☐	☐	☐	☐	☐
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐

8e. A typical **FRIDAY** *(Tick one response for EACH time period)*

	Length of time spent outside				
	Never	<15 minutes	15-30 minutes	30-45 minutes	45-60 minutes
Morning (am)					
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐
7-8	☐	☐	☐	☐	☐
8-9	☐	☐	☐	☐	☐
9-10	☐	☐	☐	☐	☐
10-11	☐	☐	☐	☐	☐
11-12	☐	☐	☐	☐	☐
Afternoon (pm)					
12-1	☐	☐	☐	☐	☐
1-2	☐	☐	☐	☐	☐
2-3	☐	☐	☐	☐	☐
3-4	☐	☐	☐	☐	☐
4-5	☐	☐	☐	☐	☐
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐

8f. A typical **SATURDAY** *(Tick one response for EACH time period)*

	Length of time spent outside				
	Never	<15 minutes	15-30 minutes	30-45 minutes	45-60 minutes
Morning (am)					
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐
7-8	☐	☐	☐	☐	☐
8-9	☐	☐	☐	☐	☐
9-10	☐	☐	☐	☐	☐
10-11	☐	☐	☐	☐	☐
11-12	☐	☐	☐	☐	☐
Afternoon (pm)					
12-1	☐	☐	☐	☐	☐
1-2	☐	☐	☐	☐	☐
2-3	☐	☐	☐	☐	☐
3-4	☐	☐	☐	☐	☐
4-5	☐	☐	☐	☐	☐
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐

8g. A typical **SUNDAY** *(Tick one response for EACH time period)*

	Length of time spent outside				
	Never	<15 minutes	15-30 minutes	30-45 minutes	45-60 minutes
Morning (am)					
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐
7-8	☐	☐	☐	☐	☐
8-9	☐	☐	☐	☐	☐
9-10	☐	☐	☐	☐	☐
10-11	☐	☐	☐	☐	☐
11-12	☐	☐	☐	☐	☐
Afternoon (pm)					
12-1	☐	☐	☐	☐	☐
1-2	☐	☐	☐	☐	☐
2-3	☐	☐	☐	☐	☐
3-4	☐	☐	☐	☐	☐
4-5	☐	☐	☐	☐	☐
5-6	☐	☐	☐	☐	☐
6-7	☐	☐	☐	☐	☐

9. Is the PATTERN of sun exposure described above FOR THE PAST MONTH similar to your child's pattern of exposure over the previous months?

Yes ☐ *Go to Question 11*
No ☐

10a. Does your child usually spend:

MORE time in the sun? OR ☐
LESS time in the sun? ☐

10b.Please tell us WHY your child's pattern of sun exposure has been DIFFERENT over the PAST MONTH?

Was he in hospital or respite care?	☐	*If yes,* What was the duration of the stay?
Was he on holidays? *(describe time and location)*	☐	*If yes,* Was his sun exposure while on holidays: More than usual? ☐ Less than usual ? ☐
Did his daily routine change so that he is:		
more outdoors	☐	
less outdoors	☐	
Other	☐	Please describe:

SECTION 4: SUN-PROTECTIVE BEHAVIOUR

The following questions ask about how you protect your child from the sun.

11.We are interested in the CLOTHING AND SUN PROTECTION that your child may have worn in the PAST MONTH. During the past month, *when your child was outside*, your child would …

	Never/Rarely	Less than half the time	More than half the time	Always/almost always
11a.Wear a broad-brimmed hat?	☐	☐	☐	☐
11b. Wear a cap?	☐	☐	☐	☐
11c. Wear any other head covering?	☐	☐	☐	☐
11d. Wear a shirt with long sleeves?	☐	☐	☐	☐
11e. Wear long trousers or clothing that covers all or most of your legs?	☐	☐	☐	☐
11f. Wear sunglasses?	☐	☐	☐	☐

12. Has your child used sunscreen in the PAST MONTH?

Yes ☐
No ☐ *Finish questionnaire here*

12a. What is the sun-protection factor (SPF) of the sunscreen that your child has usually used? (e.g. SPF 30+)

SPF? ☐

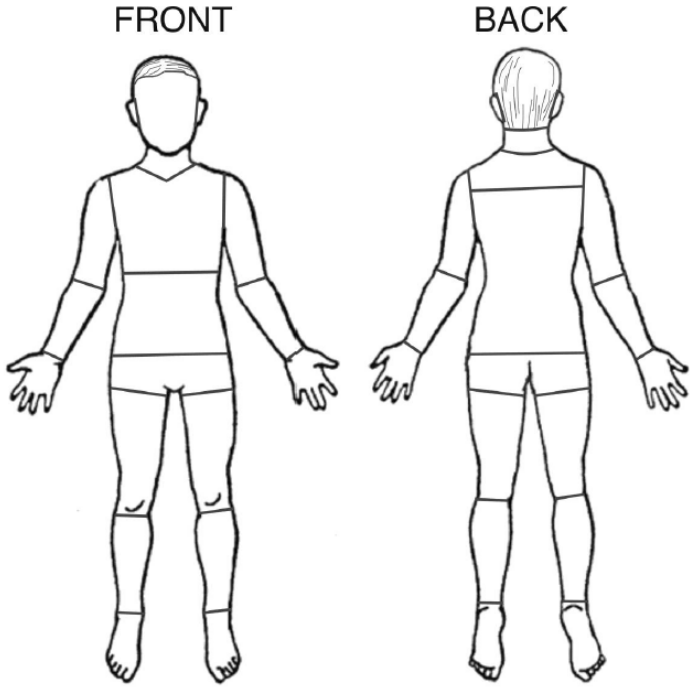
12b. Over the PAST MONTH, how often on average has your child used sunscreen?

Every day ☐
5-7 days/week ☐
3-4 days/week ☐
1-2 days/week ☐
Less than once a week ☐

12c. On the days that your child has used sunscreen in the PAST MONTH, *how often* did they apply it?

☐ Times per day

12d. Over the **PAST MONTH**, when your child used sunscreen, **WHERE** did you/they usually apply it?
(Please shade the parts of the body where sunscreen is usually applied).



Appendix 11

The **Royal Children's Hospital** Melbourne

RCH HUMAN RESEARCH ETHICS COMMITTEE APPROVAL

HREC REF. No:	**30022 A**
PROJECT TITLE:	Nutriceuticals in Duchenne muscular dystrophy: a randomised controlled trial of a multicomponent nutritional supplement
DOCUMENTS APPROVED:	P/GIS & Consent Form v5 dated 19 May 2010 Recruitment Letter/Email v1 dated 17 February 2010 Phone Recruitment Script v1 dated 17 February 2010 Food Diary v1 dated 17 June 2009 PedsQL Parent Report for Young Children (ages 5-7) (validated) PedsQL Parent Report for Children (ages 8-12) (validated) PedsQL Parent Report for Teens (ages 13-18) (validated) PedsQL NM Parent Report for Young Children (ages 5-7) (validated) PedsQL NM Parent Report for Child (ages 8-12) (validated) PedsQL NM Parent Report for Teens (ages 13-18) (validated) ACTIVLIM (validated)
APPROVED PROTOCOL:	Technical Protocol v4 dated 21 May 2010
PRINCIPAL INVESTIGATOR:	Monique Ryan
DATE OF ORIGINAL APPROVAL:	**20 October 2010**
DURATION:	[redacted]
DATE OF	[redacted]
SIGNED:	[redacted] 21/10/10.

COMMITTEE REPRESENTATIVE

APPROVED SUBJECT TO THE FOLLOWING CONDITIONS:

ALL PROJECTS

1. Must comply with the Investigator's Responsibilities in Research available at http://www.rch.org.au/emplibrary/ethics/Investigators_Responsibilities_in_Research.pdf
2. Any proposed change in the protocol or approved documents or the addition of documents (including flyers, brochures, advertising material etc) must be submitted to the Human Research Ethics Committee (HREC) for approval prior to implementation.
3. The Principal Investigator must notify Ethics & Research of:
   - Any serious adverse effects of the study on participants and steps taken to deal with them.
   - Any unforeseen events (e.g. protocol violations or complaints).
   - Investigators withdrawing from or joining the project.
4. A progress report must be submitted annually and at the conclusion of the project.
5. RCH HREC approval must remain current for the entire duration of the project. If the project is not completed in the allocated time a renewal request must be submitted to the Ethics & Research Department. Investigators undertaking projects without current HREC approval risk their indemnity, funding and publication rights.

CLINICAL TRIALS

6. Must comply with Good Clinical Practice (GCP) available at http://www.tga.gov.au/docs/pdf/euguide/ich/ich13595.pdf
7. Must report all internal (occurring in RCH participants) Serious Adverse Events (SAE) to the sponsor and the RCH HREC within 72 hours of occurrence.
8. Must report all Suspected Unexpected Serious Adverse Reactions (SUSARS) to the Therapeutic Goods Administration (TGA) (for sponsored studies the sponsor may take this responsibility).

stock no 2478
ERC 080426

Appendix 12

MONASH University

Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

Date:	**17 November 2010**
Project Number:	**2010001692**
Project Title:	**Nutriceuticals in Duchenne muscular dystrophy: a randomised controlled trial of a multicomponent nutritional supplement**
Chief Investigator:	**Prof Helen Truby**
Approved:	**From: 17 November 2010 To: 17 November 2015**

Terms of approval

1. The Chief investigator is responsible for ensuring that permission letters are obtained, if relevant, and a copy forwarded to MUHREC before any data collection can occur at the specified organisation. **Failure to provide permission letters to MUHREC before data collection commences is in breach of the National Statement on Ethical Conduct in Human Research and the Australian Code for the Responsible Conduct of Research**.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by MUHREC.
4. You should notify MUHREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. The Explanatory Statement must be on Monash University letterhead and the Monash University complaints clause must contain your project number.
6. **Amendments to the approved project (including changes in personnel):** Requires the submission of a Request for Amendment form to MUHREC and must not begin without written approval from MUHREC. Substantial variations may require a new application.
7. **Future correspondence:** Please quote the project number and project title above in any further correspondence.
8. **Annual reports:** Continued approval of this project is dependent on the submission of an Annual Report. This is determined by the date of your letter of approval.
9. **Final report:** A Final Report should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected date of completion.
10. **Monitoring:** Projects may be subject to an audit or any other form of monitoring by MUHREC at any time.
11. **Retention and storage of data:** The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.

[redacted]

Professor Ben Canny
Chair, MUHREC

cc: Dr Monique Ryan; Assoc Prof Andrew Kornberg; Ms Zoe Davidson

Postal – Monash University, Vic 3800, Australia
Building 3E, Room 111, Clayton Campus, Wellington Road, Clayton
Telephone +61 3 9905 5490 Facsimile +61 3 9905 3831
Email muhrec@adm.monash.edu.au www.monash.edu/research/ethics/human/index/html
ABN 12 377 614 012 CRICOS Provider #00008C

Appendix 13

The Royal Children's Hospital, Melbourne

Flemington Road, Parkville
Victoria, Australia, 3052

Telephone (03) 9345 5522
ISD (+613) 9345 5522
Facsimile (03) 9345 5789
Web www.rch.org.au

PARENT/GUARDIAN INFORMATION STATEMENT AND CONSENT FORM

HREC Project Number: **30022**

Research Project Title:
- **Nutriceuticals in Duchenne muscular dystrophy (DMD): Investigating the effectiveness of a multicomponent nutritional supplement**

Thank you for taking the time to read this Information Statement. This Information Statement and Consent Form is 6 pages long. Please make sure you have all the pages.

Your child is invited to participate in a research project that is explained below.

What is an Information Statement?
These pages tell you about the research project. It explains to you clearly and openly all the steps and procedures of the project. The information is to help you to decide whether or not you would like your child to take part in the research.

Please read this Information Statement carefully. You can ask us questions about anything in it. You may want to talk about the project with your family, friends or health care worker.

Participation in this research project is voluntary. If you don't want your child to take part, you don't have to. You can withdraw your child from the project at any time without explanation and this will not affect their access to the best available treatment options and care from The Royal Children's Hospital.

Once you have understood what the project is about, if you would like your child to take part please sign the consent form at the end of this information statement. You will be given a copy of this information and consent form to keep.

---

1. What is the research project about?
This study tests the use of a nutritional supplement over a 10-month period in boys with DMD to see if it can improve or maintain functional ability and muscle mass. Nutritional supplements together with steroid therapy could provide a simple and effective strategy to maintain muscle function and mobility and enhance quality of life for children with DMD.

The supplements that we plan to use in this study are creatine monohydrate (CrM), glutamine and beta-hydroxy-beta-methylbutyrate (HMB) (contained within a high protein shake), and multivitamin, vitamin D and fish oil tablets.

These supplements have been chosen because some evidence exists of their potential benefit on their own. However they have not been tested together.

This is a pilot project. We aim to recruit 40 boys with DMD aged around 5 – 13 years who are attending the neuromuscular clinic at The Royal Children's Hospital. We may recruit boys from other centres as well.

The CrM, glutamine, HMB, multivitamin, vitamin D and fish oil supplements are listed on the Australian Register of Therapeutic Goods. The listings do not provide any warnings about the age groups these products should be used in. However, the use of these supplements together is experimental.

2. Who are the researchers?

- Professor Helen Truby is a children's dietitian who works at Monash University.
- Dr Andrew Kornberg is a children's doctor at The Royal Children's Hospital.
- Dr Monique Ryan is a children's doctor at The Royal Children's Hospital.
- Zoe Davidson is a children's dietitian. This project is part of her PhD program which she is completing through Monash University.
- Aimee Segman is a children's dietitian. This project is part of her honours program which she is completing through Monash University.

3. Who is funding this research project?

This study is partly funded by Monash University. The supplements have been donated to the study by Blackmores and Musashi.

4. Why is my child being asked to be in this research project?

We are asking your son to take part in this study because he has DMD.

5. What does my child need to do to be in this research project?

Your son will be involved in the study for 12 months. The first 2 months will consist simply of monitoring. The active diet supplement duration period is 10 months.

All boys who participate in the study will receive the standard supplement which contains a protein shake, a multivitamin supplement, a vitamin D supplement and fish oil for the entire 10-month supplementation period.

Randomisation:

At the start of the study we will place your son into one of two groups. This will be done by chance, similar to tossing a coin, so your son will have an equal chance of being in either group.

One group will take the standard supplement with CrM/glutamine/HMB and the other group will take the standard supplement with a placebo powder (maltodextrin) added to it. You will not know which group your son has been placed in, however the research team will know. Your son will take their supplements for 5 months, then the two groups will "cross over" or swap, so that if your son was taking the standard supplement with CrM/glutamine/HMB supplement he will now take the standard supplement with placebo and vice versa.

There will be a washout period of two weeks when the groups cross over. During this two weeks, your son continue to take the standard supplement.

Tests and procedures:

At the start of the study we will ask you about:

- your son's genotype (if this has been analysed)
- your son's medical history
- medications that your son is taking
- pubertal status

If your son is taking a complementary medicine, he will need to stop taking it for the duration of the study.

During the course of the study, your son will need to visit The Royal Children's Hospital on 7 occasions. Each visit will take between 1-2 hours and if possible, will be organised with other hospital appointments. The visit schedule is summarized in the table below. Most supplements will be collected at study visits, however the research dietitian will home deliver supplements at week 20 and week 42 in the study,

	Visit 1 Week 0	Visit 2 Week 8	Visit 3 Week 12	Visit 4 Week 28	Visit 5 Week 30	Visit 6 Week 34	Visit 7 Week 50
Step Activity Monitoring	●	●		●	●		●
Fasting blood sample	●	●	●	●	●	●	●
6-minute walk test	●	●		●	●		●
Height and weight measurement	●	●		●	●		●
DXA scan	●			●			●
Assessment by doctor		●	●	●	●	●	●
Food Diary	●	●		●	●		●
Parent questionnaires	●	●		●	●		●

Step Activity Monitoring:
Your son will wear a StepWatch™ monitor strapped to his ankle for five days. This is a light weight monitor that records the number of steps he does in a day. We do not expect your son to notice he is wearing it. The monitor will need to be worn for the entire day, that is from when he gets out of bed in the morning until he goes to bed, with the exclusion of showers/swimming. At five points in the 12 month study, your son will be asked to wear an activity monitor for 5 consecutive days.

Fasting blood sample:
Your son will need to fast (no food) from 12am the night before their visit. At the visit a 5ml (1 teaspoon) sample of blood will be taken. Once the blood has been analysed it will be destroyed. There will no storage of blood involved with this study.

DXA scan:
This scan is painless, and will provide a measure of body composition (fat and muscle in the body), as well as information about your child's bone mineral density – a measure of bone health. When your son has a scan, he will be asked to lie on the bed. A scanner will move up and down his body. It is similar to having an x-ray.

Doctor assessment:
This will involve a doctor from RCH taking your son's blood pressure, heart rate and feeling their stomach.

Food Diary
A three day food diary will need to be completed at five separate time points during the study. This involves writing down all the food and drink your son has had over these 3 days.

Parent questionnaires:
You will be asked to complete three questionnaires asking about activity quality of life and one questionnaire asking about functional ability. The questionnaires can be completed at home after your son's visit. You will be provided with a stamped envelope to return the questionnaires. We estimate the total time to complete ALL questionnaires to be 20 minutes maximum. The questionnaires are multiple choice so you are not required to write detailed answers.

Supplementation:
Each day, during the 10-month supplementation period, all boys will be receiving the standard supplement. Based on randomization (see above) your son will or will not have the active ingredients included in his shake. Your son will need to consume:

- Protein shake
  - The shake is provided in the form of a powder and can be mixed with milk and flavoured as desired.
  - The shake may be a meal replacement eg. Breakfast. However, a dietitian will instruct you on what other foods should be eaten at the meal with the protein shake, depending on your son's requirements.
  - The base shake contains 22g whey protein powder, maltodextrin powder and sucralose (artificial sweetener). The shake will also contain the active mixture of creatine monohydrate/glutamine for 5 months throughout the study period.

- HMB capsules
    - The HMB will be provided in the form of capsules. Your son will need to take between 1 and 3 of these tablets depending on his weight.
    - When in the enhanced supplement phase, these capsules will contain HMB powder.
    - When in the standard supplement phase, these capsules will contain maltodextrin powder.

- Multivitamin tablets, vitamin D and fish oil.
    - Depending on your child's age, this will involve consumption of between 4 and 6 tablets across the day.
    - The multivitamin and fish oil tablets are chewable.

6. What are my child's alternatives to taking part in this project?
Your child doesn't have to take part if you don't want him to. If you and your child decide to participate, you are free to withdraw at any time without giving a reason. If your son withdraws from the study, we will not use data collected from your son in the analysis at the end of the study, unless some of the data relates to why he has withdrawn. We will record the reason why he has withdrawn if you wish to provide it. If your son does not take part or withdraws it will not affect the standard of care he receives or any future relationship with The Royal Children's Hospital.

7. What are the possible benefits for my child?
We are unable to say if your son will receive any benefit or not. We cannot guarantee any benefit. However, your son will be provided with a 10 month supply of a nutritional supplement that we hope may help his functional ability together with his standard therapies such as physiotherapy and/or steroid treatment.

8. What are the benefits for other people in the future?
There is very little information to support the use of nutritional supplements in children with neuromuscular disease. By participating in this study you and your child can contribute to improving our understanding of the usefulness of nutritional supplements in DMD. The information from this study will help guide future research in this area, as well as helping health professionals provide the best dietary advice for boys with DMD.

9. What are the possible risks, side-effects and/or discomforts?
There is a low risk associated with participation in this project. The risk is that your son might have a reaction when taking the supplement which could include a rash, a stomach upset or a change in blood markers of kidney function. It is unlikely that these side effects will occur at the doses used in the study however if they did, your son might require increased medical attention and possibly a hospital stay. If these events did occur, we expect that they would be resolved by stopping the supplement and we do not expect there to be any long term effects. There have been no reports of changes in kidney function in studies using CrM and glutamine in boys with DMD. A safety assessment will occur at five points through the study period to monitor your son's wellbeing. These assessments will include physical exam conducted by an RCH doctor and blood testing.

DXA Scan:
This research study involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisieverts (mSv) each year. The effective dose from this study is about 0.070mSv. At the dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. The risk is believed to be minimal.

Has your son participated in other studies that involve radiation? If so, please tell us.

Blood tests:
Some children may be nervous and anxious about the blood test and it may cause some physical discomfort. To help overcome this staff highly experienced in taking blood from children will be taking the blood sample and anaesthetic cream can be used where requested. Some children may experience bruising following a blood test. A bandaid can be provided for this.

There may be additional unforeseen or unknown risks that are not known to us. If new information about risks becomes known during the project you will be informed of this.

10. What are the possible inconveniences?
Possible inconveniences may include time off school and work to attend the visit at the hospital. Also, there is a small amount of time required for questionnaire completion at home after your visit. The cost of travel may also be an inconvenience.

It should be noted that the supplements used in this study are expensive. If you wish to continue the supplements when the study has finished, we are unable to provide you with money to do so, even if some benefit is shown. This may be an inconvenience experienced by families in the future.

11. What will be done to make sure my child's information is confidential?
Any information we collect from you and your child will remain confidential. We will use your child's information only for this research project. Only the researchers involved with this project and The Royal Children's Hospital Ethics Committee will have access to this information. As a notification will be made to the Australian Therapeutic Goods Administration (TGA) that this research project is occurring, they will also have access to the information.

We can disclose the information only with your permission, except as required by law.

You have the right to access and request correction of your son's information in accordance with the Freedom of Information Act 1982 (Vic).

Blood samples will be collected in an identifiable format and will be destroyed immediately after testing. Only researchers involved with this project and approved scientists performing the required testing will have access to the samples.

Information will be re-identifiable. This means we will remove names from the information and use a special code number. Only the research team can match names to code numbers, if it is necessary to do so.

Paper questionnaires will be stored in a locked filing cabinet at Monash University. Information will also be stored on a password-protected database. Only members of the research team and the Ethics Committee can have access to this information.

Information will be kept until the youngest participant turns 25 years old. After this time it will be destroyed. A copy of your son's assessment results will be placed in his medical record which is kept until he turns 25 years old.

Results will be published in professional journals and presented at conferences. Names will never be used in any publications, reports or presentations.

12. Will we be informed of the results when the research project is finished?
We will send you a summary of the overall project results. We will also send you a summary of your sons' test results.

13. What happens if my child is injured during the project?
In the unlikely event that your child suffers an injury as a result of taking part in this project, hospital care and treatment will be provided by the public health service at no cost to you.

14. Will my child be able to claim any compensation if injured?
No funds have been set aside to compensate your child in the event of injury. However, by signing this form, you are not giving up any legal rights to seek compensation for injury.

If you would like more information about the project or if you need to speak to a member of the research team in an emergency please contact:
Name: Zoe Davidson

Contact telephone: [redacted]

If you have any concerns about the project or the way it is being conducted, and would like to speak to someone independent of the project, please contact:

Head of Department, Ethics and Research Department, Human Research Ethics Committee
The Royal Children's Hospital, Telephone: (03) 9345 5044

The Royal Children's Hospital, Melbourne

Flemington Road, Parkville
Victoria, Australia, 3052

Telephone (03) 9345 5522
ISD (+613) 9345 5522
Facsimile (03) 9345 5789
Web www.rch.org.au

CONSENT FORM FOR PARENT/GUARDIAN TO GIVE INFORMED CONSENT FOR THEIR CHILD TO TAKE PART IN A RESEARCH PROJECT

HREC Project Number:

Research Project Title: ▪ **Nutriceuticals in Duchenne muscular dystrophy: Investigating the effectiveness of a multicomponent nutritional supplement**

I (Parent/Guardian name) _____

of (child's name) _____

voluntarily consent for my child to take part in the above research project

- I believe I understand the purpose, extent and possible effects of my child's involvement in this project.
- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I understand that this project has been approved by The Royal Children's Hospital Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007).
- I understand I will receive a copy of this Parent/Guardian Information Statement and Consent Form.

Parent/Guardian Signature _____ **Date** _____

Print name of witness to parent/guardian's signature

_____

Witness Signature _____ **Date** _____

I have explained the project to the parent/guardian who has signed above, and believe that they understand the purpose, extent and possible effects of their child's involvement in this project.

Researcher Signature _____ **Date** _____

Note: All parties signing the Consent Form must date their own signature.

Appendix 14

MONASH University

Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

Date:	**19 May 2010**
Project Number:	**CF10/0741 - 2010000344**
Project Title:	**A comparison of dietary intake and physical activity in children with Muscular Dystrophy versus healthy controls**
Chief Investigator:	**Prof Helen Truby**
Approved:	**From: 19 May 2010 To: 19 May 2015**

Terms of approval

1. The Chief investigator is responsible for ensuring that permission letters are obtained, if relevant, and a copy forwarded to MUHREC before any data collection can occur at the specified organisation. **Failure to provide permission letters to MUHREC before data collection commences is in breach of the National Statement on Ethical Conduct in Human Research and the Australian Code for the Responsible Conduct of Research**.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by MUHREC.
4. You should notify MUHREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. The Explanatory Statement must be on Monash University letterhead and the Monash University complaints clause must contain your project number.
6. **Amendments to the approved project (including changes in personnel):** Requires the submission of a Request for Amendment form to MUHREC and must not begin without written approval from MUHREC. Substantial variations may require a new application.
7. **Future correspondence**: Please quote the project number and project title above in any further correspondence.
8. **Annual reports**: Continued approval of this project is dependent on the submission of an Annual Report. This is determined by the date of your letter of approval.
9. **Final report**: A Final Report should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected date of completion.
10. **Monitoring**: Projects may be subject to an audit or any other form of monitoring by MUHREC at any time.
11. **Retention and storage of data**: The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.

[redacted]

Professor Ben Canny
Chair, MUHREC

cc: Ms Aimee Segman, Ms Zoe Davidson

Postal – Monash University, Vic 3800, Australia
Building 3E, Room 111, Clayton Campus, Wellington Road, Clayton
Telephone +61 3 9905 5490 Facsimile +61 3 9905 3831
Email muhrec@adm.monash.edu.au www.monash.edu/research/ethics/human/index/html
ABN 12 377 614 012 CRICOS Provider #00008C

Appendix 15

MONASH University

Explanatory Statement - Control group

Title: A comparison of dietary intake and physical activity in children with Muscular Dystrophy versus healthy controls

This information sheet is for you to keep. These pages tell you about the research. It explains to you clearly and openly all the steps and procedures of the project. The information is to help you to decide whether or not you would like your child to take part in the research.

My name is Aimee Segman and I am conducting a research project towards my Honours at Monash University, with Professor Helen Truby who is the head of the Nutrition and Dietetics Department. This means that I will be writing a thesis which is the equivalent of a short book.

Why did you choose this particular person/group as participants?

We want to find out more about nutrition in boys with Duchenne muscular dystrophy (DMD) living in Australia. There is currently very little known about nutrition in DMD and there have been no studies that look at nutrition of boys with DMD living in Australia. We want to look at nutritional intake and physical activity in boys with DMD compared to healthy boys of similar age.

It is hoped that this information will lead to a better understanding of the impact that DMD has on nutritional status. The findings in this study will help inform doctors and dietitians of the best dietary management options in this group.

Your child has been invited to participate in this study as a healthy 'control'. This means his measurements will be compared to the measurements of young boys who have DMD. Control data is used in research to look at differences between groups. This would help us to understand the differences in nutritional status and physical activity between children who have neuromuscular disease and those who do not.

Your contact details were obtained from the Monash University staff email list or you replied to an advertisement, and you were selected as your child met the inclusion criteria for this study.

Inclusion/Exclusion criteria

DMD only affects boys therefore the control group will be made up solely of boys. Boys will be unable to take part if they are unable to walk or play, have any medical condition such as diabetes, if they do not agree to take part in this study, and/or if parent/guardian consent is unable to be obtained.

The aim/purpose of the research

The aim of this study is to investigate physical activity levels and dietary patterns of healthy boys between 5 and 13 years of age. Your son's results will form part of a healthy control group, which means his activity patterns will be compared to the same measurements collected from young boys with Duchenne Muscular Dystrophy (DMD) or those with other long term diseases which affect their energy needs.

Possible benefits

By participating in this study you and your son will get to find out precisely how much physical activity he is going, using the latest cutting edge technology in physical activity measuring devices. By

participating in this study, you will also be helping to further research in DMD, a severe neuromuscular disorder characterised by progressive muscle wasting.

What does the research involve?

Monash University:
The study involves one visit to Monash University (Clayton Campus) to complete the following tests:

The 6 minute walk test; this is a simple test that measures the distance that your child can walk in a period of six minutes.

Height and weight will be measured

OPTIONAL Doubly labelled water (DLW) procedure. DLW is water that has been labelled with two naturally occurring and stable isotopes; deuterium and oxygen. By giving water with extra deuterium and oxygen, it boosts the levels which are already present in the body. These isotopes are non-toxic and the water is not dangerous or harmful to your child in anyway. DLW has been used on pre-term babies, infants, children and adolescents. Your child will need to give a spot urine sample (10mls) and then drink a small glass of the DLW which is tasteless. The DLW stays in your child's body for 10 days. The urine samples will then need to be sent to the University of Queensland for analysis, as the necessary equipment to do this is not available in Victoria. The results of this won't be available for many months as the analysis is complex.

At home:
Complete a food diary; you will need to fill out a food diary for 5 days recording everything that your son eats and drinks. Completion of the food diary will take approximately 10 minutes per day.

Complete a physical activity diary; you will need to complete the diary for 5 days recording your son's physical activity.

Complete a food frequency questionnaire; this is a simple 5 minute questionnaire that asks you about types of food your son eats.

Wear a StepWatch™ Activity monitor; a small accelerometer to be worn around your son's ankle for 5 days to measure physical activity. You will receive instruction on use when you are at Monash University.

OPTIONAL Doubly labelled water procedure: a single spot of urine (10mls) will be collected from your son 5 hours after he has drunk the dose of DLW (which is tasteless), and then daily for the 10 consecutive days following. This procedure is a very expensive and precise way to determine total energy expenditure. It is also a very safe procedure. We ask you to freeze the urine samples at home and then we will collect them from you. At the time of collection we will also weigh your son using digital scales. THIS SECTION OF THE STUDY IS OPTIONAL AND ONLY AVAILABLE FOR 5 SUBJECTS.

After the study:
Information collected from you and your son may be used as 'control' information in other studies. In such cases, active participation would NOT be required. You are able to consent to this separately. If you do consent to this, we will not contact you before using the information. Confidentiality will be maintained as discussed below

.
How much time will the research take?

Your child's visit to Monash University will take approximately 1 hr and can be arranged at a convenient time for yourself and your child. Additionally, there is a small amount of time required for questionnaire completion, and possibly urine collection whilst at home.

Inconvenience/discomfort

Possible inconveniences may include time off school and work to attend the appointment at Monash University. However you will be able to organise a time after school hours or on a Saturday.

The risks of taking part in the study are minimal. There may be additional unforeseen or unknown risks that are not known to us. If new information about risks becomes known during the project you will be informed of this.

Payment

There is no financial reward for volunteering in this research project. Although your child may not get any direct benefit from taking part, we will give you the results of all the tests your child is completing.

Can I withdraw from the research?

Being in this study is voluntary and you are under no obligation to consent to participation. If you and your child decide to participate, you are still free to withdraw at any time without giving a reason.

Confidentiality

Any information we collect from you and your child will remain confidential. We will use your child's information only for this research project, unless you have consented for us to use the information in other studies for 'control' purposes. Only the researchers involved with this project Monash University's ethics committee will have access to this information. We can disclose the information only with your permission, except as required by law. You have the right to access and request correction of your information in accordance with the Freedom of Information Act 1982 (Vic).
Results may be published in professional journals and presented at conferences. Names will never be used in any publications, reports or presentations.

For DLW only: When the urine samples are sent to the University of Queensland for analysis, the samples will be sent in 'coded' form. This means that the scientists looking at the samples will not be able to identify who they came from. This is to maintain confidentiality during the process. The urine samples will be the only personal data collected from you and your son that will be transferred to the University Queensland. Results of the analysis will be sent, via registered post, to Monash, Victoria for interpretation. At all stages, data will remain in the 'coded' form.

Storage of data

Paper questionnaires will be stored in a locked filing cabinet at Monash University for 5 years. Information from the tests will also be stored on a password-protected database through Monash University. Only members of the research team and the Ethics Committee can have access to this information.

Results

If you would like to be informed of the aggregate research finding, please contact Aimee Segman on [redacted] or email [redacted]

If you would like to contact the **researchers** about any aspect of this study, please contact the Chief Investigator:	If you have a **complaint** concerning the manner in which this research is being conducted, please contact:
Professor Helen Truby Head of Nutrition and Dietetics Telephone: [redacted] Email: [redacted]	Executive Officer, Human Research Ethics Monash University Human Research Ethics Committee (MUHREC) Building 3e Room 111 Research Office Monash University VIC 3800 Tel: +61 3 9905 2052 Fax: +61 3 9905 3831 Email: muhrec@adm.monash.edu.au

Thank you.

Consent Form – Healthy Control Group
Title: A comparison of dietary intake and physical activity in boys with Muscular Dystrophy versus healthy controls

NOTE: Signed written consent will remain with the Monash University researcher for their records.

I agree that _____ may take part in the above Monash University research project. The project has been explained to _____ and to me, and I have read the Explanatory Statement, which I keep for my records. Being in this study is voluntary and you are under no obligation to consent to participation. If you and your child decide to participate, you are still free to withdraw at any time without giving a reason.

I understand that agreeing to take part means that I am willing to allow _____ to:

- Complete a 6-minute walk test ☐ Yes ☐ No
- Allow height and weight measurements to be taken ☐ Yes ☐ No
- Wear a StepWatch™ Activity monitor for 3 consecutive days ☐ Yes ☐ No
- Complete a 3 day food diary and food frequency questionnaire ☐ Yes ☐ No

I agree that the above information collected from my son _____ to be used as control data in other studies conducted by the researchers. I understand that I will not be contacted again for consent to use this information.

☐ **Yes** ☐ **No**

OPTIONAL:

I agree for my son, _____ to provide urine samples once a day for 10 consecutive days after he has drunk a dose of doubly labelled water (which tastes like water).

☐ **Yes** ☐ **No**

I agree for the urine samples collected from my son, _____, to be sent to the University of Queensland in a 'coded' form for analysis.

☐ **Yes** ☐ **No**

I understand that my son's participation is voluntary, that I can choose not to participate in part or all of the project, and that I can withdraw at any stage of the project without my healthcare being penalised or disadvantaged in any way. I understand that data from the tests will be kept in a secure storage and accessible to the research team. I also understand that the data will be destroyed after a 5 year period.

Participant's name……..

Participant's Age…….

Parent's / Guardian's Name…….

Parent's / Guardian's relationship to participant.....

Parent's / Guardian's Signature……Date……..

Child section

I agree to take part in the described study and understand the activities I will be doing.

Participant (Child) signature: ……Date……..

Witness Name:…….. ……..……..

Witness signature: ……..Date ……..

Nutriceuticals in DMD HREC #30022
6MWT

Participant Name:_____ Date of evaluation: _____

Randomisation No.: _____ Hospital URN: _____ Study Visit_____

Evaluators: _____ Time of evaluation: _____

Lap	Distance (M)	Time (m:ss)	Lap	Distance (M)	Time (m:ss)
0	0	0:00	22	550	
1	25		23	575	
2	50		24	600	
3	75		25	625	
4	100		26	650	
5	125		27	675	
6	150		28	700	
7	175		29	725	
8	200		30	750	
9	225		31	775	
10	250		32	800	
11	275		33	825	
12	300		34	850	
13	325		35	875	
14	350		36	900	
15	375		37	925	
16	400		38	950	
17	425		39	975	
18	450		40	1000	
19	475		41	1025	
20	500		42	1050	
21	525		END		

	Distance travelled prior to fall (M)	Time of Fall (m:ss)	Time of recovery (m:ss)
Fall A			
Fall B			
Fall C			
Fall D			
Did the participant fall during the test? If yes, please provide details in the table above		☐ YES	☐ NO

NOTES:

1 Minute Distance (M)		4 Minute Distance (M)	
2 Minute Distance (M)		5 Minute Distance (M)	
3 Minute Distance (M)		6 Minute Distance (M)	

Was this a valid test?	☐ YES ☐ NO
If "NO" check the primary reason why the test was not valid	
Subject failed to follow instructions	☐
Subject did not remain on the course for the duration of the test	☐
Subject moved in reverse direction for more than a few steps	☐
Subject discontinued test due to injury caused by fall	☐
Subject sat down but not due to exhaustion	☐
Other (Please Specify)	☐

ROYAL CHILDREN'S HOSPITAL & HEALTH SERVICE DISTRICT ETHICS COMMITTEE

Professor John Pearn (Chair) 3365 5323
Mrs Amanda Smith (Ethics Officer) 3636 9167

Queensland **Health**

Level 3, RCH Foundation Building
Royal Children's Hospital
Herston QLD 4029 Australia
Telephone (07) 3636 9167
Facsimile (07) 3365 5455

14th May 2007

Ms Helen Truby
Senior Research Fellow
Children's Nutrition Research Centre
Discipline of Paediatrics & Child Health
Royal Children's Hospital
Level 3, Foundation Building
Herston QLD 4029

Dear Ms Truby,

An examination of body composition and energy expenditure in boys with neuromuscular disease.

Many thanks for your letter of the 1st May together with the above project for consideration by the Ethics Committee.

I have now been through this and am happy to give the project Executive approval on behalf of the Committee. This will be tabled at our next meeting and I will write again if there are any further queries.

Please do not hesitate to contact me should you have any queries.

With kindest regards,

[redacted]

Professor John Pearn
Chair
Royal Children's Hospital & Health Service District Ethics Committee

Cc: Ethics Committee Files
Members of the Ethics Committee

ROYAL CHILDREN'S HOSPITAL & HEALTH SERVICE DISTRICT ETHICS COMMITTEE

Professor John Pearn (Chair) 3365 5323
Mrs Amanda Smith (Ethics Officer) 3636 9167

Queensland **Health**

Level 3, RCH Foundation Building
Royal Children's Hospital
Herston QLD 4029 Australia
Telephone (07) 3636 9167
Facsimile (07) 3365 5455

Ethics Number: 2007/119

19th February 2008

Ms Helen Truby
Senior Research Fellow
Children's Nutrition Research Centre
Discipline of Paediatrics & Child Health
Royal Children's Hospital
Level 3, Foundation Building
Herston QLD 4029

Dear Ms Truby,

2007/119: An examination of body composition and energy expenditure in boys with neuromuscular disease.

Royal Children's Hospital & Health Service District Ethics Number: 2007/119

We are in the process of inputting all projects into our Ethics Database.

The above titled project has been allocated the number 2007/119. Please quote this number on any future correspondence regarding this project.

Please do not hesitate to contact me should you have any queries.

With kind regards,

[redacted]

Mrs Amanda Smith
Ethics Co-ordinator
Royal Children's Hospital and Health Service District Ethics Committee

Appendix 18

THE UNIVERSITY OF QUEENSLAND

Institutional Approval Form For Experiments On Humans Including Behavioural Research

Chief Investigator:	Dr Helen Truby
Project Title:	Energy Requirements And Body Composition In Children With Neuromuscular Disease, A Cross Sectional Study
Supervisor:	None
Co-Investigator(s):	Dr Kate Sinclair, Dr Geoff Wallace, Helen Posselt, Sarah Elliott
Department(s):	Children's Nutrition Research Centre, Paediatrics and Child Health, School of Medicine
Project Number:	2007000797
Granting Agency/Degree:	
Duration:	31st August 2010

Comments:

Approval subject to ethical clearance from the Royal Children's Hospital HREC.
Please forward the approval letter when available.

Name of responsible Committee:-
Medical Research Ethics Committee
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Research Involving Humans* and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:-
Professor Bill Vicenzino
Chairperson
Medical Research Ethics Committee

Date: 1 August 2007 Signature: [redacted]

Appendix 19

Children's Nutrition Research Centre
Discipline of Paediatrics and Child Health
The University of Queensland

Associate Professor Peter SW Davies (Director)

Department of Neurology
Royal Children's Hospital
Herston, QLD, 4029

Dr Kate Sinclair

Parent / Guardian Information Sheet

Dear Parent/Guardian

Study Title: Energy requirements and body composition in children with neuromuscular disease

Investigators: Dr Helen Truby (Senior Research Fellow and Paediatric dietitian, University of Queensland); Sarah Elliott (Nutritionist, PhD Scholar, University of Queensland) and Drs Kate Sinclair and Geoff Wallace (Department of Neurology, Royal Children's Hospital), Helen Posselt (Clinic Coordinator, Montrose Access) and Zoe Davidson (Dietitian, PhD Scholar, University of Queensland).

Your child has been invited to take part in the above research study. Before you decide whether you would like your child to participate or not, it is important that you understand why the research is being undertaken and what it will involve.

Please take the time to read the following information carefully and discuss this with your family and your child. Please do not hesitate to contact us if you need clarification on any of the details or if you require additional information. We feel that it is important that you understand why we wish your child to take part in this research study and therefore would like you to browse through this information at your leisure.

What is the purpose of the study?
In this study we want to find out more about the nutritional status of boys with neuromuscular disease (NMD) living in Queensland. We want to evaluate both body composition and energy requirements of boys with NMD compared to healthy boys of similar age. It is hoped that this information will lead to a better understanding of the impact NMD has on body composition. The findings from this study will help inform health professionals in the future of the best dietary management strategy in this group.

What does the study involve?
The amount of energy your child takes in each day (energy intake) is decided by how much they eat and drink. The amount of energy they need and use up (energy expended) depends on the amount of activity they undertake each day.

We usually estimate energy requirements using standard equations. However, for children with NMD we are not aware of exactly how much energy is needed due to changes in their body composition. Our study is trying to investigate these needs. This would greatly help the dietary advice given and mean an individualised dietary 'prescription' may be made. To achieve this we need to measure energy expenditure, metabolic rate and, if appropriate, physical activity.

Metabolic rate can be recorded by measuring the basal metabolic energy or the resting energy expenditure (REE) of your child's body. This is the amount of energy used when we are resting and usually accounts for approximately 70% of the energy we get from diet alone. Usually the remaining energy from the diet goes into physical activity and growth. If we eat more than is required by our bodies we gain weight.

In those boys that are ambulatory (i.e. not in a wheelchair) we will measure energy expenditure with doubly labelled water. Doubly labelled water (DLW) is the 'gold standard' method of measuring energy expenditure. DLW is water that has been 'labelled' with two naturally occurring and stable isotopes (a different form of the elements, Hydrogen and Oxygen); deuterium and $Oxygen_{18}$. By giving water with extra deuterium and oxygen, it boosts the levels which are already present in the body. These isotopes are non toxic and the water is not dangerous or harmful to your child in anyway. DLW has been used on pre-term babies, infants, children, and adolescents. Your child will need to give a spot urine sample and then asked to drink a small glass of the DLW water which is tasteless. The DLW stays in your child's body for 10 days and we would collect urine samples 5 hours and daily for 10 days after administration. You will be provided with instructions and containers for the urine collection. We will organise for the urine samples to be picked up from your home.

An assessment of your child's body composition will also be made using several different techniques (discussed overleaf).

Does my child have to take part in the study?
It is for you and your child to decide whether or not you both wish to participate. If you decide to take part you will be asked to sign and return the consent form enclosed with this information sheet. If you and your child decide to participate, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your child receives or any future relationship with the Royal Children's Hospital or University of Queensland.

What will happen to my child if I take part?
Your child will be invited to attend an extended hospital out-patient appointment at the Body Composition Laboratory, Children's Nutrition Research Centre, Royal Children's Hospital. Before this appointment your child will be sent an activity and a food diary to be completed which we will ask you to return on the appointment day. The appointment will take about half a day and will be from 08.00am to 12.00pm approximately. It is very important that **your child has nothing to eat or drink (except water) before coming** to the appointment because a fasting blood sample is required. You will be given a voucher to buy breakfast for your child. The schedule for this half day appointment is outlined below.

1) Measurement of your child's resting energy expenditure. This involves him/her lying under a special hood that collects and analyses some of the gases your child breathes in and out. This should take about half an hour and is not unpleasant.
2) Your child will have a fasting blood sample collected by a professional experienced in taking blood from children.
3) Your child will have breakfast.

4) Your child will then be asked to provide a spot urine sample (10ml) for the measurement of energy expenditure by DLW and asked to drink a small glass of the DLW. A second urine sample will be collected five hours later.
5) Height, weight and waist circumference will be measured.
6) We would also like to assess the amount of fat, muscle, bone and water that makes up your child's body. This will be measured by three methods:
   - The first is a special machine that measures the amount of potassium in your child's body. Your child's body is scanned while they are lying down and it is completely painless. This test is not an X-ray and there is no radiation dose.
   - The second is a sophisticated machine (BodPod) that measures the amount of air your child's body takes up. Your child will need to sit in a spaceship-like chamber, with a clear Perspex front. It will take a few minutes to measure the air in the chamber and in your child's body. Your child will need to wear a swimsuit and if possible, a swimming cap so that air trapped in clothes and hair does not affect the reading. They will also need to sit still while the measurements are being taken.
   - The third is a special machine that measures 'bioelectrical impedance'. A small harmless electrical current runs through the body for a few seconds. It is completely painless.
7) Children will also be asked to provide some information on their dietary and if ambulatory, physical activity patterns. This will require the completion of the following before coming in for your child's appointment:
   a. Food diary
   b. Physical activity diary/questionnaire

Also, if your child has had their genes analysed, we would like to utilise this information. We would like to see if there is any relationship between genotype and nutritional status. The information can be accessed from medical records held at Royal Children's Hospital and will stored in a secure file.

Parental Questionnaire
We would also like to know what parents see as the main nutritional concerns for their children. So we would like to ask you a few questions whilst you are at Royal Children's Hospital.

Your Expenses
Your car parking expenses will be reimbursed for your visit.

What are the possible disadvantages and risks of taking part?
We cannot think of any possible disadvantages or risks to taking part in the study.
Some children may be nervous and anxious about the blood test. To help overcome this staff highly experienced in taking blood from children will be taking the blood sample.

There is also the possibility that your child may feel unfamiliar in the study environment. We will be using the Body Composition Laboratory which has child friendly facilities and staff who are experienced in taking these measurements and working with children. You will be welcome to stay with your child during all the measures and can provide reassurance if this is required. There is also a portable DVD player available so children are allowed to bring a suitable DVD to watch (this should be age appropriate and not over excite your child).

What are the possible benefits of taking part?
Currently there is no information collected routinely about the nutritional status of boys in QLD with neuromuscular disease. By participating in this study you and your child can contribute to improving our understanding of body composition change in NMD and how this affects energy requirements. You will also be given the results of the measurements taken by state of the art equipment. There is no charge associated with taking part in the study.

The information gathered in this study will help guide future research in this area as well as helping health professionals provide optimal dietary treatment for boys with NMD in the future.

Will my child taking part in this study be kept confidential?
All information which is collected about your child during the course of the research will be kept strictly confidential. Participants will be identified by a number only and names will never be used in any subsequent publications or reports. Information regarding the study will be available to you on completion of the data analysis period on request. The health professional that referred you will be informed of the results of the assessments and a copy put in your child's hospital chart. The data collected will form part of a database of nutritional status of children in QLD with NMD.

Who is organising and funding the research?
The study is being organised and funded by the Children's Nutrition Research Centre, University of Queensland.

Contact for further information
Thank you for taking the time to read this information and we hope that after careful consideration, you agree for your child to participate in this study. Helen Truby or Sarah Elliott will contact you in a couple of weeks to see if you want to take part in the study.

In the meantime if you have any questions that have not been answered in this information sheet, or would like to discuss anything in more detail, please don't hesitate to contact Sarah Elliott or Helen Truby on 33655476.

Thank you for your attention.

Dr Kate Sinclair
Consultant Neurologist
RCH
Phone: [redacted]
Email:
[redacted]

Dr Helen Truby
Children's Nutrition Research Centre
RCH
Phone: [redacted]
Email:
[redacted]

Sarah Elliott
Children's Nutrition Research Centre
RCH
Phone: [redacted]
Email:
[redacted]

Zoe Davidson
Children's Nutrition Research Centre
RCH
Phone: [redacted]
Email:
[redacted]

This study has been cleared by one of the human ethics committees of the University of Queensland in accordance with the National Health and Medical Research Council's guidelines. You are of course, free to discuss your participation in this study with project staff (contactable on 3636 9271.). If you would like to speak to an officer of the University not involved in the study, you may contact the Ethics Officer on 3365 3924.

Appendix 20

Do you think that you have eaten as you would do usually? YES ☐
NO ☐

If NO, then why not? e.g. were you ill, on holiday, etc.

……

……

……

Please look after your food dairy.

It is important that you bring it back to RCH appointment.

Thank you for completing the food diary, it is really appreciated and we hope that you found it interesting.

FOOD DIARY

DMD Study

Name: ……

Date of Birth …….

Day 1: ……

Day 2: ……

Day 3: ……

For Office use

Id No ……. / ……	Food diary no. ……..

A food diary is a really useful way of learning about your food habits. When you are making changes to your diet it is a way of checking how you are going.

INSTRUCTIONS

1. Write down **everything** (except plain water) that you **eat and drink**, no matter how small the amount.
2. Give as much information as possible about the foods and drinks you eat. It is very useful if you include:
   - Brand name e.g. Home Brand chips, Cadburys Rocky Road chocolate bar
   - Food weight where known. Try looking on the packaging for this.
   - How the food was cooked e.g. fried, grilled, raw etc. You may need to ask your parents for help with this.
3. Estimating food weights:
   - Give an idea of the amount of the food eaten e.g. cheese cubes-2 x matchbox size
   - Use household measures such as spoonfuls e.g. teaspoon, serving spoon, or cups. If you are eating at home then ask your parents to help with this.

Day and date_____

Food and drink Please include as much detail as possible e.g brand name, flavour, packet weight, cooking method	Amount eaten Remember to include leftovers

Day and date_____

Food and drink Please include as much detail as possible e.g brand name, flavour, packet weight, cooking method	Amount eaten Remember to include leftovers

REMEMBER!!!

It is very easy to forget what you have eaten so carry your diary with you and try to write things down while eating.

Day and date_____

Food and drink Please include as much detail as possible e.g brand name, flavour, packet weight, cooking method	Amount eaten Remember to include leftovers

Day and date_____

Food and drink Please include as much detail as possible e.g brand name, flavour, packet weight, cooking method	Amount eaten Remember to include leftovers

Appendix 21

Protein Shake Recipe Form
Clinical Trial # 30022: Nutriceuticals in DMD
Phase 2 and 3

RECIPE FOR BATCHES 1, 2, 3, 4

Patient Name: _____ Patient Randomisation No.: _____ UR No.: _____

Weight: _____ (/ /) Fat Free Mass: _____ (/ /)

Calculations by: _____ Sign _____ Date: _____

Enhanced/Standard: _____ Sign _____ Date: _____

ENHANCED SHAKE – BATCH 1, 2, 3

RECIPE FOR 1 SACHET

Protein Powder: **22g**

Creatine Monohydrate: **5g**

Glutamine:

_____

Maltodextrin: **8g (~1 scoop)**

Splenda: **1 teaspoon (0.5g)**

ACTIVE HMB CAPS (500mg):

HMB Dose:

_____

HMB Caps:

_____

HMB Caps for Batch 1:
30 x HMB Caps dose = _____

HMB Caps for Batch 2:
60 x HMB Caps dose = _____

HMB Caps for Batch 3:
60 x HMB Caps dose = _____

STANDARD SHAKE – BATCH 1, 2, 3

RECIPE FOR 1 SACHET

Protein power: **22g**

Maltodextrin: **16g (~2 scoops)**

Splenda: **1 teaspoon (0.5g)**

MALTODEXTRIN CAPS (500mg):

Maltodextrin Dose:

_____

Maltodextrin Caps:

_____

Maltodextrin Caps for Batch 1:
30 x Maltodextrin Caps dose = _____

Maltodextrin Caps for Batch 2:
60 x Maltodextrin Caps dose = _____

Maltodextrin Caps for Batch 3:
60 x Maltodextrin Caps dose = _____

WASH OUT SHAKE– BATCH 4

RECIPE FOR 1 SACHET

Protein power: **22g**

Calculations: Active

Glutamine: 0.6g/kg fat free mass/day
(rounded up to 0.5g)

HMB Dose: 38mg/kg/day = (38xweight)/1000
(rounded up to 0.5g)

HMB Caps: HMB dose (g)/0.5

Calculations: Placebo

Maltodextrin Dose:
38mg/kg/day = (38xweight)/1000
(rounded up to 0.5g)

Maltodextrin Caps:
Maltodextrin dose (g)/0.5

Protein Shake Recipe Form
Clinical Trial # 30022: Nutriceuticals in DMD
Phase 4

RECIPE FOR BATCHES 5, 6, 7

Patient Name: _____ Patient Randomisation No.: _____ UR No.: _____

Weight: _____ (/ /) Fat Free Mass: _____ (/ /)

Calculations by: _____ Sign _____ Date: _____

Enhanced/Standard: _____ Sign _____ Date: _____

ENHANCED SHAKE – BATCH 5, 6, 7

RECIPE FOR 1 SACHET

Protein Powder: **22g**

Creatine Monohydrate: **5g**

Glutamine:

_____

Maltodextrin: **8g (~1 scoop)**

Splenda: **1 teaspoon (0.5g)**

ACTIVE HMB CAPS (500mg):

HMB Dose:

_____

HMB Caps:

_____

HMB Caps for Batch 5:
30 x HMB Caps dose = _____

HMB Caps for Batch 6:
60 x HMB Caps dose = _____

HMB Caps for Batch 7:
60 x HMB Caps dose = _____

STANDARD SHAKE – BATCH 5, 6, 7

RECIPE FOR 1 SACHET

Protein power: **22g**

Maltodextrin: **16g (~2 scoops)**

Splenda: **1 teaspoon (0.5g)**

MALTODEXTRIN CAPS (500mg):

Maltodextrin Dose:

_____

Maltodextrin Caps:

_____

Maltodextrin Caps for Batch 5:
30 x Maltodextrin Caps dose = _____

Maltodextrin Caps for Batch 6:
60 x Maltodextrin Caps dose = _____

Maltodextrin Caps for Batch 7:
60 x Maltodextrin Caps dose = _____

Calculations: Active

Glutamine: 0.6g/kg fat free mass/day
(rounded up to 0.5g)

HMB Dose: 38mg/kg/day = (38xweight)/1000
(rounded up to 0.5g)

HMB Caps: HMB dose (g)/0.5

Calculations: Placebo

Maltodextrin Dose:
38mg/kg/day = (38xweight)/1000
(rounded up to 0.5g)

Maltodextrin Caps:
Maltodextrin dose (g)/0.5

Appendix 22

ACTIVLIM - Activity Limitations Measure
English version

Name:_____ Date:_____

	How difficult are the following activities?	Impossible	Difficult	Easy	?
1	Putting on a T-shirt				
2	Washing one's upper body				
3	Dressing one's lower body				
4	Taking a shower				
5	Sitting on the toilet				
6	Taking a bath				
7	Walking dowstairs				
8	Stepping out of a bath tub				
9	Opening a door				
10	Walking outdoors on level ground				
11	Washing one's face				
12	Hanging up a jacket on a hatstand				
13	Wiping one's upper body				
14	Walking upstairs				

To evaluate an **adult** patient (age 16-80), please answer to the following questions.

To evaluate a **child** patient (age 6 -15), please mark the following questions with the "?"

15	Carrying a heavy load	A				
16	Getting into a car	A				
17	Standing for a long time (± 10 min)	A				
18	Walking more than 1 kilometre	A				

To evaluate a **child** patient (age 6-15), please answer to the following questions.

To evaluate an **adult** patient (age 16-80), please mark the following questions with the "?"

19	Closing a door	C				
20	Hopping on one foot	C				
21	Putting on a backpack	C				
22	Running	C				

Order 1

Faculté de Médecine, Unité de Réadaptation et de Médecine Physique, UCL5375, Avenue Mounier 53, 1200 Bruxelles, Belgium. www.rehab-scales.org

ID# _____

Date: _____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

PARENT REPORT for **CHILDREN** (ages **8-12**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

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01/00

*In the past **ONE month,** how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores around the house	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or blue	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Getting along with other children	0	1	2	3	4
2. Other kids not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other children	0	1	2	3	4
4. Not able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

SCHOOL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with schoolwork	0	1	2	3	4
4. Missing school because of not feeling well	0	1	2	3	4
5. Missing school to go to the doctor or hospital	0	1	2	3	4

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01/00

ID# _____

Date:_____

PedsQL™
Neuromuscular Module

Version 3.0

PARENT REPORT for CHILD (ages **8-12**)

DIRECTIONS

Children with neuromuscular disorders sometimes have special problems. Please tell us **how much of a problem** each one has been for your child during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

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*In the past **ONE month**, how much of a **problem** has this been for your child …*

ABOUT MY CHILD'S NEUROMUSCULAR DISEASE *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for my child to breathe	0	1	2	3	4
2. My child gets sick easily	0	1	2	3	4
3. My child gets sores and/or rashes	0	1	2	3	4
4. My child's legs hurt	0	1	2	3	4
5. My child feels tired	0	1	2	3	4
6. My child's back feels stiff	0	1	2	3	4
7. My child wakes up tired	0	1	2	3	4
8. My child's hands are weak	0	1	2	3	4
9. It is hard for my child to use the bathroom	0	1	2	3	4
10. It is hard for my child to gain or lose weight when he or she wants to	0	1	2	3	4
11. It is hard for my child to use his or her hands	0	1	2	3	4
12. It is hard for my child to swallow food	0	1	2	3	4
13. It takes my child a long time to bathe or shower	0	1	2	3	4
14. My child gets hurt accidentally	0	1	2	3	4
15. My child takes a long time to eat	0	1	2	3	4
16. It is hard for my child to turn him or herself during the night	0	1	2	3	4
17. It is hard for my child to go places with his or her equipment	0	1	2	3	4

COMMUNICATION *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for my child to tell the doctors and nurses how he or she feels	0	1	2	3	4
2. It is hard for my child to ask the doctors and nurses questions	0	1	2	3	4
3. It is hard for my child to explain his or her illness to other people	0	1	2	3	4

ABOUT OUR FAMILY RESOURCES *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for our family to plan activities like vacations	0	1	2	3	4
2. It is hard for our family to get enough rest	0	1	2	3	4
3. I think money is a problem in our family	0	1	2	3	4
4. I think our family has a lot of problems	0	1	2	3	4
5. My child does not have the equipment he or she needs	0	1	2	3	4

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Nutriceuticals in DMD HREC # 30022
Supplement Evaluation Form

Name: _____ Randomisation No.: _____

1. What did you think about the TASTE of the protein shake?

☹ 😐 ☺

1	2	3	4	5
YUK		OK		YUM

2. How did the protein shake FEEL in your mouth?

☹ 😐 ☺

1	2	3	4	5
YUK		OK		NICE

3. What did you think about the LOOK of the protein shake?

☹ 😐 ☺

1	2	3	4	5
YUK		OK		NICE

4. How easy was it to drink the protein shake every day?

☹ 😐 ☺

1	2	3	4	5
VERY HARD		OK		VERY EASY

5. How easy was it to take the tablets every day?

☹ 😐 ☺

1	2	3	4	5
VERY HARD		OK		VERY EASY

6. Could you taste a difference between the protein shake you had at the start of the study and the protein shake you just had?

YES NO

→ If YES: Which protein shake did you like the better?
a) The protein shake I had first
b) The protein shake I just had

7. Is there anything else you would like to tell me about the protein shake?

_____
_____
_____
_____
_____
_____
_____
_____
_____

8. Is there anything else you would like to tell me about the tablets?

_____
_____
_____
_____
_____
_____
_____
_____
_____

Thank you for completing this evaluation form

QLD CHILDREN'S HEALTH SERVICES (RCH)
HUMAN RESEARCH ETHICS COMMITTEE

Professor John Pearn (Chair) 3365 5323
Mrs Amanda Smith (Co-ordinator) 3636 9167

Queensland **Health**

Level 3, RCH Foundation Building
Royal Children's Hospital
Herston QLD 4029 Australia
Telephone (07) 3636 9167
Facsimile (07) 3365 5455

2nd February 2011

Professor Helen Truby
Nutrition and Dietetics, Monash University
Faculty of Medicine, Nursing and Health Sciences
Monash Medical Centre, Level 5, Block E
246 Clayton Rd
Clayton, VIC 3168

Dear Professor Truby,

HREC Reference number: HREC/11/QRCH/2
Project title: Nutriceuticals in Duchenne muscular dystrophy: a randomised controlled trial of a multicomponent nutritional supplement

Many thanks for your application of the above project. This was tabled and reviewed at our meeting of the 31st January 2011.

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) *National Statement on Ethical Conduct in Human Research (2007), NHMRC and Universities Australia Australian Code for the Responsible Conduct of Research (2007)* and the *CPMP/ICH Note for Guidance on Good Clinical Practice.*

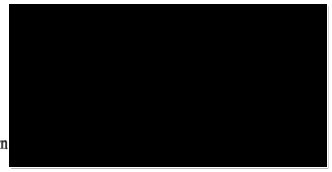
I am pleased to advise the proposal meets the requirements of the National Statement on Ethical Conduct in Human Research and the Committee is happy to give approval. The documents reviewed and approved include:

Document	*Version*	*Date*
Investigator's Brochure: Blackmores product information		12 March 2010
Investigator's Brochure: TGA Summary Musashi Creatine Monohydrate		12 July 2010
Investigator's Brochure: TGA Summary Musashi Glutamine		12 July 2010
Investigator's Brochure: TGA Summary Musashi HMB Capsules		12 July 2010
Investigator's Brochure: TGA Summary Blackmores Fruity Fishies		23 April 2010
Investigator's Brochure: TGA Summary Kids Multi Vitamin		23 April 2010
Investigator's Brochure: TGA Summary Vitamin D		23 April 2010
Letter of invitation to participant: Initial letter of invitation	2	17 November 2010
Protocol	4.1	17 November 2010
Questionnaire: ACTIVLIM questionnaire		14 December 2010
Questionnaire: PedsQL Core module 5-7 yrs	4	01 January 2000
Questionnaire: PedsQL core module 8-12yrs	4	01 January 2000
Questionnaire: PedsQL core module 13-18yrs	4	01 January 2000
Questionnaire: PedsQL Neuromuscular module age 5-7yrs	3	01 January 2005
Questionnaire: PedsQL Neuromuscular module 8-12yrs	3	01 January 2005
Questionnaire: PedsQL Neuromuscular module 13-18yrs	3	01 January 2005
Food Diary	1	17 June 2009
Peer Review: Monash University ethics approval		17 November 2010
radiation assessment		13 July 2010
Peer Review: The Royal Children's Hospital ethics approval		20 October 2010
~~Peer Review: Amend approval from Royal Children's Hospital~~		~~06 December 2010~~
Signed NEAF from Victorian organisations.		14 December 2010
Investigator CV: Associate investigator Davidson CV		16 December 2010
Covering Letter: Signed cover letter		16 December 2010
Patient Information Sheet/Consent Form: Parent information sheet and consent form	7	14 December 2010
Investigator CV: CV for Prof Helen Truby		17 December 2010
Research Funding Schedule		16 December 2010
Copy of signatures for QLD NEAF		06 January 2011
Application		

A copy of this approval must be submitted to the District Executive with a completed Institutional Approval Form for authorisation from the CEO or Delegate to conduct this research within the Children's Health Service District.

The HREC wishes you every success in your research.

With kind regards,



Professor John Pearn
Chair
QLD Children's Health Services (RCH) Human Research Ethics Committee

Cc: Ethics Committee Files

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MATER HEALTH SERVICES HUMAN RESEARCH ETHICS COMMITTEE

27th June 2011

Professor Helen Truby
C/O- Miss Zoe Davidson
Monash Medical centre
Level E Block E
Clayton VIC 3168

Dear Prof Truby

Re: Protocol *Ref №. 1730C* Nutriceuticals in Duchenne Muscular Dystrohy (DMD): Investigating the effectiveness of a multicomponent nutritional supplement

I write to advise that the Mater Health Services Human Research Ethics Committee considers the above study to meet the requirements of the *National Statement on Ethical Conduct in Human Research* (2007) and has granted ethical approval for your research proposal. Please accept our very best wishes for the success of this study. *In all future correspondence with the Committee please quote the Mater reference number.*

Documents reviewed and approved include:

- NEAF
- Protocol V4.1 Dated 17/11/2010
- PICF – Parent version 7.0 Dated 14/12/2010
- Recruitment Letter
- Staff Information Sheet
- Monash University Human Ethics Certificate of Approval Dated 17/11/2010
- Royal Children's Hospital (QLD) Human Ethics Certificate of Approval Dated 2/2/2011
- Royal Children's Hospital (VIC) Human Ethics Certificate of Approval (AMEND) dated 6/12/2010
- Blackmores Product Information
- TGA Summary Musashi Creatine Monohydrate Dated 12/7/2010
- TGA Summary Musashi Glutamine Dated 12/7/2010
- TGA Summary Musashi HMB Capsules Dated 12/7/2010
- TGA Summary Blackmores Fruity Fishies Dated 23/4/2010
- TGA Summary Blackmores Kids Multi Vitamiin Dated 23/4/2010
- TGA Summary Blackmores Vitamin D Dated 23/4/2010
- Questionnaires:
  - ACTIVLIM
  - Ped QL Core Module 5-7 years Version 4 Dated 2000
  - Ped QL Core Module 8-12 years Version 4 Dated 2000
  - Ped QL Core Module 13-18 years Version 4 Dated 2000
  - Ped QL Neuromuscular Module 5-7 years Version 3 Dated 2005
  - Ped QL Neuromuscular Module 8-12 years Version 3 Dated 2005

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  - Ped QL Neuromuscular Module 13-18 years Version 3 Dated 2005
- Food Diary version 1.0 17/6/2009

This approval is valid until **27th June 2014**. Please note the following conditions of approval.

- Any departure from the protocol detailed in your proposal must be reported immediately to the Committee.
- When you propose a change to an approved protocol, which you consider to be minor, you are required to submit a written request for approval to the Chairperson, through the Secretary. Such requests will be considered on a case by case basis and interim approval may be granted subject to ratification at the next meeting of the Committee.
- Where substantial changes to any approved protocol are proposed, you are required to submit a full, new proposal for consideration by the Human Research Ethics Committee.
- You are required to advise the Research Ethics Coordinator immediately of any complaints made, or expressions of concern raised, in relation to the study, or if any serious or unexpected adverse events occur.
- Under the *NHMRC National Statement on Ethical Conduct in Research Involving Humans*, research ethics committees are responsible for monitoring approved research to ensure continued compliance with ethical standards, and to determine the method of monitoring appropriate to each project. You are required to provide written reports on the progress of the approved project annually, the first report being due on **date** and finally on completion of the project. *(The Progress Report is located at http://www.mater.org.au/Home/Research/Human-Research-Ethics-Committee.aspx or can be accessed through the Mater Intranet, Applications, Research Register then under the project name or alternately can be emailed to you).* Please inform the Committee of publications, presentations at Conferences, education and quality improvement outcomes from this study. The Committee may also choose to conduct an interim audit of your research.
- Please be aware that all study procedures including follow up of participants and data analysis should be completed within the approval time frame or an extension should be requested.

You are reminded that this letter constitutes ethical approval only. You must not commence this research project until authorisation from the Research Governance Office has been obtained.

Please contact the Executive Director in the participating hospital/hospitals prior to commencing of the study. To access medical records, for the purpose of this study, please provide a copy of this approval letter to the Corporate Health Information Manager. I would also be grateful if you could confirm the date of commencement. (All correspondence should be directed to the Mater Research Ethics Coordinator.)

Yours sincerely

[redacted]

A/Prof Andrew Crowden
Chairperson
Mater Health Services Human Research Ethics Committee

Research Ethics Secretariat Room 57 Level 3 Quarters Building Ph: 07 31631585 Fax: 07 31631571 Email: research.ethics@mater.mmri.org.au

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