

THE INFLUENCE OF COMBINED VITAMIN D3
SUPPLEMENTATION AND RESISTANCE EXERCISE
TRAINING ON MUSCULOSKELETAL HEALTH IN OLDER
MEN AND WOMEN

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ABSTRACT

Sarcopenia, defined as the loss of muscle mass, strength and function has serious personal and societal implications. This is mainly via the loss of independence and decreased quality of life, and the prevalence of sarcopenia is known to increase with age. Although resistance exercise training is known to combat sarcopenia, improvements observed in older adults are blunted in comparison to younger adults. Some observational and interventional evidence suggest that vitamin D is associated with aspects of sarcopenia, therefore, the aim of this thesis was to determine if vitamin D3 supplementation combined with resistance exercise training was more effective in improving musculoskeletal health than resistance exercise training alone.

Chapter 2 presented the first systematic review and meta-analysis of the effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health in older adults. A total of 7 studies including 792 participants were reviewed. A small but significant improvement in muscle strength of the lower limb and further improvements in muscle function parameters and bone mineral density was evidenced as a result of the combined intervention from a subgroup of 3 studies (n = 518) adopting a similar methodology. The review highlighted the lack of studies implementing an appropriate study design (i.e. the combined intervention compared to exercise alone) and sample size to investigate the role of vitamin D in improving aspects of sarcopenia.

Chapter 3 presents a secondary data analysis examining the association between seasonal serum 25(OH)D concentration, lean mass and strength and the novel outcome of seasonal-dependent sarcopenic status in a subgroup of postmenopausal women from the Vitamin D, Food Intake, Nutrition and Exposure to Sunlight in Southern England (D-FINES) cohort. No association between lean mass and 25(OH)D concentration was observed, however, muscle strength and serum 25(OH)D concentrations were positively associated across all 4 seasons. Sarcopenic status was found to be transient in a very small sample of sarcopenic participants (n = 10).

The Exercise and vitamin D study (EXVITD) is presented in Chapters 4 to 6. This was a 6-month double-blinded, randomized controlled trial of twice weekly resistance exercise training and daily supplemental vitamin D3 (800 IU) or placebo tablet. This study recruited 24 community-dwelling older adults (mean age = 70.83 years), with 19 participants completing the intervention. Between-group differences were not detected in any of the outcome measures assessed, most likely due to the small sample size of recruited participants. The results presented here suggest that further research is warranted and thus, recommendations for future studies are presented in Chapter 7.

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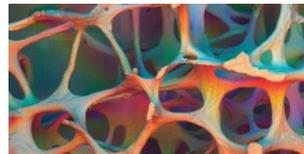
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LIST OF ABBREVIATIONS

1,25(OH)D – 1,25-hydroxyvitamin D

1-RM – 1 repetition maximum

25(OH)D – 25-hydroxyvitamin D

4EBP-1 – 4E binding protein 1

ADL – Activities of daily living

ADNFS – Allied Dunbar National Fitness Survey

ALM - Appendicular lean mass

ASM – Appendicular skeletal muscle mass

BIA – Bioelectrical impedance

BMC – Bone mineral content

BMD – Bone mineral density

BMI – Body mass index

CI – Confidence interval

COPD – Chronic obstructive pulmonary disease

CRP – C-reactive protein

CSA – Cross-sectional area

CT- Computerized tomography

CV – Coefficient of variation

DBP – Vitamin D binding protein

DXA – Dual-energy X-ray absorptiometry

EAA – Essential amino acids

EE – Energy expenditure

ESCEO – European Society for Clinical and Economical aspects of Osteoporosis and Osteoarthritis

EWGSOP – European Working Group on Sarcopenia in older People, 2010

EWGSOP2 - European Working Group on Sarcopenia in older People, 2018

FFM – Fat free mass

FNIH – Foundations for the National Institute of Health

FSA – Food Standards Agency

GH – Growth hormone

HR – Hazard ratio

HSE -Health Survey for England

ICC – Intraclass correlation coefficient

IGF – Insulin-like growth factor

IL – Interleukin

IU – International Units

MAPK – MAP Kinase

MCAR – Missing completely at random

MDD – Minimum detectable dose

MPB – Muscle protein breakdown

MPS – Muscle protein synthesis

MRI – Magnetic resonance imaging

mTOR – mammalian receptor of rapamycin

NHANES – National Health and Nutritional Examination Survey

OR – Odds ratio

p70s6K – ribosomal protein S6 kinase beta-1

PLC – Phospholipase C

PTH -Parathyroid hormone

RASM – Relative appendicular skeletal muscle mass

RCT – Randomized controlled trial

RDA – Recommended daily allowance

RET – Resistance exercise training

RNI – Reference Nutrient Intake

RXR – Retinoic X receptor

SACN – Scientific Advisory Committee

SARC-F – SARC-F questionnaire

SASP – Senescent associated sensory phenotype

SAT – Subcutaneous adipose tissue

SD – Standard deviation

SMC – Swedish Mammography Cohort

SMD – Standardized mean difference

SMI – Skeletal Muscle Index

SMM – Skeletal muscle mass

SPPB – Short physical performance battery

TNF- α – Tumour necrosis factor alpha

TUG – Timed up and go test

UV – Ultraviolet

VAT – Visceral adipose tissue

VDR – Vitamin D receptor

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1.1. An ageing population

The UK has an ageing population. It is projected that, by 2041, older people of pensionable age (defined as those aged 67 years and older) will constitute 22.3% of the total population, and people within this age category will outnumber the under 16s by 3.6 million (Office for National Statistics, 2017). The changing demographic profile of the UK population has social implications as increasing strain is being placed on the pension system, the National Health Service, social care and an increased number of people are providing informal care for family members (Rutherford, 2012).

The consequences of an ageing population are not confined to the UK; an ageing population is a global phenomenon, with the number of people aged 60 years and older expected to almost triple across the world by 2050 (Rutherford, 2012), and countries in Europe, North America, China, Iran, Korea and Russia are expected to have over 30% of their populations aged 60 and over (World Health Organization, 2015). Although life expectancy at birth is increasing, there is also an increasing number of years that men and women can expect to live in poor health; in 2010 this was 7.7 years and 8.7 years, respectively (Office for National Statistics, 2014b).

Babies born in the UK between 2009-2011 are expected to live around 20% of their lives in poor health, and for women, disability rates at age 65 years have worsened (Office for National Statistics, 2014b). Participation in physical activity and maintaining adequate nutrition are essential for healthy older age (World Health Organization, 2015), with a large systematic review (n= 83,740) reporting that regular

aerobic physical activity conferred a 30-50% reduction in risk of functional limitation and disability (Paterson and Warburton, 2010).

1.1.1. Physiological characteristics of older age

Ageing is typified by progressive losses in muscle mass and strength, leading to reduced physical functioning and performance (Trombetti et al., 2016). Increased amounts of adipose tissue further characterise the ageing process, with intramuscular adipose deposits resulting in further detriments to muscle strength and function (Visser et al., 2002) . Changes in muscle are accompanied by losses in bone mineral density, strength and changes to bone architecture, resulting in fragility and an increased risk of fracture (NIH Consensus Development Panel on Osteoporosis Prevention and Therapy, 2001). Finally, ageing is also associated with a decreased vitamin D status, for which there is evidence of both a direct and indirect influence on muscle and bone health (Sanders et al., 2014).

1.1.2. Sarcopenia: Definition and current consensus

Sarcopenia was first described by Irwin Rosenberg in 1989 as the term used to define the age-associated decline in muscle mass (Rosenberg, 1989). In 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) categorised sarcopenia as a geriatric syndrome, multifactorial in nature, encompassing losses in muscle strength and function alongside losses in muscle mass (Cruz-Jentoft et al., 2010c). The rationale for the new working definition was thus; losses in muscle strength and function are more clinically significant than losses in muscle mass alone, as previously described by (Rosenberg, 1989), and losses in muscle mass, strength and function are often disproportionate (Marcell, 2003).

The EWGSOP met most recently in 2018 (EWGSOP2) to revise the 2010 consensus definition and diagnosis. Sarcopenia is now recognised as a muscle disease by the EWGSOP2, and designated an ICD-10-CM Diagnosis Code in the USA in 2019 (International Classification of Diseases, 2019), the EWGSOP2 describe sarcopenia with an operational algorithm, comprising muscle strength to assess suspected cases of sarcopenia, muscle quality/quantity to confirm clinical suspicions and functional performance assessment to categorise severity (Writing Group for the European Working Group on Sarcopenia in Older People 2 et al., 2018). Muscle strength is the primary focus of the algorithm, with the EWGSOP2 citing muscle strength as the most reliable measure of muscle function (Writing Group for the European Working Group on Sarcopenia in Older People 2 et al., 2018).

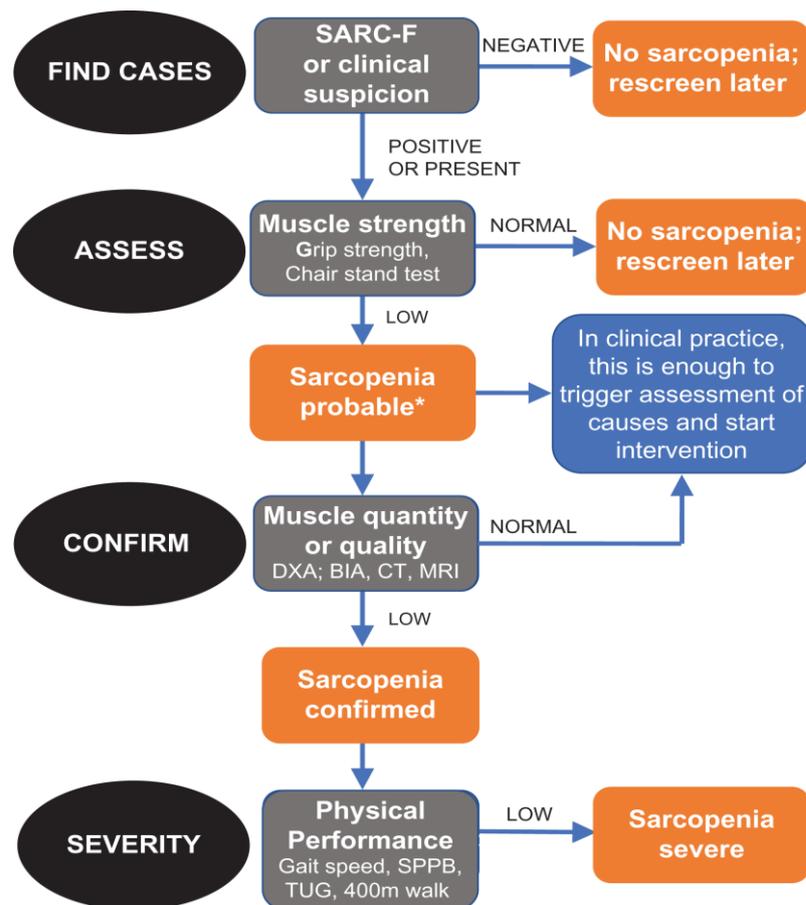


Figure 1.1: EWGSOP2 algorithm for sarcopenia diagnosis and categorisation

SARC-F: SARC-F questionnaire, DXA: Dual energy X-ray absorptiometry, BIA: Bioelectrical impedance, CT: Computerised Tomography, MRI: Magnetic resonance imaging, SPPB: Short physical performance battery, TUG: Timed up and go.

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1.1.3. Measurement of sarcopenia

As shown in Figure 1.1, the EWGSOP2 recommends grip strength, isometric lower limb strength and the chair rise test to measure muscle strength (Writing Group for the European Working Group on Sarcopenia in Older People 2 et al., 2018).

Four main techniques are currently used to assess muscle quality and quantity; i) bioelectrical impedance (BIA), which is founded on the principle that specific tissue types have differences in conductivity. This technique is safe and inexpensive, and a

previous validity study (n = 484, mean age = 63.5 years) concluded excellent agreement with dual energy x-ray absorptiometry (DXA) for whole lean body mass (intraclass correlation coefficient (ICC) female = 0.95, men = 0.96), fat mass (ICC female = 0.97, men = 0.93) and percentage fat mass (ICC female = 0.93, men = 0.88). However, a direct-segmental multi-frequency BIA system using an eight-point tactile electrode system measuring the 5 body components at six different frequencies (In-body 720) systematically underestimated segmental lean body mass (trunk lean body mass ICC female = 0.73, male = 0.69) (Ling et al., 2011). Although ii) Magnetic resonance imaging (MRI) and iii) computed tomography (CT) are considered to be the gold standards for measuring muscle mass and quality, expense, operator expertise and access currently make extensive use unfeasible (Beaudart et al., 2016a). Additionally, CT exposes individuals to higher levels of radiation compared with DXA (Buckinx et al., 2018a).

Conversely, iv) DXA is more widely available, produces lower radiation doses and measurements of muscle mass and quality are highly correlated with both CT (multi-slice thigh fat-free mass, $r^2 = 0.96$) (Levine et al., 2000) and MRI (whole body lean mass $r = 0.94$) (Chen et al., 2007). Although DXA does have a number of limitations, such as the inability to measure intramuscular fat and reported differences in results between devices (Buckinx et al., 2018a), it has been cited as a reliable method of indirectly estimating muscle mass in older adults (Chen et al., 2007).

Muscle quantity is traditionally reported as appendicular skeletal muscle mass (ASM). ASM is measured as the sum of lean mass of the arms and legs, and a result of 2 standard deviations (SD) below that of a young reference population is categorised as sarcopenic (Baumgartner et al., 1998).

ASM can be used to additionally calculate skeletal muscle mass index (SMI); $SMI = ASM/body\ mass \times 100$. SMI is widely used to define sarcopenia (Rubbieri et al., 2014), adjusting for the fundamental correlation between body size and muscle mass. Sarcopenia is divided into 2 classes; class I if the SMI is 1 to 2 SDs below young adult reference values, and class II if the SMI is over 2 SDs below young adult values (Janssen et al., 2002). The EWGSOP2 classify sarcopenia as “severe” if muscle mass, strength and performance are all determined to be low (Writing Group for the European Working Group on Sarcopenia in Older People 2 et al., 2018)

1.1.4. Prevalence of sarcopenia

Prevalence rates of sarcopenia vary extensively dependent on the operational definition applied and the assessment tools utilised. For example, of 250 men and women aged 65 years and older, regarding the measurement of muscle mass, sarcopenia prevalence was estimated to be 12.8% using BIA and 21% when measured using DXA (Beaudart et al., 2015b). Similarly, when assessing the component of muscle strength, use of a pneumatic dynamometer diagnosed almost twice the proportion of sarcopenic participants than a hydraulic dynamometer (22% vs 11.4% (Beaudart et al., 2015b)). No differences were observed between gait speed and the SPPB as methods to assess physical performance (Beaudart et al., 2015b).

However, regardless of methodology, prevalence rates of sarcopenia increase with increasing age (Baumgartner et al., 1998), with studies reporting prevalence rates approaching (Berger and Doherty, 2010) or exceeding 50% in people older than 80 years (Kenny et al., 2002). A systematic review of 18 publications (n = 15,824) in adults aged ≥ 50 years reported sarcopenia prevalence rates using the EWGSOP

definition to be 1 – 29% for community dwelling older adults, 14 – 33% for older adults living in care homes (as high as 68% in men) and 10% for older adults receiving acute hospital care (Cruz-Jentoft et al., 2014a). A more recent study using the EWGSOP definition of sarcopenia within a UK cohort published prevalence rates of 21% in men and women of mean age 86.5 years (Dodds et al., 2017).

1.1.5. Aetiology of sarcopenia

As a geriatric syndrome, sarcopenia is multifaceted and complex in nature. The key factors believed to be involved are cited in a review study as age, malnutrition, lifestyle, hormonal changes, neuromuscular impairments, changes in muscle protein synthesis and a decline in motor unit number (Narici and Maffulli, 2010). These factors are represented in Figure 1.2 and are discussed in the following sections.

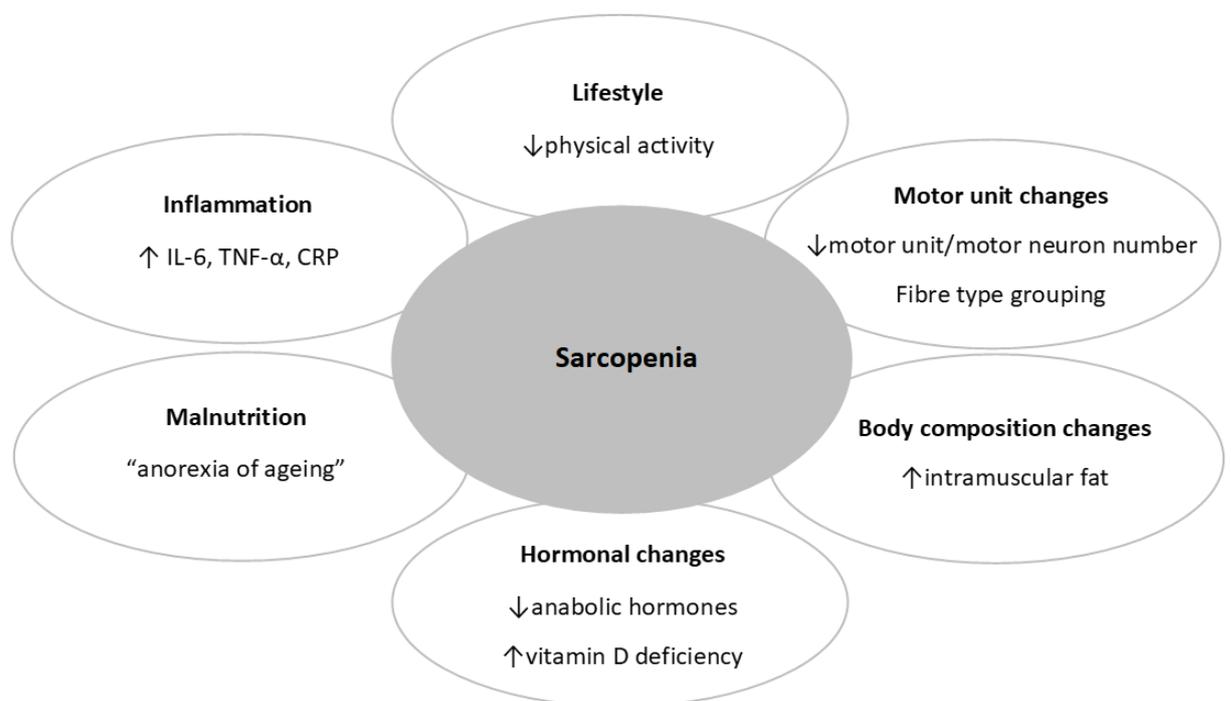


Figure 1.2: The multifaceted aetiology of sarcopenia (adapted from (Walston, 2012)).

1.1.6. Lifestyle

Significant changes in body composition are known to occur with age, due in part to an increasingly sedentary lifestyle (Kyle et al., 2001). Accelerated declines in ASM and fat free mass (FFM) have been reported in older (>75 years) compared to younger (18-35 years) adults; ASM -16.4% and -12.3% (men and women respectively), and FFM -11.8% and -9.7% (men and women respectively) (Kyle et al., 2001). However, losses in muscle mass may not manifest as weight loss, and body mass index (BMI) may remain stable or non-significantly altered due to increases in fat mass, potentially concealing sarcopenia (Gallagher et al., 2000). Furthermore, one study of men and women aged 70 – 79 years (n = 2,307) reported that greater fat mass predicted accelerated losses of lean mass in men and women; this was not explained by insulin resistance, although may be mediated via increased local inflammation as a result of greater adipose tissue and/or physical inactivity. Greater fat mass was also significantly related to lower muscle quality at baseline (Koster et al., 2011).

An overall decrease in subcutaneous fat is a feature of advancing age, with increases in fat mass mainly visceral and intramuscular (Stenholm et al., 2008). Infiltration of fat into the muscle tissue is known to negatively impact muscle strength and performance, independent of total body fat and muscle area, particularly within the lower limbs (Visser et al., 2002); in a 13 year longitudinal study of 3075 older men and women (mean age = 74.2 years), every 1 SD increase in thigh intramuscular adipose tissue area correlated with a 37% and 8% increased risk for mobility limitation (2 consecutive reports of difficulty walking 0.25 miles or ascending 10 steps) in men and women, respectively (Murphy et al., 2014).

This relationship appears to be true of muscles with secondary roles supporting and stabilising force production and movement. The Framingham Heart study assessed paraspinal muscles via 8-slice multidetector CT in 1152 older men and women (mean age = 66.2 years) and found every 1 SD decrement in muscle attenuation (i.e. more intramuscular fat) was associated with an increased odds of walking speed ≤ 1 m/s of 1.29 (95% confidence interval (CI) 1.11, 1.50, $p = 0.00009$); the relationship was still significant after adjustment for BMI, visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) volume (Therkelsen et al., 2016). Furthermore, every 1 SD decrement in muscle attenuation was associated with a - 1.29kg decrement in strength (handgrip strength; 95% CI -2.01, -0.57, $p = 0.0005$), although this relationship was observed in men only (Therkelsen et al., 2016).

Additionally, those with both sarcopenia and obesity, known as sarcopenic obesity, have higher levels of onset of activities of daily living disability than those with either sarcopenia or obesity alone (Baumgartner et al., 2004). The two diseases working synergistically are known to be associated with poor health outcomes, such as frailty and metabolic disorders (Zamboni et al., 2008). Additionally, in a meta-analysis of 12 cohort studies including 35,287 men and women aged 45 and over, sarcopenic obesity was associated with an increased risk of all-cause mortality (Hazard ratio (HR) 1.24, 95% CI 1.12–1.37, $P < 0.001$); heterogeneity was no longer significant following a subgroup analysis by sarcopenia definition, however the increased risk of all-cause mortality remained (Tian and Xu, 2016)

Physical activity levels are negatively associated with age (Milanović et al., 2013). Reductions in physical activity are responsible for approaching 50% of the decrease in energy expenditure (EE) observed in older people (Elia et al., 2000), and a sedentary lifestyle (assessed by the International Physical Activity Questionnaire) is

associated with obesity (Silva et al., 2013) and losses in muscle mass and physical functioning (Gianoudis et al., 2015); in a cross-sectional study of 162 older men and women (mean age = 67.5 years), every 1 hour increase in daily sitting time was associated with a 33% increased risk of being sarcopenic (relative appendicular skeletal muscle mass (RASM) estimated by DXA) (Odds ratio (OR) 1.33, 95% CI 1.05, 1.68, $p < 0.01$) (Gianoudis et al., 2015).

In a multi-continent study including 18,363 adults aged 65 years and over, low levels of self-reported physical activity were significantly associated with an increased risk for both sarcopenia (OR 1.36, 95% CI 1.11, 1.67, $p < 0.01$) and sarcopenic obesity (OR 1.80, 95% CI 1.23, 2.64, $p < 0.01$), but not skeletal muscle mass (SMM) (Tyrovolas et al., 2016). Percentage body fat (indirectly estimated using specific population formulas) was significantly associated with lower SMM (OR -0.005, 95% CI -0.006, -0.005, $p < 0.001$) and an increased risk for sarcopenia (OR 1.03, 95% CI 1.02, 1.04, $p < 0.001$) (Tyrovolas et al., 2016).

1.1.7. Hormonal changes

Also contributing towards a change in body composition are the hormonal changes observed with ageing. The observed age-related declines in anabolic hormones, including testosterone, growth hormone (GH) and insulin-like growth factor 1 (IGF-1) (Kamel et al., 2002) are proposed as a potential factor influencing the pathogenesis of sarcopenia.

Serum testosterone concentrations are known to decline during healthy ageing; decreased testosterone production results in a decline of approximately 25% in serum free testosterone between the ages of 25 – 75 years (Kaufman et al., 2018). The reduction in both free and total testosterone concentrations in older men is

known to be associated with negative body composition outcomes (decreased muscle mass and increased fat mass), along with diminished muscle strength and physical function (Bhasin et al., 2010); longitudinally, higher concentrations of testosterone are associated with smaller losses in both lean mass and lower limb function in older men losing weight (LeBlanc et al., 2011).

A recent review of systematic reviews investigating possible pharmacological interventions to improve sarcopenia domains in older people concluded, from high quality evidence, that testosterone supplementation may be one possible intervention to improve muscle mass and strength in older men with androgen deficiency (De Spiegeleer et al., 2018). Individual studies included in the review report gains of up to 4kg in lean body mass, with duration and type of testosterone regimen noted as influencing intervention response (O'Connell et al., 2011). There was a less prominent effect of testosterone supplementation on muscle strength, with supplementation producing a moderate increase in overall muscle strength of approximately 19.3% (Ottenbacher et al., 2006); a smaller effect still was observable on physical functioning (De Spiegeleer et al., 2018).

The Testosterone Trial (Snyder et al., 2016) and the TEAAM trial (Storer et al., 2016) were recent randomized controlled trials (RCTs) supplementing with testosterone. The Testosterone Trials were a series of 7 studies recruiting men aged 65 years and older with low serum testosterone (<9.5nmol/L) who were randomized to receive testosterone or placebo gels for 1 year. Serum testosterone concentration was assessed periodically, and testosterone gel dose adjusted to maintain concentrations within the normal range for young men. The Physical Function Trial arm included a subgroup of 387 older men, and it was concluded that there was no significant difference in the percentage of men whose 6-minute walking distance improved by at

least 50 meters between the testosterone and placebo groups, although self-reported walking ability score was higher in the testosterone group (Snyder et al., 2016).

The TEAAM trial recruited 256 older men (mean age = 66.6 years) with low or low normal serum testosterone concentrations (3.5 – 13.9 nmol/L) whom were randomized to receive 7.5g of 1% testosterone gel or placebo gel daily for 3 years; dosage was adjusted as required to maintain normal serum testosterone concentrations. Chest-press strength (number of presses +16.3), chest-press (+22.5 W) and leg-press peak power (+83.8 W), stair-climb power (loaded = +22.4 W, unloaded = +10.7 W) and lean body mass estimated by DXA (arms = +0.9kg) were significantly improved within the testosterone group compared to the placebo group. However, no significant differences were observed in leg-press strength, chest-press or leg-press fatiguability between groups (Storer et al., 2016).

Although testosterone supplementation has been shown to improve sarcopenia in men with low serum testosterone concentrations, and (via a much smaller body of evidence) in women (Morley and Perry, 2003), concern of adverse effects, such as the risk and exacerbation of some cancers (Dimopoulou et al., 2016), and a substantially increased risk of erythrocytosis (Ponce et al., 2018), means that its use remains controversial. Alongside testosterone, vitamin D is the only other pharmacological intervention that would be “clinically justifiable” in the improvement of sarcopenia (De Spiegeleer et al., 2018), with one study stating that “no other drugs have been shown to be clinically more therapeutically effective” than vitamin D (Morley, 2016).

A further factor proposed to influence age-related loss of skeletal muscle mass is insulin resistance. Hyperinsulinaemia is known to stimulate the accretion of muscle

mass via the activation of p38 mitogen-activated 154 protein kinase (MAPK) and the mammalian target of rapamycin (mTOR) pathway (Guillet and Boirie, 2005), however, the anabolic action of insulin is blunted in older compared to younger adults, resulting in a reduction in muscle mass, strength and function (Rasmussen et al., 2006).

In a study of 399 men and women aged 60 years and over, the homeostasis model assessment of insulin resistance (HOMA-IR; calculated as fasting plasma glucose x fasting insulin) was found to be inversely associated with ASM (BIA; $\beta=0.47$, $p = 0.001$) following adjustment for body weight (Lee et al., 2015). Furthermore, it has been suggested that sarcopenia may contribute towards insulin resistance, in turn exacerbating skeletal muscle loss (Lee et al., 2015).

1.1.8. “Inflammaging” and its associations with sarcopenia

The term “inflammaging” was conceived to describe the persistent, chronic low-grade inflammation characteristically observed with ageing, which is believed to result from an increase in pro-inflammatory markers resulting in a vulnerability to antigenic stressors (e.g. malnutritional, physical and psychological trauma) (Franceschi et al., 2000), which can be positively or negatively affected by lifestyle factors (e.g. physical activity) (Abramson and Vaccarino, 2002).

The Senescent-Associated Sensory Phenotype (SASP) acquired by accumulating senescent cells triggers the production of pro-inflammatory cytokines such as interleukine-6 (IL-6) and tumour necrosis factor-alpha (TNF- α), which have both positive (e.g. promoting tissue repair) and negative (e.g. inflammation and ageing) effects (see Figure 1.3) (Lopes-Paciencia et al., 2019). The subsequent chronic inflammation has been proposed as one factor contributing to the dysregulation

between muscle protein synthesis (MPS) and muscle protein breakdown (MPB) observed with chronological ageing, resulting in direct catabolic effects and a loss in skeletal muscle mass (Dalle et al., 2017). Alternatively, it has been suggested that inflammation may indirectly contribute towards sarcopenia via its negative association with anabolic hormones such as GH and IGF-1; for example, the Women’s Health and Aging Study, a study of 718 community-dwelling women aged over 65 years concluded that women in the both the lowest quartile for IGF-1 and the highest quartile for IL-6 had significantly greater limitations in walking, disability in mobility tasks and instrumental activities of daily living (ADL) and a 2-fold higher risk of mortality during the 5 year follow-up compared with those in the high IGF-1/low IL-6 group (Cappola et al., 2003). However, whether the role of inflammation in muscle atrophy and sarcopenia may be causal is not well understood.

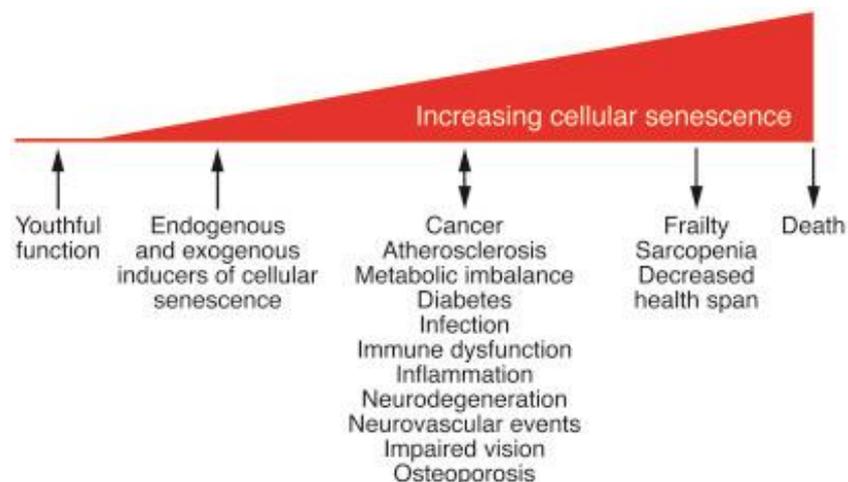


Figure 1.3: Cellular senescence, frailty and sarcopenia

Increasing cellular senescence, the SASP and a possible predisposition towards frailty and sarcopenia. Increasing numbers of senescent cells across the lifespan, further increased by age-related disease and the spread of senescence to healthy cells in close proximity. Reproduced with permission from (Tchkonia et al., 2013). Published by the American Society for Clinical Investigation on behalf of the Journal of Clinical Investigation. Copyright license number 4625940756077.

IL-6, TNF- α and C-reactive protein (CRP) are the markers most frequently associated with chronic diseases and physical disability (Singh and Newman, 2011), with cross-

sectional studies consistently reporting these markers to be negatively associated with muscle mass, strength, physical function and frailty (Beyer et al., 2012). A 2016 meta-analysis of 17 studies including 11,249 sarcopenic (mean age = 66.3) and non-sarcopenic (mean age = 66.2) control participants concluded that sarcopenic participants experienced significantly higher CRP concentrations than controls (Standardised Mean Difference (SMD) = 0.51, 95% CI 0.26, 0.77, $p < 0.0001$). No differences in IL-6 nor TNF- α concentrations were observed between sarcopenic and control participants, although studies assessing these markers were underrepresented within the sample (Bano et al., 2017).

Inflammatory markers were associated with muscle strength in the Longitudinal Aging Study Amsterdam; every 1 SD increment in IL-6 resulted in a 3.21kg decrease in muscle strength assessed by grip strength, and high CRP concentrations ($>6\mu\text{g/ml}$) and high IL-6 concentrations ($>5\text{pg/ml}$) were associated with a 2 and 3-fold greater risk of strength loss, respectively, in the 986 older men and women (mean age = 74.6) included (Schaap et al., 2006). Inflammatory markers have also been negatively associated with knee extension strength (Zembron-Lacny et al., 2019), appendicular skeletal muscle mass (Alemán et al., 2011) and activities of daily living (ADL) (Sujarwoto and Tampubolon, 2015) in older adults.

In addition, obesity is an important risk factor for chronic inflammation; obesity-related inflammation acts synergistically with senescent cells to produce a pro-inflammatory environment (Kalinkovich and Livshits, 2017). Increases in fat mass result in the enlargement of adipocytes and an accumulation of macrophages, which secrete a number of pro-inflammatory markers such as IL-6 and TNF- α (Tilg and Moschen, 2006). In a cross-sectional study of 130 postmenopausal women (mean age = 66.7 years), individuals with sarcopenic obesity demonstrated higher serum

concentrations of CRP and TNF- α and significantly higher concentrations of IL-6 in comparison to non-sarcopenic obese controls; additionally IL-6 and CRP were also significantly associated with waist circumference, percentage body fat and fat mass in all participants (Dutra et al., 2017). In a further study, sarcopenic older adults over 60 years demonstrated significantly higher serum concentrations of IL-6 and TNF- α than non-sarcopenic controls, with BMI and visceral fat area found to be predictive factors of this difference (Bian et al., 2017).

Exercise has been found to have a positive influence on inflammatory status; in a study including 16 type II diabetic men (aged 55 – 70 years) randomized to 3 sessions of combined aerobic and resistance exercise training (RET) for 16 weeks or a usual care control, the exercise group not only significantly improved their aerobic (VO_2max), muscular (strength and endurance) fitness and body composition parameters, but also significantly reduced their TNF- α (-19.8%) and IL-6 (-25.3%) and significantly increased their IGF-1 (+16.4%) concentrations; CRP was also reduced by a greater amount than the control group (-12.5%), although this was non-significant (Annibalini et al., 2017).

Furthermore, there may be an association between vitamin D deficiency and inflammatory markers; in a cohort of 23 older men and women (mean age = 68.8 years), vitamin D deficiency (defined as $<50nmol/L$) was significantly correlated with TNF- α concentrations (Elizondo-Montemayor et al., 2017). In addition to TNF- α concentrations, IL-6 and CRP concentrations were significantly higher with vitamin D deficiency (defined as $<25nmol/L$) and vitamin D deficient participants were 3-times more likely to have a IL-6:interleukin-10 (IL-10) ratio of 2:1 in a sample of 957 older adults (mean age =70.5 years) (Laird et al., 2014). These studies raise the possibility that vitamin D deficiency predisposes older adults to a pro-inflammatory environment.

1.1.9. Changes in motor unit number

With chronological ageing, losses in muscle mass associated with sarcopenia are due to a combination of muscle fibre atrophy and muscle fibre loss (Narici and Maffulli, 2010); and although fibre atrophy may be partially overcome with particular interventions (evidence presented in section 1.3), hypoplasia remains unaffected (Piasecki et al., 2018). A motor unit consists of an alpha motor neuron and all of the muscle fibres that it innervates; ageing is associated with a net loss in motor units (Hepple and Rice, 2016). Post mortem anatomical studies have demonstrated progressive losses in motor neuron cells after the age of 60 years, with the average 75 year old displaying approximately 30% fewer motor neuron cells in the lower limbs than younger subjects, although this loss reached 50% in several older subjects (Tomlinson and Irving, 1977).

In a study comparing younger (n = 22, mean age = 25.3 years) and older (n = 20, mean age = 71.4 years) habitually active men exceeding Department of Health physical activity guidelines, older men had 50-60% fewer motor units than younger men (Piasecki et al., 2016). Additionally, the remaining motor units observed in the older men were larger with significantly slower discharge rates than the younger men; these changes were observed before the onset of any muscle weakness, according to grip-strength cut-offs, or functional impairments, as none of the men were classified as sarcopenic, and 95% of the cohort scored maximum marks in the SPPB test (Piasecki et al., 2016).

Cycles of denervation and reinnervation result in fewer but larger surviving motor units (Jones et al., 2009), fibre type grouping and selective hypoplasia of fast type fibres (Vandervoort, 2002); denervated cells are left vulnerable to atrophy and loss

(Wilkinson et al., 2018). Furthermore, Piasecki et al concluded that in sarcopenic older men, there was a failure to reinnervate fibres compared with non-sarcopenic older men, with fibre loss occurring when reinnervation could no longer keep pace with denervation (Piasecki et al., 2018).

1.1.10. Malnutrition

Food intake may be reduced by as much as 25% in older adults (Robinson et al., 2012a). The prevalence of malnutrition and those at risk of malnutrition aged over 65 years and living within the community have been reported to be 4.3% and 25.4%, respectively, with much higher prevalence rates observed in residential care (Nieuwenhuizen et al., 2010). The reasons behind the so called “anorexia of ageing” are complex and multifactorial, but include physical impairments such as losses in sight, smell and taste perception (Methven et al., 2012), dentition status, impaired masticatory ability and dysphagia, all of which may negatively influence food choices (Mann et al., 2013). Appetite is known to be decreased in older age, due to a reduction in the secretions that regulate appetite (Tsutsumimoto et al., 2018), and changes to the gastrointestinal tract and gut such as delayed gastric emptying, affect postprandial anorexia (Morley, 2017). Chronic disease, medications, altered absorption of essential nutrients and social determinants are additional factors effecting nutritional status in older adults (Brownie, 2006).

Protein and vitamin D are among the nutrients most consistently associated with sarcopenia (Robinson et al., 2012a), although the influence of dietary vitamin D intake on sarcopenia is not clear (Cruz-Jentoft et al., 2017) (Section 1.6 discusses vitamin D in more detail). Approximately 20-35% of amino acids required to maintain the balance between MPS and MPB are supplied through the diet (Mader, 1988),

and in the Maastricht Sarcopenia Study of older people (n= 227, age range = 65 – 95 years), the lowest quartiles for essential amino acids, total branched-chain amino acids and leucine blood concentrations were associated with significantly lower SMI (assessed by bioelectric impedance), muscle strength (grip strength) and function (chair stand time) (Ter Borg et al., 2019).

There is currently much debate as to whether current guidelines for protein intakes are sufficient for older people; a Recommended Daily Allowance (RDA) of $0.8 \text{ g.kg}^{-1} \text{ .day}^{-1}$ (Food and Nutrition Board, 2005) has remained unchanged since the 1985 joint World Health Organization/FAO/UNU Expert Committee and is the minimum recommendations to avoid progressive lean mass loss based on nitrogen balance. Some studies advocate that current RDAs need to be almost doubled to protect against losses in muscle mass and function (Wolfe et al., 2008, Phillips et al., 2016). There is evidence suggesting that the postprandial protein anabolic response is blunted in older adults compared to younger, which may increase the threshold of protein intake necessary to produce the desired stimulation of MPS; 43 older men (mean age =71.1 years) required significantly greater amounts of absolute protein to maximally stimulate MPS (up to an additional 140%) compared to 22 younger men (mean age = 22 years) (Moore et al., 2014). In addition to quantity, the quality, timing and distribution of protein intake have also been identified as other important factors influencing optimal MPS (Lonnie et al., 2018). For example, the consumption of protein and carbohydrate together may blunt the expected synthetic response (Robinson et al., 2017).

Vitamin D has been stated by the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) as important for “optimizing the effect of dietary protein on skeletal muscle anabolism” (Rizzoli et al., 2014). This was

demonstrated via post-hoc analysis of an earlier RCT study (Baier et al., 2009); older men and women (mean age = 76 years) were assigned to an active (N=40, supplemented with β -hydroxy- β -methylbutyrate, arginine and lysine) or control group (N=37). After one year, active supplementation resulted in a significant increase in FFM, however, it was only those within the active group with serum 25(OH)D₃ concentrations ≥ 75 nmol/L, that demonstrated significant increases in muscle strength measured as isokinetic knee extension strength (Fuller et al., 2011) Additionally, the PROVIDE study determined that in older adults with sarcopenia (N = 380, >65 years), sufficient baseline concentrations of 25(OH)D and protein intake may be required to respond to supplemental vitamin D and protein and increase muscle mass; the 13 week double-blind RCT of supplementary protein and vitamin D or isocaloric control found that male and female participants with higher baseline vitamin D (≥ 50 nmol/L) and higher baseline protein intakes (>1.0 g/kg/d) had a significantly larger gain in ASM (+0.59kg, 95% CI: 0.29, 0.90, $p < 0.001$), SMI and relative skeletal muscle mass than those in the intervention group with low baseline vitamin D and protein intake (Verlaan et al., 2018).

One mechanism for this action may be that the active form of vitamin D, 1,25(OH)₂D₃ sensitizes the Akt/mTOR-dependent pathway to the anabolic effects of proteins; 10nM of 1,25(OH)₂D₃ in addition to leucine and insulin enhanced protein fractional synthetic rate by 14 – 16% in murine C2C12 myotubes and also increased vitamin D receptor (VDR) expression (Salles et al., 2013).

1.1.11. Consequences of sarcopenia

The personal and societal costs of sarcopenia are serious. One of the major consequences of sarcopenia is the significant association with functional impairment and disability (Janssen et al., 2002). Sarcopenia is associated with a loss of independence, poorer quality of life, depression, admissions to care homes, an increased risk for hospitalization and even death (Leveille et al., 2000, Vaughan et al., 2015). Additionally, sarcopenia is associated with number of comorbidities such as diabetes, obesity and osteoporosis (Kim et al., 2014). Losses of muscle mass have further detrimental effects, since the tissue contributes towards many other roles, such as blood glucose and body temperature homeostasis (Vandervoort, 2002).

Sarcopenia represents a substantial burden to the healthcare system; a previous study reported that elderly hospitalized sarcopenic patients had a longer length of stay by 4 days, and the risk of non-elective readmission was significantly higher in these patients (Gariballa and Alessa, 2013). Furthermore, both short and prolonged periods of disuse have been shown to exacerbate muscle atrophy in older persons with sarcopenia (Wall et al., 2013); moreover, Welch et al coined the term “acute sarcopenia” to denote disuse atrophy and associated losses in function due to hospitalization (Welch et al., 2018). The cost of treating a sarcopenic patient older than 65 years is estimated to be increased by 34% compared with non-sarcopenic patients (Sousa et al., 2016), and sarcopenic patients have increased rates of surgical complications and mortality (Friedman et al., 2015).

1.2. Osteopenia and osteoporosis: Definition

Alongside a decline in muscle mass and strength, ageing is defined by a reduction in bone mass and strength parameters. Osteopenia and osteoporosis describe decreased bone density, resulting in adverse changes to the skeleton (Karaguzel and Holick, 2010). Compromising alterations in bone structure (geometry, microarchitecture, porosity) and bone material (mineralisation, collagen composition and damage accumulation) reduce the strength and integrity of bone, resulting in fragility and a predisposition towards an increased risk of fracture (NIH Consensus Development Panel on Osteoporosis Prevention and Therapy, 2001), see Figure 1.4.

Clinically, osteopenia and osteoporosis are assessed by comparing the results of a T-or Z-score via reference to a population of the same age and sex or a young adult population. Osteopenia is present in an individual with a T-score of between -1.0 and -2.5 and osteoporosis is confirmed in an individual with a T-score of 2.5 or more SDs below that of a young adult reference population (World Health Organization, 1994). The measurement of bone mineral density (BMD) (a proxy of bone strength) at the hip using DXA is considered to be the gold standard method of osteoporosis diagnosis, with accuracy at this site exceeding 90% (Kanis, 2002).

Growth and Bone

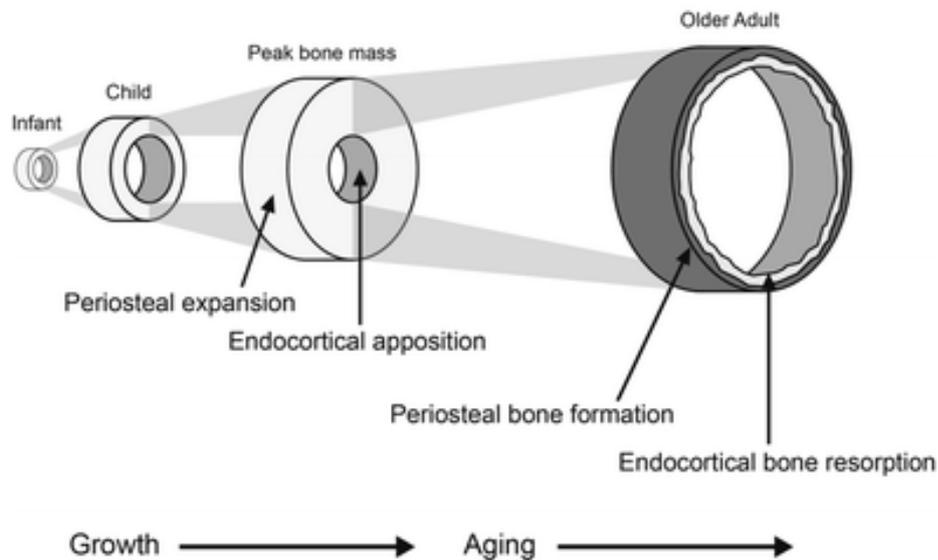


Figure 1.4: Changes in the structural composition of bone from infancy to older age.

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1.2.1. Prevalence and consequences of osteoporosis

As with sarcopenia, the incidence rate of osteoporosis continues to rise concomitantly with an ageing population; globally, it is estimated that 200 million people have osteoporosis (Reginster and Burlet, 2006). The most profound outcome of osteoporosis is the prevalence rate of fractures and their consequences; 1 in 3 women and 1 in 5 men over the age of 50 years will experience an osteoporotic fracture in their lifetime, and globally, an estimated 9 million osteoporotic fractures are reported per annum, equating to one fracture every 3 seconds (International Osteoporosis Foundation, 2017). 1 in 5 people suffering an osteoporotic hip fracture will die within the following year (Klibanski et al., 2001), and two thirds of those who survive never fully regain their pre-fracture function (Lane, 2006). In addition to

clinical consequences, osteoporosis is also the source of significant disability, pain, depression, anxiety and fear (Gold, 1996).

On a societal level, the financial cost of osteoporosis is considerable. In 2010 the medical cost attributed to treating osteoporosis in the European Union was an estimated €37 billion; nearly €25 billion of this amount was ascribed to fractures (Hernlund et al., 2013). Osteoporosis also significantly increases the risk of hospitalization, and a hip fracture increases average length of stay by 8.7 days (Becker et al., 2010).

1.2.2. Aetiology and risk factors for osteoporosis

As with sarcopenia, osteoporosis is a result of disequilibrium; in this instance, bone resorption rate outpaces formation rate. There are several factors which influence the decline in bone mass, such as the impaired functionality of osteoblast cells observed in older adults (Kveiborg et al., 2000). Peak bone mass is an important determinant of bone mass and fracture risk, with a failure to accrue a strong skeleton in younger life indicative of fragility in later life (Raisz, 2005).

Sexual dimorphism influences peak bone mass; in a 2-year longitudinal study of 620 men and women (age range = 20 – 89 years), peak BMD (estimated via DXA) in men was approximately 12-25% higher than in women (Warming et al., 2002). Similarly, a study of 51 male (mean age = 22.2 years) and female (mean age = 22.4 years) students found that BMD (estimated via DXA) was higher, and bone mineral content (BMC) significantly higher ($p < 0.01$) in the total femur, lumbar spine and distal radius and bone area significantly higher ($p < 0.0001$) at the lumbar spine and distal radius in males than in females (Avdagić et al., 2009).

A 4 year longitudinal study demonstrated much higher absolute mean percentage losses in BMD at all sites in older women than in older men of mean age 74 years (Hannan et al., 2000). Sex hormones such as oestrogen play a substantial role on BMD, primarily in women, but also in men; the withdrawal of oestrogen signals a period of accelerated bone loss in women, with cumulative BMD losses at the femoral neck and lumbar spine approximating 9.1% and 10.6%, respectively in the decade following the menopause (Cauley, 2015). Additionally, undetectable total serum oestradiol concentrations (<18pmol/L) were associated with a 2.5-fold greater risk for subsequent hip and vertebral fractures compared to concentrations >18pmol/L; 33% of the cohort of 247 older women (mean age = 72 years) were found to have undetectable oestradiol concentrations (Cummings et al., 1998). Oestrogen deficiency is known to influence bone mass by i) increasing rates of bone turnover, ii) increasing bone resorption by lengthening the lifespan of osteoclasts and increasing osteoclastogenesis (Seeman, 2002) and iii) decreasing bone formation by shortening the lifespan of osteoblasts (Lane, 2006). A low BMI (De Laet et al., 2005) and weight loss are significant indicators of bone loss; older women who lost weight also lost significantly more bone mass than those whose weight remained stable in a study of 1134 women over 60 years with an average follow-up of 2.7 years (Nguyen et al., 1998).

Low BMD has been associated with a number of inflammatory conditions, including systemic lupus erythematosus (Alele and Kamen, 2010) and rheumatoid arthritis (Heinlen and Humphrey, 2017). Additionally, chronic inflammation may contribute to the aetiopathology of osteoporosis by inducing adverse effects on bone metabolism (Lorenzo et al., 2008). Numerous inflammatory cytokines have been found to be associated with bone resorption, including IL-1, TNF and IL-6 (Lorenzo, 2000); bone

turnover is amplified via mechanisms including increased bone resorption by increasing the formation of osteoclasts, inhibiting osteoblast activity and therefore bone formation and by the stimulation of prostaglandin synthesis which may stimulate or inhibit bone resorption dependent on local conditions (Blackwell et al., 2010). In a 2.9 year longitudinal study of 168 older adults (mean age 63 years), the highest quartile of circulating IL-6 (>4pg/ml) and CRP (>4.2mg/L) concentrations at baseline were significantly associated with greater loss of total body BMD compared to the lower quartiles (Ding et al., 2008). Additionally, serum concentrations of interleukin-1-beta (IL-1 β) and IL-6 were found to be significantly higher in postmenopausal women with osteoporosis (N=100, mean age = 56.2 years) than controls (N=100, mean age = 55.2 years) (Al-Daghri et al., 2017). In a case-cohort study of men aged 65 years and older who were followed-up for an average period of 6.13 years, men in the highest quartile for serum TNF- α concentrations had a 2.0 – 4.2 fold greater risk of experiencing a hip or vertebral fracture than men in the lowest quartile (Cauley et al., 2016). Furthermore, men with 3 or more inflammatory markers in the top quartile (IL-6, CRP, interleukin-soluble receptor 6, TNF- α , TNF- α soluble receptors 1 and 2) were more likely to be older, less physically active and have lower appendicular lean mass (ALM), along with an increased risk for hip (2.03; 95% CI: 1.11, 3.71) and vertebral fractures (3.06; 95% CI: 1.66, 5.63) in comparison to men in the lowest quartile, although being in the highest quartile for IL-10 was protective against vertebral fracture (Cauley et al., 2016).

Physical activity is known to be beneficial for bone health, both during the development of peak bone mass and in maintenance in the period following. Strong evidence shows physical activity to be the most important lifestyle factor, along with adequate calcium intake, for bone mass and density in people \leq 21 years of age

(Weaver et al., 2016), and in reducing the loss of bone mass in later years; physically active older women lost significantly less BMD at follow-up than habitually inactive older women (assessed by nurses during interview) ((-0.5% vs -1.4% per year, respectively (Nguyen et al., 1998)).

1.3. The influence of resistance exercise training (RET) on muscle and bone health

1.3.1. Influence of RET on muscle size and function

Physical activity, specifically resistance exercise training (RET), has long been established as the gold standard for maintaining and improving musculoskeletal health in older adults (Marcell, 2003), and is the only existing intervention proven to treat sarcopenia (Offord and Witham, 2017). In a recent systematic review and meta-analysis of cross-sectional studies including 40,007 sarcopenic and non-sarcopenic people (age range = 40 – 106 years), physical activity significantly reduced the risk of developing sarcopenia in later life (OR = 0.45; 95% CI 0.47 – 0.55) (Steffl et al., 2017).

Additionally, RET increases anabolic hormone production; concentrations of free testosterone in men, and GH in both men and women are acutely elevated following exercise (Nascimento et al., 2018). RET also confers benefits on the cardiovascular system, endurance and falls prevention (Hurley and Roth, 2000).

It is well documented that physical activity declines with increasing age; two thirds of people aged 65 years and older reportedly do not take part in any form of leisure time physical activity (Nascimento et al., 2018). RET has been shown to increase muscle strength, mass and function even in very old adults; nonagenarians increased their quadriceps one-rep max (1-RM) strength by 175%, their thigh muscle area by 9% and their gait speed by 48% after 8 weeks of high-intensity RET (Fiatarone et al.,

1990). A systematic review including 6700 participants from 121 trials of RET in people aged 60 and older concluded that RET significantly improved functional ability and muscle strength. Importantly, no serious adverse events were reported directly related to the exercise programme, as have been reported with pharmacological interventions, and adverse events were related to mild musculoskeletal discomfort (Liu and Latham, 2009).

These results are concurred by various groups; Taaffe and colleagues demonstrated that once-weekly RET sessions significantly improved muscle strength and function in adults aged over 65 years (Taaffe et al., 1999b); Lindenberger et al. reported significant gains in strength and cross-sectional area (CSA) of type I and II fibres after 30 weeks of RET (Lindenberger et al., 1994) and Frontera et al found significant muscle strength increases and hypertrophy following 12 weeks of RET (Frontera et al., 1988). A dose-response relationship appears to occur between muscle strength and RET intensity; meta-analysis of studies suggests high intensity training confers more benefit than moderate or low intensity training (Steib et al., 2010). For example, 64 older men and women (mean age 70.8 years) were randomized to 16 weeks of low, moderate or high intensity resistance training or a control group, the high intensity training group demonstrated the greatest increases in strength at low velocities in comparison to the other groups (Beneka et al., 2005).

Conversely, there is evidence to suggest that the response to high intensity RET may not be universally beneficial in males and females. For example, following 12 weeks of progressive RET, female participants experienced the greatest benefits on tendon stiffness at lower forces (<40% maximum voluntary isometric force), whilst male participants exhibited most benefit at higher forces (>40% maximum voluntary isometric force) (Onambele-Pearson and Pearson, 2012), with tendon stiffness

impacting the speed at which muscle can generate force (Brumitt and Cuddeford, 2015).

Mode, rather than frequency of exercise appears to be most important when designing a training programme to improve strength in older adults (Taaffe et al., 1999a, Henwood and Taaffe, 2006, DiFrancisco-Donoghue et al., 2007), with resistance training concluded to be superior to strength training at improving functional ability in 67 older men and women aged 65 – 84 years (Henwood and Taaffe, 2006), although little information is available in this area.

1.3.2. Influence of chronic exercise on muscle health

The chronic effects of exercise are demonstrated in studies involving Master athletes, who maintain high levels of function across the lifespan. Lifelong participation in physical activity appears to conserve muscle mass and prevent fat infiltration into the muscle when compared with untrained older adults (Sipilä and Suominen, 1993). Although increases in FM were still observed in the Master athletes, no decline in absolute muscle mass with age was reported (Wroblewski et al., 2011). Concerning muscle strength, Master athletes displayed significantly higher isometric knee flexion and extensor strength (66% and 38%, respectively) than healthy age-matched controls (McCrorry et al., 2009). In a recent systematic review and meta-analysis, maximal voluntary contraction was significantly higher in Master strength/power athletes and young healthy controls in comparison to Master endurance athletes, older controls and young endurance trained individuals (Mckendry et al., 2018) . Additionally, Master athletes maintained body fat percentage comparable to untrained young controls, whereas older controls exhibited higher body fat percentages (Mckendry et al., 2018).

Importantly, in Master athletes aged 40 to 81 years, no differences in quadriceps muscle strength were observed until participants entered the 60 years and older age category where upon there was a decline in strength. However this decline was not further increased with additional ageing (Wroblewski et al., 2011).

1.3.3. Influence of RET on bone health

Peak bone mass of the total skeleton occurs at around 30 years of age, as shown in Figure 1.5, with much of this mass attributed to periods of rapid growth and remodelling, e.g. puberty (Baxter-Jones et al., 2011). Since it is understood that individuals who accrue higher levels of peak bone mass in earlier life generally exhibit higher levels of bone mass in later life (Heaney et al., 2000), many interventions are aimed at younger generations and optimising peak bone mass. It has been suggested that osteoporosis susceptibility may be detectable in childhood (Bonjour et al., 1998). The aims of exercise interventions change across the lifespan; the goal during childhood is achieving optimum peak bone mass via growth and acquisition, maintenance of mass during adulthood and lowering the rate of bone loss and falls risk in older adults (Beck and Snow, 2003)

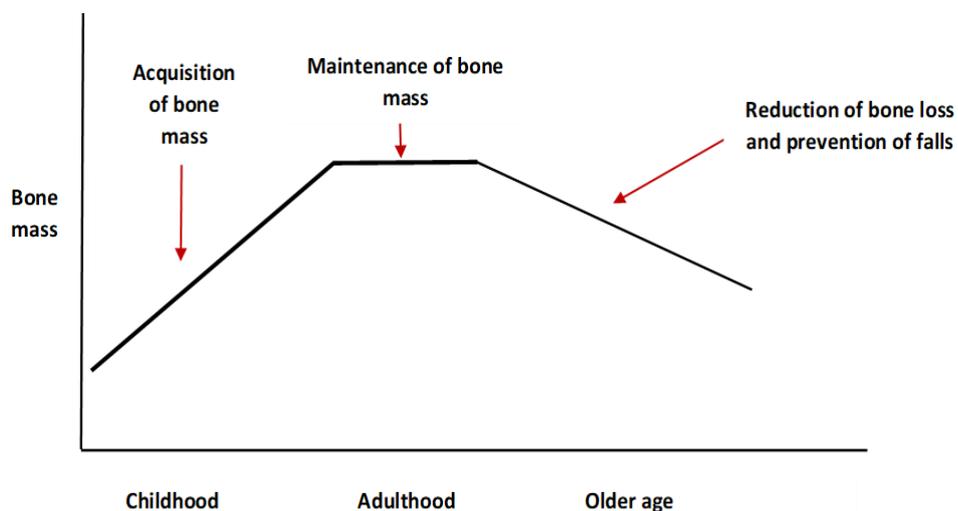


Figure 1.5: The role of exercise in maintaining bone health throughout the life span. Adapted with permission from (Beck and Snow, 2003). Copyright license number 4634180942587

Physical activity, such as RET, plays a central role in bone health, with the Royal Osteoporosis Society recommending a combination of weight-bearing and muscle strengthening exercises for the maintenance of bone strength and management of osteoporosis (The Royal Osteoporosis Society, 2019a). Moreover, exercise such as RET has the additional benefit of impacting all domains of fracture risk such as falls risk parameters (e.g. balance) and bone strength, which pharmacological interventions alone do not (Kemmler et al., 2016).

RET has been shown to exert modest benefits on BMD and bone architecture, although these results are not unanimous. For example, large strength gains and gains in muscle CSA and functional capacity but not BMD were reported after 42 weeks of RET in older adults (McCartney et al., 1995). Regional changes in BMD have been reported in other studies; improvements in femoral neck BMD (+1.96%) in older men and women were observed after 6 months of high intensity resistance exercise (Vincent and Braith, 2002). Similarly, BMD at the total and inter-trochanter hip significantly increased after 2 years of strength training in 126 postmenopausal women (mean age = 60), although no effect was seen at the forearm, lumbar spine or whole body regions (Kerr et al., 2001).

Trochanteric BMD was significantly increased and femoral neck BMD decreased in both older men and women after 6 months of high intensity RET, however significant BMD gains at the spine were only documented in men (Maddalozzo and Snow, 2000). Conversely, no sex or age differences were observed in the BMD gains reported at the femoral neck, Ward's triangle or the greater trochanter between young (mean age = 25) and older (mean age = 69) men and women following a 6 month RET regime (Ryan et al., 2004).

When considering BMD in athletes, high intensity resistance training and impact sports are the optimum modes of physical activity to stimulate an osteogenic effect (Kohrt et al., 2004). In addition to exhibiting higher bone mass, weightlifting athletes in all age groups have superior bone structure compared with untrained individuals, with higher cortical thickness and CSA of long bones observed in the athletes (Suominen, 2006). Maintaining long-term participation in physical activity appears to confer benefits into older age; chronic, regular participation in sport and leisure activities, including impact and loading, is positively associated with bone size, quality and strength, and reduces the risk of low bone mass in the mid-femur of older adults (Daly and Bass, 2006).

Conversely, participation in non-impact activity negatively affects bone health. Male Master cyclists demonstrated significantly higher losses of BMD after a 7-year follow up compared with non-athletic active controls. Master cyclists were also significantly more likely to meet the criteria for clinical osteopenia and osteoporosis compared with controls; however, participation in weight training or impact sports had a protective effect on the loss of BMD at the spine and femoral neck (Nichols and Rauh, 2011).

1.3.4. Influence of RET on falls and fractures

Falls and fall-related injuries are a principal cause of hospitalization and death in older adults (Gerards et al., 2017), and a fear of falling represents a source of significant physical and psychosocial ill health (Scheffer et al., 2008). Figures suggest at least 33% of community-dwelling adults over 65 years fall each year (Sherrington et al., 2019); fall rates are much higher in care facilities, where 62% of

older adults were found to fall in one year (Kennedy et al., 2015), and in hospitals, up to 8.9 falls per occupied bed days have been reported (Oliver et al., 2010).

Intervention studies comprising of strength and balance training unanimously report reductions in the number of falls and fall-related injuries in older people; a meta-analysis pooling data from 1116 men and women over 65 years taking part in a home-based strength and balance program reported a 35% lower rate of falling in the exercise group and fewer injurious falls than observed in the controls (Robertson et al., 2002). Supervised, individually tailored exercise programmes and also untargeted group-based exercise programmes have both been shown to reduce falls (Sherrington et al., 2004, Kannus et al., 2005); 163 people aged 65 years randomized to 1 year of group exercise comprising strength and balance work demonstrated a 40% lower rate of falls and improved balance in comparison with the control group (Barnett et al., 2003).

A number of Cochrane systematic reviews report on the relationship between exercise and falls prevention; a large systematic review (n = 23,407 from 25 countries) concluded exercise reduced rate of falls, and specifically a programme containing RET, balance and functional exercises was more effective at reducing the rate of falls in community-dwelling older adults (mean age = 77 years) than exercises such as Tai Chi and balance and functional exercises alone (reduction of 34%, 19% and 24%, respectively) (Sherrington et al., 2019). A further review including 138,164 participants reported on the effect of interventions for preventing falls in older men and women in care home facilities (mean age = 84 years) and hospitals (mean age = 78 years); conclusions as to the effect of exercise on falls in these settings were not possible due to the very low quality of available evidence, however, moderate quality evidence supported the role of vitamin D supplementation in reducing the rate, but

not risk, of falls in individuals with low vitamin D concentrations (Cameron et al., 2018). Exercise was associated with a small to moderate reduction in fear of falling following the intervention, and a small reduction in fear of falling both up to and beyond the 6 months post-intervention in a review of 2878 community-dwelling older adults (mean age range 68 – 85 years); however, the latter was concluded from very low quality evidence (Kendrick et al., 2014).

1.4. Age and the musculoskeletal response to stimuli

1.4.1. Blunted anabolic synthetic response to anabolic stimuli in older adults

When muscle protein synthesis (MPS) and degradation (MPD) are not balanced, muscle loss occurs; the consequence of an accumulating disequilibrium over time is sarcopenia (Bowen et al., 2015). Longitudinally, rates of muscle mass loss have been reported to be as high as 0.7% and 0.98% per year in women and men aged 75 years and over, respectively (Mitchell et al., 2012). Losses in muscle strength have been shown to be three times higher than losses in mass (Schwartz et al., 2006), with low muscle strength a strong predictor of decreased functional ability and disability in older people (Hairi et al., 2010). The disparity observed between losses in muscle strength and mass in older adults may be explained by the accumulation of non-contractile materials within the muscle which are not as efficiently removed with age (Marcell, 2003), contributing to overall CSA but not force production. Additionally, intramuscular fat and strength are known to be negatively correlated (Akazawa et al., 2017), further reducing the quality of aged muscle.

RET interventions have demonstrated significant gains in muscle strength and/or mass in older adults, with evidence suggesting modality of loading may be a differentiation factor in degree of hypertrophic and strength response to training. Intuitively, it is assumed that training involving eccentric contractions produce a

greater hypertrophic and strength response than concentric contractions, since lengthening skeletal muscle results in a higher generation of force at a lower metabolic cost than shortening or concentric contractions, as kinetic energy is converted to elastic energy of tendons and regained during limb support (Vogt and Hoppeler, 2014). This theory is supported by some reviews and intervention studies; a systematic review and meta-analysis of 20 RCT studies of adults aged 18-65 years concluded that high velocity eccentric training resulted in superior total muscle strength (WMD = 7.84 Nm; 95% CI 3.14, 12.54, $p = 0.001$) and mass (measured as girth: WMD = 1.47 cm², $p = 0.001$) gains than concentric training (Roig et al., 2009). Similarly, an RCT of 62 older adults (mean age = 80.6 years) completing 12 weeks of eccentric ergometer or traditional concentric RET found that maximal isometric leg extension strength was significantly improved only within the eccentric training group (+8.4% Vs +2.3%, $p < 0.01$; adjusted for body mass). Leg lean mass significantly increased within both groups, although this increase was highest within the eccentric training group (+2.5% Vs +2.0%, $p < 0.01$) (Mueller et al., 2009). Conversely, a literature review found that eccentric and concentric training resulted in similar hypertrophic gains when matched for maximum load (Franchi et al., 2017).

Improvements in skeletal muscle parameters as a result of RET are blunted in comparison with those observed in younger adults. For example, following 12 weeks of RET, muscle volume (+6.2% and +2.5%) and strength (+27% and +16%) increased in both young (median age, 26y) and older (median age, 80y) women respectively, but improvements were significantly higher in the younger women (Greig et al., 2011a). Similarly, after 12 weeks of high intensity RET, muscle strength increased significantly in both young ($n = 9$, mean age = 21y) and elderly women ($n = 6$, mean age = 85y), although improvements were greater in young women (+36% vs

+26%). Additionally, although thigh muscle CSA was significantly increased by +5% in young women, no change in muscle size was observed in the elderly women (Raue et al., 2009).

In general, studies have shown a blunting of the anabolic response following RET in older adults; myofibrillar fractional protein synthesis rate was shown to be 27% slower in older versus younger adults following 3 months of RET and the percentage of total protein synthesised after a single session of resistance exercise was concluded to be 30% lower in older versus younger men (Welle et al., 1995). Since general consensus is that basal (i.e., postabsorptive) rates of MPS are equal in both young and older adults (Cuthbertson et al., 2005, Kumar et al., 2009), the deficit in the response could be due to alterations in anabolic signalling with age, such as the blunted phosphorylation (Welle et al., 1995), of ribosomal protein S6 kinase beta-1 (p70s6K) and 4E binding protein-1 (4EBP1), downstream effectors of the mammalian target of rapamycin (mTOR) (Kumar et al., 2009).

In addition to attenuating the MPS response to RET, ageing has been shown to diminish the anabolic response to feeding. A cross-sectional retrospective study of young (mean age, 22y) and older (mean age, 75y) men concluded that post-prandial MPS rates were significantly reduced, less responsive and the increase in MPS from the post-absorptive to post-prandial state was greatly diminished in older men in comparison to their younger counterparts following ingestion of an anabolic stimulus (Wall et al., 2015). Following ingestion of differing amounts of essential amino acids (EAA), older men demonstrated a reduction in the degree of anabolic sensitivity and responsiveness to stimuli, again perhaps the result of reduced phosphorylation of mTOR and downstream translational regulators (Cuthbertson et al., 2005). Additionally, the anabolic effects of insulin were diminished and leg protein

breakdown suppression was attenuated to a smaller degree in older vs younger men during moderate insulin availability (Kumar et al., 2009).

1.4.2. An age-related blunting in the osteogenic response to loading?

The blunting of the anabolic muscle synthetic response to stimuli in older adults has been previously described, but is this effect also observed in bone; i.e. is there an age-related blunting in the osteogenic response to mechanical stress?

Few data exist in this area, but animal studies appear to suggest a relationship between age and response. For example, bone CSA in young turkeys increased by 30.2% but remained relatively unchanged in older animals after an eight week loading regimen (Rubin et al., 1992). After 2 weeks of tibial loading, both young and old mice increased their trabecular thickness and number of trabecular connections, however, the latter was diminished in the old mice (Meakin et al., 2014). Older animals were shown to be less responsive to mechanical stimuli by Turner et al; older rats required a higher amount of mechanical loading to exhibit bone formation than younger rats, and even after higher mechanical loading, bone formation in old rats was 16-fold lower than in younger rats (Turner et al., 1995). Additionally, as reported in muscle, VDR expression in the bone cells of older mice was significantly reduced in comparison to younger mice (Duque et al., 2002).

In humans, 12 months of jumping exercises significantly increased proximal femur BMD in premenopausal women after 6 months, although in postmenopausal women there was no change in BMD after 12 months of jumping (Basseley et al., 1998). The ability to improve total and/or regional BMD is retained in older men (Kukuljan et al., 2011, Allison et al., 2015, Kemmler et al., 2018) and women (Wallace and Cumming, 2000, Kerr et al., 2001, Suominen, 2006, Huovinen et al., 2016, Zhao et al., 2017),

thus the capacity to respond to mechanic stimuli must be attenuated. It has been suggested that the blunting of the osteogenic response in older adults may be due to reductions in muscle mass and strength with age resulting in lower force production than required to overcome the remodelling threshold (Sinaki, 1998). Indeed, in a study of 112 healthy, independently-living older adults (mean age = 72.5 years), physical activity, determined objectively by 7-day accelerometry, had a negligible effect on regional and total-body BMD, with the authors suggesting that participants did not take part in activities breaching the threshold of impact required to stimulate bone formation (Onambele-Pearson et al., 2019). It was also suggested that high levels of physical activity were not enough for BMD maintenance; a reduction in sedentary behaviours may also be needed (Onambele-Pearson et al., 2019).

Furthermore, declining concentrations of hormones which act directly on bone and influence turnover, for example oestrogen, may alter the mechanical signalling sensitivity to loading (Kohrt, 2001).

1.5. Muscle-bone relationship: Osteosarcopenia

Muscle and bone work together towards a shared functional purpose; force production, movement and structure (Bruyère et al., 2017), and are linked not only by proximity but also by endocrine and developmental factors, as shown in Figure 1.6 (Edwards et al., 2015). The most obvious way in which muscle and bone are related is via the mechanostat theory (Frost, 2003); the mechanical loading of bone by muscle contraction stimulates osteogenesis, and was described as the most important factor effecting bone strength (Frost, 2003). However, several other factors positively effect both muscle and bone, such as vitamin D status (Sanders et al.,

2014), hormones, for example growth hormone, and physical activity (Laurent et al., 2016).

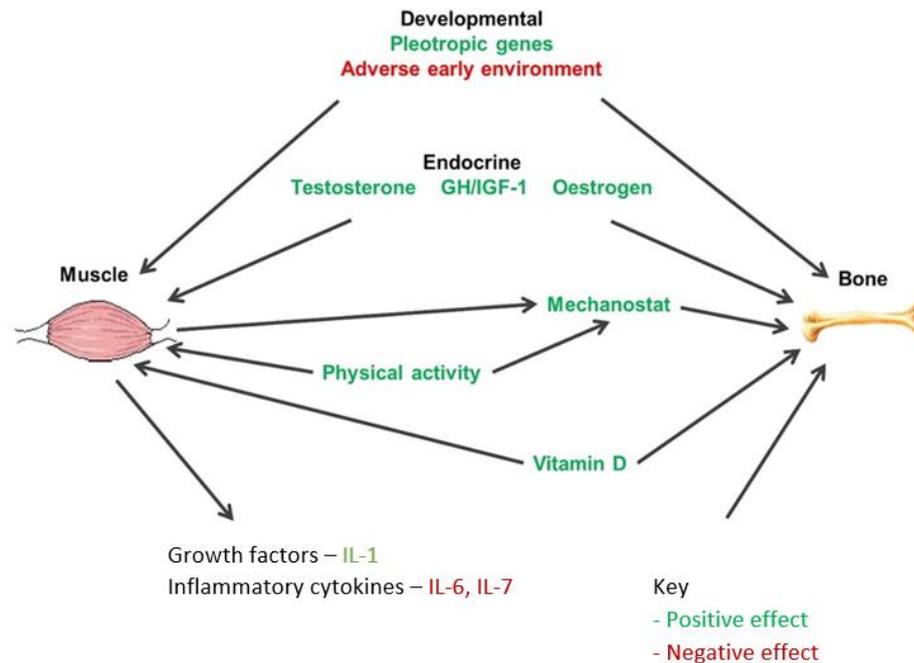


Figure 1.6: Relationship between muscle and bone

GH: Growth hormone, IGF-1: Insulin-like growth factor 1, IL-6: Interleukin 6, IL-7: Interleukin 7. Adapted with permission from (Edwards et al., 2015). Copyright licence number 4580150253064.

The aetiology of sarcopenia and osteoporosis have factors which intersect, and both have a similar patient demographic, with prevalence increasing with age. In a cross-sectional study of 17,891 African-American, Caucasian and Chinese adults (age range 18 – 97 years), participants with sarcopenia defined by relative appendicular skeletal muscle mass (RASM), and by the EWGSOP definition were 2 times and 1.8 times, more likely to be osteopenic or osteoporotic than non-sarcopenic participants (He et al., 2016). Additionally, BMD was significantly associated with lean mass, grip strength was significantly associated with BMD at all sites, and each SD increase in RASM resulted in a 37% decreased risk for osteopenia or osteoporosis (He et al., 2016).

The presence of both osteoporosis and sarcopenia may present a cumulative risk, resulting in worse health outcomes than either condition alone (Drey et al., 2016); this “hazardous duet” (Crepaldi and Maggi, 2005) has been termed osteosarcopenia in the literature. However, a lack of clear definition guidelines for the condition has resulted in high levels of heterogeneity and therefore difficulty in comparisons between studies (Bruyère et al., 2017).

Nevertheless, individual studies provide evidence for a link between sarcopenia and osteoporosis; a study examining physical performance and bone health in 68 community-dwelling older adults (aged 65 – 94 years) found that osteosarcopenia had an additive effect on fracture risk, with osteosarcopenic men at a 3.5-fold higher risk for fracture than men without osteoporosis or sarcopenia (Yu et al., 2014). An additional cross-sectional study of 313 women who had suffered a hip fragility fracture (mean age = 79.7 years) found a significant association between sarcopenia and osteoporosis, and a significant increase in risk for osteoporosis was found in sarcopenic women (odds ratio = 1.8) (Di Monaco et al., 2011).

1.6. Vitamin D: Background, status and influence on musculoskeletal health and function

1.6.1. What is vitamin D?

Rickets, the epidemic disease of the 19th century, provided the catalyst for the discovery of vitamin D. As a consequence of the industrial revolution, high levels of air pollution and low levels of sunlight contributed to as many as one third of children under the age of 2 years suffering from rickets in England (Hess, 1930). In 1922, a new vitamin was announced to have cured rickets and was named “vitamin D” (McCollum et al., 1922). It is now recognised that the “vitamin” in vitamin D was a misnomer; vitamin D is more appropriately labelled a biologically active hormone with

numerous roles, including calcium and phosphorus homeostasis and with a range of target tissues including skeletal muscle and bone (Girgis, 2015).

1.6.2. Synthesis of vitamin D

The two main forms of vitamin D are vitamin D2 and vitamin D3. Vitamin D2 (ergocalciferol) is synthesised by plants and fungi after UV exposure or created by irradiating food sources, for example mushrooms (Lips, 2006). Vitamin D3 (cholecalciferol) is synthesised cutaneously upon exposure to UV radiation and ingested from dietary sources. Under ideal conditions the skin is able to produce enough vitamin D3 to fulfil 80-100% of vitamin D requirements (Glerup et al., 2000c).

Synthesis of vitamin D begins with exposure to sunlight; UVB radiation converts provitamin D3 (7-dehydrocholesterol) in the skin to previtamin D3. Since previtamin D3 is highly unstable, conversion to fat-soluble vitamin D3 is rapid. Circulating vitamin D3 is then bound to the vitamin D binding protein (DBP), and together with dietary sources of vitamin D2 and D3 bound to chylomicrons, transported to the liver and hydroxylated to 25-hydroxyvitamin D. 25-hydroxyvitamin D, or 25(OH)D, is the major circulating form of vitamin D used to assess vitamin D status and is biologically inactive. 25(OH)D is converted to the biologically active metabolite 1,25hydroxyvitamin D, or 1,25(OH)₂D₃, in the proximal tubule of the kidneys. From here, 1,25(OH)₂D₃ is transported to tissue such as bone, where it binds to the vitamin D receptor (VDR) to exert genomic effects.

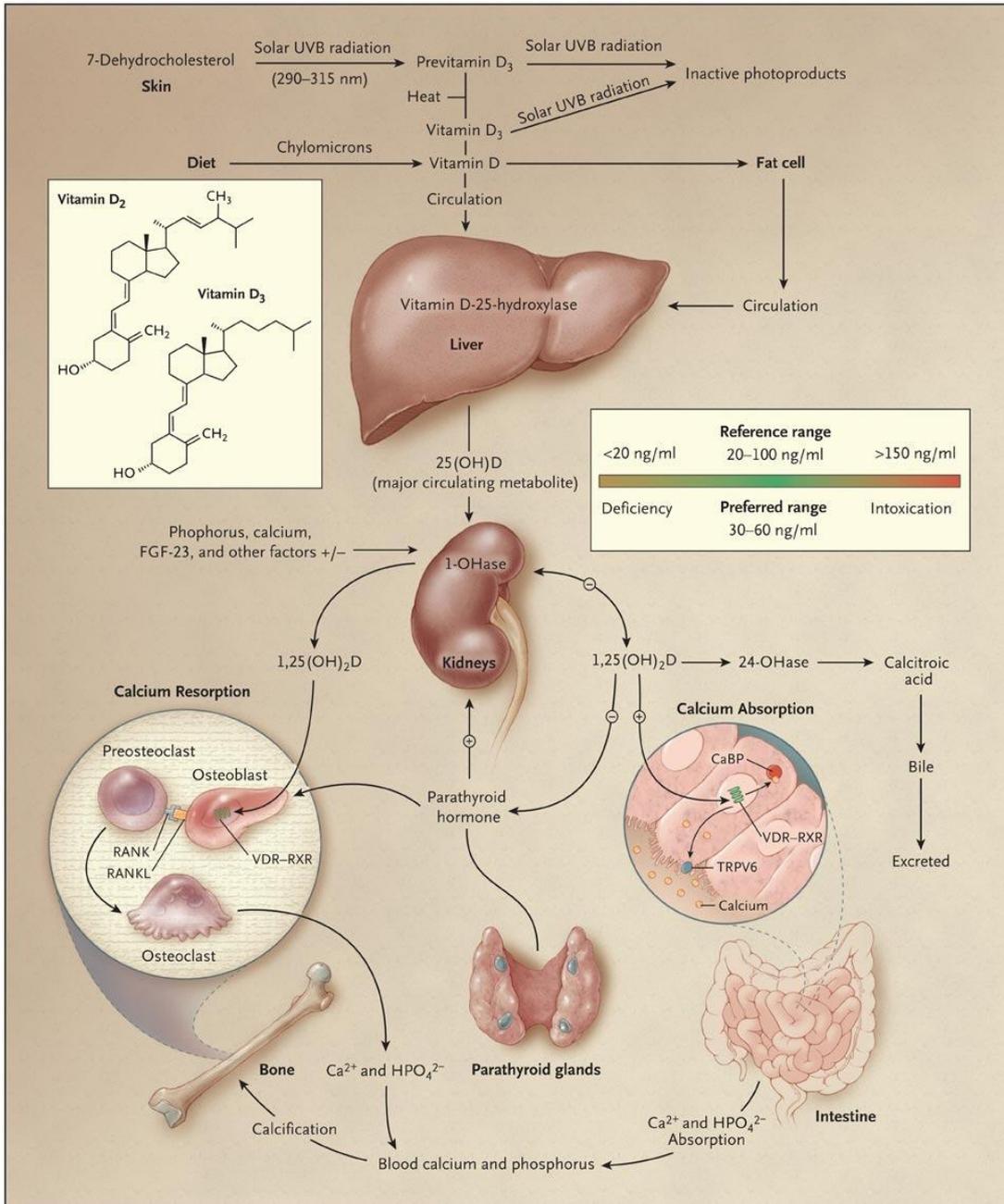


Figure 1.7: Synthesis of vitamin D and the regulation of calcium and bone metabolism

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1.6.3. Vitamin D2 or D3?

When compared with vitamin D₂, D₃ possesses an additional methyl group and double bond, and, as depicted in Figure 1.7, both D₂ and D₃ are hydroxylated in the

liver and kidney. There is some evidence that D3 is the 'preferred' metabolite in the liver, since hydroxylation of D3 has been found to be over 4 times as fast as that of D2 (Holmberg et al., 1986).

Previously vitamin D2 and D3 were assumed to be of equal potency in humans; this is now a subject of some contention. Although no difference in serum 25(OH)D concentration was reported after 1000 IU of D2 and D3 was administered daily for 11 weeks (Ameri et al., 2008), the majority of available literature supports the theory that D3 is more effective than D2, with no studies suggesting D2 is more efficient than D3 (Wilson et al., 2017).

Vitamin D3 supplementation has been shown by a number of studies to be more efficient at increasing serum 25(OH)D; serum concentrations in vitamin D3 supplemented participants increased by 1.7 times (Trang et al., 1998) to over 3 times (Armas et al., 2004) as much in those provided with equal amounts of D2.

Additionally, serum 25(OH)D concentrations continued to rise 14 days after administration of 50,000 IU D3, and remained elevated after 28 day; in participants supplemented with identical amounts of D2, serum 25(OH)D was no different from baseline after 14 days (Armas et al., 2004).

Supplementation with D3 (1000 IU) appeared to be protective of serum 25(OH)D concentrations over the winter months; D2 supplementation (1000 IU) resulted in a significant decrease in total serum 25(OH)D, however, this was unaltered in the D3 group (Logan et al., 2013). Furthermore, total calciferol storage in subcutaneous fat was found to be 3 fold higher in participants supplemented with 50,000 IU D3 per week for 12 weeks in comparison with equivalent doses of D2 (Armas et al., 2011).

The differing plasma half-lives of 25(OH)D₂ and 25(OH)D₃ may provide one possible explanation for the reason equivalent doses of D₃ and D₂ do not raise serum 25(OH)D to equivalent concentrations; the half-life of 25(OH)D₂ was found to be significantly shorter than that of 25(OH)D₃ (Harnpanich et al., 2014). This is suggestive of a higher affinity of 25(OH)D₂ for the vitamin D binding protein (DBP) or perhaps a greater rate of catabolism of 25(OH)D₂ within the liver (Logan et al., 2013).

1.6.4. Vitamin D status: measurement

Vitamin D status is measured as serum 25(OH)D concentration, due to the stable nature of the metabolite (Thacher and Clarke, 2011). The absence of clear international guidelines for optimal serum 25(OH)D concentration is due to widespread variation between different assays and laboratories, yielding subsequently non-comparable results. This measurement bias has been reported to be up to 25% between methods (Salti et al., 2012), and importantly may underestimate the proportion of the population requiring treatment for vitamin D deficiency (Power et al., 2007).

However, the general consensus is that a serum 25(OH)D concentration of <50nmol/L can be considered as insufficient (Lips, 2004) (Ross et al., 2011a), with 25(OH)D below 25nmol/L associated with bone disease (Thacher and Clarke, 2011). The current gold standard methodology for assessing 25(OH)D is liquid chromatography-tandem mass spectrometry, and is the technique favoured by the National Health and Nutritional Examination Survey (NHANES) and the UK Food Standards Agency (FSA) (Volmer et al., 2015).

1.6.5. Vitamin D deficiency: prevalence

Despite a lack of standardisation between assays making population studies of serum 25(OH)D status difficult, over 50% of the worldwide population are considered to have inadequate vitamin D3 in winter (<50nmol/L) (van Schoor and Lips, 2018), with clinical deficiency widely accepted as serum 25(OH)D concentration <25nmol/L (van Schoor and Lips, 2018). Older people are one of the population groups regarded to be most at risk for deficiency due to changes in the skin, diet and behaviour; Figure 1.8 depicts the prevalence of worldwide low serum 25(OH)D status in adults aged 50 – 105 years.

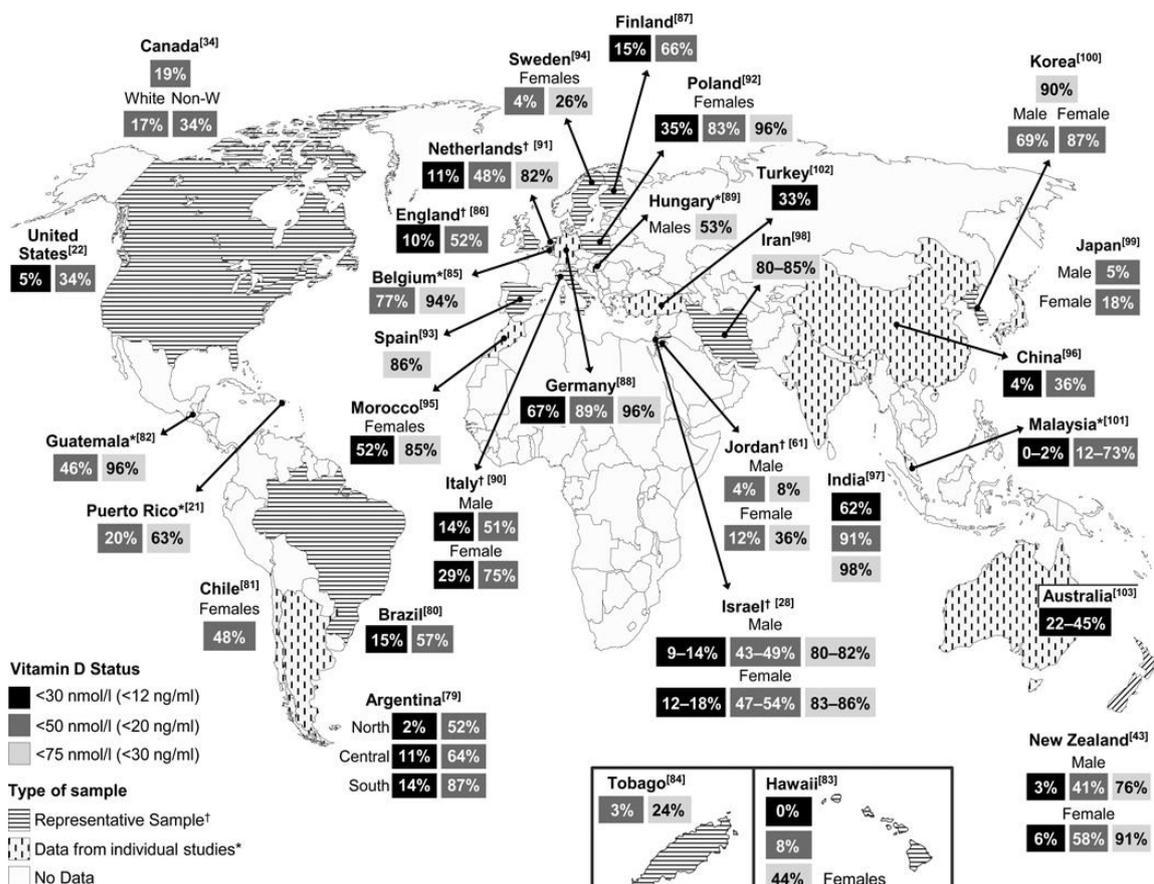


Figure 1.8: Prevalence of worldwide low serum 25(OH)D status in adults aged 50 – 105 years
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Comparison of 6 young (20-30) and 6 older (62-80) participants receiving whole body phototherapy revealed serum vitamin D concentrations in the younger participants increased over three times as much as older participants within 24 hours (Holick et al., 1989). The same research group concluded earlier that ageing significantly reduced cutaneous production of previtamin D₃ (MacLaughlin and Holick, 1985). Additionally, older people may require higher concentrations of 25(OH)D to overcome hyperparathyroidism and the differing relationship between parathyroid hormone (PTH) and 25(OH)D in comparison to younger adults (Ladak et al., 2003).

Observational data report high levels of deficiency within older adults; a study of the 2005 Health Survey for England (HSE) reported that 8% of men and 14% of community-dwelling women aged 65 years and over were considered vitamin D deficient (<25nmol/L) and 49% of men and 58% of women over 65 were vitamin D insufficient (<50nmol/L); vitamin D concentration also decreased with increasing age (Hirani et al., 2009). Additionally, serum vitamin D concentrations were significantly lower in UK adults over 65 years living in care homes compared with those observed in community-dwelling older adults (Hirani and Primatesta, 2005). Similarly, of patients living in an elderly care rehabilitation facility (n=1578, median age = 82), 89% were reported to have hypovitaminosis D (<50nmol/L) and 67% were reported to be severely deficient (<25nmol/L) (Schilling, 2012).

In the UK, the Scientific Advisory Committee on Nutrition Vitamin D working group (SACN) published their recommendations in 2016 regarding reference nutrient intakes (RNI) of vitamin D advised for protecting musculoskeletal health. A daily supplement of 400 international units (IU) per day of vitamin D was recommended throughout the year for everyone aged 4 years and above; this was calculated to be a sufficient amount for 97.5% of the population to maintain a 25(OH)D serum

concentration ≥ 25 nmol/L (Scientific Advisory Committee on Nutrition, 2016). This supplement is to be in addition to dietary intakes, which were found to be low; community dwelling men and women aged over 65 years had a total intake (dietary sources and supplementation) of 51% of the RNI, and institutionalised adults over 65 years had a total intake of 33% of the RNI (Scientific Advisory Committee on Nutrition, 2016).

1.6.6. Factors influencing vitamin D status

Together with age, a number of factors effect vitamin D status, including sunlight exposure, skin pigmentation, physical activity and disease. Sunlight is known to be the most important source of vitamin D, with exposure to ultraviolet (UV) radiation in the summer months responsible for 80% of annual vitamin D intakes (Macdonald et al., 2011b). The photolysis of provitamin D₃ can only occur from UVB light of a wavelength between 290 – 316nm; the relationship between serum 25(OH)D concentration and season tracks a sinusoidal pattern, with highest concentrations detected during summer, then declining until the end of winter (Grey et al., 2007). A recent study using both observational and intervention data from UK populations concluded that 9 minutes of midday sun exposure every day between March and September is required to maintain serum 25(OH)D concentration above deficiency throughout the winter months (Webb et al., 2018). However, UVB exposure is not only limited by season, but also by latitude, cloud cover, time of day (Kimlin, 2008). Additionally, cultural factors such as clothing influence vitamin D status; traditional Islamic dress covering the whole body was shown to significantly decrease 25(OH)D concentrations in comparison with women clothed in a western style; vitamin D deficiency was observed in all women in the traditional dress group (Alagöl et al., 2000).

Skin pigmentation is a further factor influencing 25(OH)D status; lightly pigmented skin can produce 6-fold the amount of vitamin D than heavily pigmented skin types (Mostafa and Hegazy, 2015), and darker skin types have been shown to exhibit significantly lower 25(OH)D concentrations than whites (Gutierrez et al., 2011, Powe et al., 2013). Additionally, physical inactivity, obesity and a number of chronic diseases are associated with lower serum 25(OH)D concentrations (Scragg et al., 1992, Scragg and Camargo, 2008, Brock et al., 2010, Tsiaras and Weinstock, 2011).

1.7. Relationship between vitamin D status and bone density

The relationship between vitamin D and bone health has long been recognised. Vitamin D during human formative years is vital for the healthy development and mineralization of the skeleton, and deficiency restricts peak bone mass and height and causes the deforming bone disease rickets (Holick, 2004). Traditionally, vitamin D is considered in association with calcium and phosphate; promoting absorption at the intestines, reabsorption at the kidneys and mobilization from the skeleton to maintain homeostasis. 1,25(OH)₂D, the biologically active vitamin D metabolite stimulates calcium absorption from the small intestine, and during periods of vitamin D insufficiency the efficiency of the intestines to absorb calcium drops by up to 35% (Holick, 1996), leading to bone loss, osteopenia and osteoporosis (Lips and Van Schoor, 2011). Additionally, vitamin D receptors (VDRs) have been identified on a number of bone cells, including osteoblasts, denoting that vitamin D plays a direct role in bone turnover and remodelling (St-Arnaud, 2008).

The European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) concluded that serum 25(OH)D concentrations <50nmol/L are detrimental to bone health and are associated with increased bone turnover,

bone loss, increased non-vertebral and hip fractures (Rizzoli et al., 2013). In a group of 549 postmenopausal women (mean age = 74.95 years) both with and without acute hip fractures, serum 25(OH)D concentrations were found to be significantly lower in the fracture group than the controls (30.78 Vs 59.70 nmol/L, $p < 0.001$), additionally, the hip fracture group consisted of a significantly higher percentage of deficient participants (defined as <50 nmol/L) than the controls (86.3% Vs 40.8%, $p < 0.001$) (Fan et al., 2018). Serum 25(OH)D was identified as an independent risk factor for hip fracture in postmenopausal women (OR = 0.878; 95% CI 0.855, 0.902, $p < 0.001$) (Fan et al., 2018).

With regards to BMD and vitamin D status, 25(OH)D concentrations were analysed in a sample of 5002 women (mean age = 68 years) from the Swedish Mammography Cohort (SMC). In the summer months (June-August), women with serum 25(OH)D concentrations <30 nmol/L ($n = 245$) had 11% lower BMD at the total hip (95% CI 3, 19, assessed via DXA), in women with concentrations of 30 – 40 nmol/L ($n = 520$) BMD at the total hip was 6% lower (95% CI 1, 11) in comparison to women with serum 25(OH)D concentrations >80 nmol/L ($n = 559$) (Michaëlsson et al., 2017). Additionally, vitamin D deficiency (<30 nmol/L) was associated with a 5-fold increased risk of osteoporosis (95% CI 2.9, 8.4) compared with concentrations >80 nmol/L (Michaëlsson et al., 2017).

Intervention studies have reported the positive effects of vitamin D supplementation on BMD, both alone (Chevalley et al., 1994, Ooms et al., 1995) and combined with calcium (Dawson-Hughes et al., 1997, Baeksgaard et al., 1998, Jackson et al., 2006). A meta-analysis of 29 studies ($n=63,897$) found that treatment with calcium, alone, or in combination with vitamin D reduced the risk of all fracture types by 12%,

and significantly reduced the rate of BMD loss at the hip (0.54%) and spine (1.19%) in men and women aged 50 years and over (Tang et al., 2007).

However, the effect of vitamin D supplementation on falls and fractures is a subject of some controversy, with many studies published within this area in disagreement.

Some studies report a beneficial effect (Bischoff-Ferrari et al., 2004, Bischoff-Ferrari et al., 2005, Kalyani et al., 2010, Bischoff-Ferrari et al., 2012). For example, a 2011 meta-analysis of 26 trials (n = 45, 782, mean age = 76 years) reported a significant reduction in risk of falls associated with vitamin D and calcium supplementation in comparison to a control group (OR = 0.86, 95% CI, 0.77 – 0.96), with a greater effect observed in participants with hypovitaminosis D at baseline (Murad et al., 2011). This reduction in risk of falls was comparable to a smaller (n = 1237), earlier review study reporting an Odds Ratio of 0.87 (95% CI, 0.80 – 0.96), equivalent to a 22% reduced risk of falling with vitamin D supplementation (Bischoff-Ferrari et al., 2004).

However, some studies report no effect of vitamin D supplementation on falls and fractures (Jackson et al., 2006, Raphael et al., 2007, Uusi-Rasi et al., 2015), although this was thought to be due to an insufficient dose of vitamin D supplement (Bischoff-Ferrari et al., 2012). One study of 36,282 women of mean age 62 years reported that although vitamin D supplementation increased hip bone density, there were no significant reductions in vertebral, lower arm, wrist or total fractures, and the 12% reduction in hip fractures was not statistically significant after a 7-year follow up period. However, adherence to supplementation was poor, and a sensitivity analysis of adherent participants revealed a significant reduction in hip fracture risk of 29% (Jackson et al., 2006).

A 2-year RCT of vitamin D3 and/or exercise found no reduction in falls in 409 women (mean age = 74.1 years); unlike many other trials, the authors noted that recruited participants unintentionally had sufficient serum 25(OH)D concentration at baseline (mean = 67 nmol/L) (Uusi-Rasi et al., 2015).

A recent systematic review and meta-analysis of 81 RCTs (n = 53,537) investigating the effect of vitamin D supplementation on fractures and falls, concluded no effect on total or hip fractures or falls. Additionally, no consistent clinically relevant effects were observed on BMD at any site, although this review included only 4 studies (n = 831) of populations with serum 25(OH)D concentration <25 nmol/L (Bolland et al., 2018b).

This finding is consistent with Cochrane reviews on interventions to prevent falls in older people (Gillespie et al., 2012) and vitamin D and its analogues to prevent fractures in postmenopausal women and older men (Avenell et al., 2014), which both concluded from high quality evidence that vitamin D alone was unlikely to prevent hip fractures (Avenell et al., 2014) and had no overall effect on reducing falls or risk of falls (Gillespie et al., 2012), although a greater reduction in rate and risk of falls was observed in participants with lower serum 25(OH)D concentration at baseline (Gillespie et al., 2012) and a small reduction in hip fracture risk and risk of any fracture type was observed with vitamin D and calcium supplementation (Avenell et al., 2014).

1.8. The association between vitamin D and skeletal muscle

Evidence of the relationship between skeletal muscle and vitamin D is emerging and, as such, is not as well understood as the long-standing association between bone and vitamin D. Currently, this evidence is four-fold, as described by (Bischoff-Ferrari, 2012); i) a myopathy is observed in cases of vitamin D deficiency which is reversed

upon treatment, ii) vitamin D receptors (VDRs) are present in skeletal muscle providing evidence for a direct relationship with vitamin D, iii) observational data report 25(OH)D concentration is correlated with muscle strength and function in older adults and iv) interventional data provides evidence of benefit of vitamin D supplementation on muscle strength, balance, falls and function.

1.8.1. i) Vitamin D deficient myopathy

Vitamin D deficiency is known to be associated with muscle myopathy, myalgia and changes in gait; symptoms which are reversed upon treatment (Ziambaras and Dagogo-Jack, 1997). Early clinical observations recorded muscle weakness in association with vitamin D deficiency and osteomalacia (Schott and Wills, 1976). Myopathy was also observed independent of osteomalacic diagnosis (Glerup et al., 2000a), and severe deficiency resulted in restricted activities of daily living, such as difficulties rising from a chair or ascending stairs, and wheelchair use in 55% of participants in one study (Al-Said et al., 2009). A clinical observation study reported severe proximal muscle weakness, chronic pain and difficulty walking short distances in association with severe vitamin D deficiency in 3 adults over 65 years. All 3 patients were treated with 50,000 IU vitamin D, and within 8 weeks regained strength, experienced reduced pain, improved mobility and were able to discard their wheelchair (Prabhala et al., 2000).

Alongside myopathy, myalgia, that is, chronic musculoskeletal pain, is known to be associated with hypovitaminosis D, with particular reference made to immigrants to western countries as a group at risk documented in the literature due to skin pigmentation, sun exposure and diet (Prabhala et al., 2000). For instance, veiled Arab women exhibited significantly lower serum 25(OH)D than Danish controls and,

subsequently, significantly more muscle pain, cramps, functional impairments and changes in gait than controls (Glerup et al., 2000c).

Misdiagnosis of vitamin D-deficient myalgia is common, due to the non-specific nature of the symptoms (Glerup et al., 2000a); for example, in a Swiss study involving 33 female asylum seekers complaining of musculoskeletal pain, 90.9% of patients received an alternative diagnosis, and reported symptoms for a mean duration of 2.53 years before severe vitamin D deficiency was confirmed (de La Jara et al., 2006). A cross-sectional study of patients presenting with non-specific musculoskeletal pain reported a 100% deficiency rate in immigrant participants, although this rate was similarly high in all participants (93%) (Plotnikoff and Quigley, 2003).

1.8.2. ii) VDR and skeletal muscle

Evidence supporting the direct effect of vitamin D on muscle was provided by the discovery of the VDR in the skeletal muscle tissue of animals (Simpson et al., 1985), cloned (Costa et al., 1986), and later, in-situ, human skeletal muscle tissue biopsies (Bischoff et al., 2001). $1,25(\text{OH})_2\text{D}_3$ is bound with high affinity to the VDR (Holick, 2006), where it is thought to exert both genomic and non-genomic effects (see Figure 1.9).

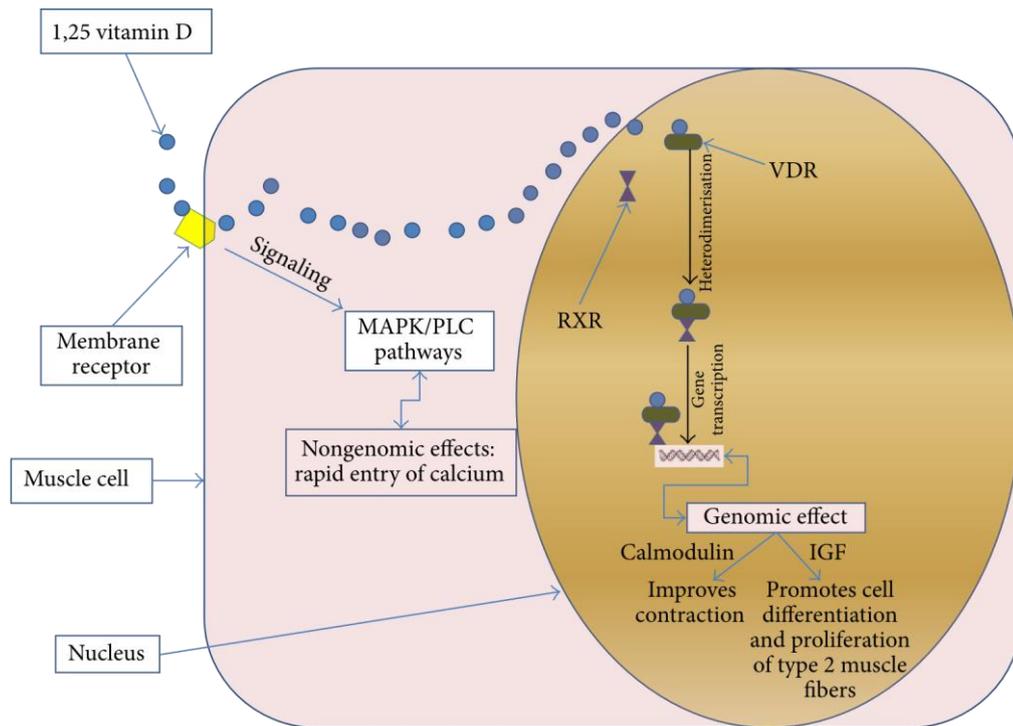


Figure 1.9: Genomic and non-genomic actions of the VDR and their relation to muscle

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MAPK: MAP Kinase; PLC: Phospholipase C; VDR: Vitamin D receptor; RXR: Retinoic X Receptor; IGF: Insulin-like growth factor

The genomic actions of vitamin D are slow acting; in the muscle cell nucleus, the $1,25(\text{OH})_2\text{D}_3$ -VDR complex stimulates both protein synthesis and transcription (via the formation of the heterodimer retinoic X receptor (RXR)), increasing calcium uptake and muscle proliferation and differentiation (Ceglia, 2008). Non-genomic actions are more rapid, including the transportation of calcium into the muscle cell following contraction (Halfon et al., 2015b).

Animal studies have shown that the deletion of VDR gene in mice results in abnormal muscle development; VDR-null mice skeletal muscle cell diameters were 20% smaller in comparison to wild-type mice (Endo et al., 2003). In humans, certain VDR polymorphisms have been shown to influence muscle strength; for example, the *bb*

Bsml genotype expressed in non-obese older women was significantly associated with an increase in quadriceps (+23%) and handgrip strength (+7%) in comparison to older women expressing the *BB* VDR genotype (Geusens et al., 1997).

Additionally, serum 25(OH)D and VDR concentrations have been shown to be significantly associated, with muscle biopsies of vitamin D sufficient older men and women exhibiting higher VDR concentrations than those who were insufficient or deficient (Pojednic et al., 2015); however, this finding was in opposition to (Bischoff-Ferrari et al., 2004).

VDR expression in skeletal muscle tissues has been shown to be significantly reduced with age (Bischoff-Ferrari et al., 2004). Over time, this is thought to contribute towards impaired protein synthesis in muscle cells (Costa et al., 1986); primarily affecting type II muscle fibre atrophy, notably the fibres first recruited to prevent a fall (Sato et al., 2005a), and which may account for the increased risk of falls in vitamin D deficient older adults (Visser et al., 2006).

The mechanism for the decrease in VDR expression with age is not yet established; it has been suggested depleted levels of vitamin D frequently demonstrated in older adults (Holick, 2006) may result in a downregulation of the receptor through lack of stimulation (Bischoff-Ferrari et al., 2004). Indeed, muscle samples from older sarcopenic patients (mean age 63 years) retrieved during distal radius fracture surgery exhibited a significant under-expression of VDR and myogenin and an over-expression of myostatin in comparison to age-matched controls (Roh et al., 2019). Although vitamin D status was not reported in this study, one further study reported that vastus lateralis muscle biopsies of older women (mean age 78.5y) supplemented with 4000 IU per day vitamin D3 for 16 weeks displayed a significant increase in VDR

gene expression in comparison to age-matched women supplemented with a placebo (Pojednic et al., 2015).

1.8.3 iii) Vitamin D and musculoskeletal health; observational evidence

Observational studies provide overwhelming evidence suggesting that serum 25(OH)D concentration is associated with muscle strength (Glerup et al., 2000a, Zivelonghi et al., 2002, Gerdhem et al., 2005, Cherubini et al., 2007, Mastaglia et al., 2011a), power (Bischoff et al., 1999), function (Boeke et al., 2007, Andersen et al., 2009, Dam et al., 2009, Okuno et al., 2010b, Cherubini et al., 2007, Mastaglia et al., 2011a, Zivelonghi et al., 2002, Glerup et al., 2000b), frailty, rate of falls (Stein et al., 1999) and fractures.

Muscle strength has also been found to be associated with vitamin D status by several studies; the InCHIANTI study (n = 976) reported that handgrip strength was significantly reduced in both men and women aged over 65 years with serum 25(OH)D concentration < 25nmol/L and also < 50nmol/L (Cherubini et al., 2007). Additionally, serum 25(OH)D concentration < 30nmol/L have been associated with significantly reduced knee flexion (Gerdhem et al., 2005) and extensor strength (Gerdhem et al., 2005, Zivelonghi et al., 2002, Mastaglia et al., 2011a, Glerup et al., 2000a), hip flexion and extensor strength (Mastaglia et al., 2011a) and arm strength (Zivelonghi et al., 2002) in women over 65 years. Leg extension power was found to be reduced in men and women with hypovitaminosis D (< 30nmol/L), and a modest but significant correlation between 25(OH)D and muscle strength was observed in older men of mean age 76 years (Bischoff et al., 1999).

Musculoskeletal function and its association with vitamin D status has been widely reported; several studies have concluded that SPPB score was significantly lower in

older adults with serum 25(OH)D < 50nmol/L (Mastaglia et al., 2011a, Cherubini et al., 2007), with functional decline at 3 year follow up significantly higher in these participants, independent of age, sex, BMI and chronic disease (Boeke et al., 2007). A study of the Rancho Bernardo cohort (n = 1065) found the TUG and timed chair stand times to be highest, and functional decline after 2.5 years greatest, in women in the lowest quartile of 25(OH)D (< 80nmol/L) compared to the highest quartile (< 337.5nmol/L); this relationship was independent of several covariates, including age and physical activity (Dam et al., 2009). Interestingly, in a study of 80 women aged over 65 years attending 3 months of weekly exercise sessions, those in the highest quartile for 25(OH)D (> 67.5nmol/L) made significantly greater improvements in the TUG, 5 meter walk, functional capacity, balance and alternate step tests than those in the lowest quartile (< 47.5nmol/L). Women in this group made no significant improvements, although there were no significant differences in baseline scores for the tests (Okuno et al., 2010b).

Conversely, one study reported no association between serum 25(OH)D status, loss of muscle strength and risk for onset of new disability in 628 moderately to severely disabled, independent-living women over 65 years. Although there was a significant decline in hip flexion, knee extension and handgrip strength, walking speed and the 5 timed chair stand test after 3 year follow up, this decline was not associated with 25(OH)D (Verreault et al., 2002).

A cohort of 1005 men and women (average age = 74.2 and 75.6 years) from the InCHIANTI study for whom frailty (defined using 5 domains) and serum 25(OH)D data were available, concluded that men with serum 25(OH)D concentrations < 50nmol/L had a 4.94 -fold greater risk for frailty than those men with serum concentrations >50nmol/L; no association was observed in women (Andersen et al.,

2009). Falls and fracture rate has been associated with vitamin D status; a study of 83 elderly nursing home residents (median age = 84 years) reported that fallers had significantly lower serum 25(OH)D concentrations and higher parathyroid hormone (PTH) concentrations than residents who did not report a fall (Stein et al., 1999). The OPRA study reported that women over 75 years (n = 986) with serum 25(OH)D concentrations < 50nmol/L were 2.04 times more likely to sustain at least one low-energy fracture during the 3 year follow up period than women with levels > 50nmol/L (Gerdhem et al., 2005). Interestingly, there was also a significant association between self-assessed physical activity and 25(OH)D concentration, with higher 25(OH)D concentrations associated with more time spent outside and higher activity levels (Gerdhem et al., 2005).

Conversely, relatively little evidence exists documenting the relationship between vitamin D status and muscle mass. Vitamin D status has been associated with muscle mass and sarcopenia; The Longitudinal Aging Study Amsterdam reported that sarcopenia was 2.57 times (based on grip strength) and 2.14 times (based on muscle mass assessed by DXA) more likely to be experienced by a large cohort (n = 1008) of men and women over the age of 65 years with serum 25(OH)D < 25nmol/L than those with serum concentrations > 50nmol/L (Visser et al., 2003c). Additionally, the odds of incident sarcopenia, defined using the more conservative Foundation for the National Institutes of Health (FNIH) criteria (grip strength <27kg in men, <16kg in women; ASM <19.75KG in men, <15.02kg in women (Studenski et al., 2014)), were significantly and independently increased in a cohort of 1705 older men over 70 years (mean age = 77.8 years) in the lowest quartiles of baseline 25(OH)D (<40 nmol/L) and 1,25(OH)D (<62 nmol/L) after a 2 and 5 year follow-up period (25(OH)D

OR = 2.53, 95% CI 1.14, 5.64 and 1,25(OH)D OR = 2.67, 95% CI 1.25, 5.60) (Hirani et al., 2017).

1.8.4. iv) Vitamin D and musculoskeletal health; interventional evidence

Evidence for the effect of vitamin D supplementation on musculoskeletal health is mixed, which is surprising considering the decisiveness of available observational data, although comparison between studies has proved difficult due to high heterogeneity and variations in methodological quality (Beaudart et al., 2014b).

Systematic reviews and meta-analyses investigate the effect of vitamin D supplementation on muscle strength and function (Muir and Montero-Odasso, 2011, Stockton et al., 2011, Beaudart et al., 2014b, Rosendahl-Riise et al., 2017), with an emphasis on the role of baseline serum 25(OH)D. 3 meta-analyses (n = 12,966) concluded that vitamin D supplementation had significant, positive effects on lower limb muscle strength (Beaudart et al., 2014b), hip muscle strength (Stockton et al., 2011), postural sway, TUG time and knee extensor strength (Muir and Montero-Odasso, 2011). These effects were dose, baseline serum 25(OH)D, living situation and age dependent; a greater effect was shown in participants supplemented with >800IU per day (Muir and Montero-Odasso, 2011), with baseline 25(OH)D concentrations <30nmol/L (Beaudart et al., 2014b) or <25nmol/L (Stockton et al., 2011), those who were institutionalized rather than community-dwelling (Beaudart et al., 2014b) and with one study reporting a significant improvement in muscle strength only in adults >65 years (Beaudart et al., 2014b).

Conversely, further systematic reviews and meta-analyses reported no significant effect of vitamin D supplementation on muscle strength (Latham et al., 2003b, Rosendahl-Riise et al., 2017) or falls (Latham et al., 2003b). In the earlier study,

(Latham et al., 2003b) examined the effect of vitamin D supplementation with and without calcium supplementation in a small number of trials (13 trials, n = 2496) including older adults (mean age = 60). No trials supplementing with vitamin D alone were found to affect muscle strength or physical performance, which were analysed using a narrative analysis due to the low quality and differences in measurement methodology, however, meta-analyses demonstrated a small effect of combined vitamin D and calcium supplementation on the number of people who fell. Of note, the authors highlighted the poor quality of the data included; small sample sizes, a high number of drop outs and use of poorly validated measurement techniques influenced quality (Latham et al., 2003b). The more recent study included 15 trials of older men and women over 65 years (n = 2866) and concluded there was no improvement in hand grip strength with vitamin D supplementation, both with and without additional calcium supplementation, however, there was a small significant improvement in TUG time with vitamin D supplementation (Rosendahl-Riise et al., 2017). Again, this review included a small number of studies in the meta-analysis, reported high heterogeneity and the authors noted the outcomes were measured using different devices and protocols, therefore adopting a random-effects model to account for this variety.

The systematic review including younger adults included 5 trials of “excellent” or “good” quality (assessed via the PEDro scale), examining the effect of vitamin D supplementation on muscle strength in young healthy athletes (aged 18 – 45 years). Interestingly, the studies using vitamin D2 supplementation reported no effect on muscle strength, however, all studies supplementing with vitamin D3 reported a positive effect of supplementation on strength; 2 studies reached significance and 2 studies demonstrated trends for increased muscle strength, improvements ranged

from +1.37% to +18.75% (Chiang et al., 2017). The variety of different measurement methodologies utilised to assess the outcome measures was listed as a limitation of the review, with the authors stating that this may have effected the “reliability and accuracy” of the results (Chiang et al., 2017).

1.9. Combined RET and vitamin D supplementation

Evidence for the effectiveness of a combined intervention on parameters of musculoskeletal health is not conclusive, nor is its relationship to sarcopenia. Although some data are suggestive of an association between serum 25(OH)D concentration and muscle weakness (Visser et al., 2003a), this association is not causal, and considering the individual benefits of RET and vitamin D3 on muscle1.7., a number of research groups have proposed a plausible additive effect may exist if the 2 interventions were combined, optimizing the potential for healthy ageing muscle, and have advocated the need for additional data to help draw conclusions (Daly, 2010, Robinson et al., 2012b, Daly et al., 2014, Denison et al., 2015, Agostini et al., 2018).

To date very few studies have been suitably designed to test the combined effects of vitamin D and exercise in older adults, and of the few studies which have, poor exercise compliance (Bunout et al., 2006b) and small sample sizes (Agergaard et al., 2015b) are reported. A recent systematic review and meta-analysis concluded that vitamin D supplementation and exercise training significantly improved muscle strength within the lower limb in comparison to exercise training alone (SMD 0.98, 95%CI 0.73, 1.24, $p < 0.001$) (Antoniak and Greig, 2017). However, the limitations of the review serve to highlight the lack of knowledge within this area; only 3 studies

were included for this analysis, and the high weighting of one particular study with a moderate sample size means that any conclusion drawn can only be tentative.

Of the sparse available data in other population groups, results are similarly mixed; 23 overweight and obese younger adults (mean age = 26.1 years) took part in 40 minutes of RET, 3 days per week for 12 weeks and were supplemented with either 4000IU vitamin D3 and 500mg calcium per day or placebo and 500mg calcium per day. Exercise (97%) and supplement (93%) compliances were high, and muscle strength (sum of chest press, leg press, and leg curl 1RMs) increased significantly in both groups, although no between group differences were observed (Carrillo et al., 2012). A group of Chronic Obstructive Pulmonary Disease (COPD) patients referred for rehabilitation aged >50 years (mean age = 67 years) were randomized to complete 3 months of rehabilitation exercises, 3 times per week incorporating 90 minutes of aerobic and strength training plus either 100,000IU vitamin D or placebo per month for 1 year. Vitamin D deficiency is highly prevalent in this group, as is skeletal muscle weakness, which was one of the main reasons for rehabilitation referral (Hornikx et al., 2012). Isometric quadriceps strength (assessed via dynamometer) was most improved in the vitamin D group, but did not reach significance (+15Nm Vs + 7Nm, $p = 0.121$), similarly, 6m walking distance was most improved in the vitamin D group, but again did not reach significance (40m Vs 11m, $p = 0.130$); in neither outcome were effects more pronounced in participants with vitamin D deficiency at baseline (Hornikx et al., 2012).

1.10. Aims and objectives of the thesis:

1. Systematically review the available literature to determine the effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health and function in older adults.
2. Complete a secondary data analysis of the Vitamin D, Food Intake, Nutrition and Exposure to Sunlight in Southern England (D-FINES) study data set to determine the relationship between serum 25(OH)D concentration and muscle mass in older men and women
3. Produce a working protocol for a randomized controlled trial of resistance exercise training and vitamin D3 supplementation
4. Conduct a randomized controlled trial of resistance exercise training and vitamin D3 supplementation to determine:
 - i) Is vitamin D3 supplementation any more effective in improving musculoskeletal function when combined with exercise training compared with exercise training alone?
 - ii) The seasonal variation in serum vitamin D3 in a population of frailer older adults, both free-living and those living in supported housing.
 - iii) The association between serum vitamin D3 and physical activity (measured using accelerometry) at baseline and responsiveness to RET.
 - iv) Between-group differences with respect to changes in falls as events and quality of life (QoL).
 - v) Between-group differences with respect to changes in fractures as events.
 - vi) Whether RET influences serum inflammatory markers in frailer older adults.
 - vii) The influence of RET on serum stress markers (serum cortisol/ DHEAS) in frailer older adults.

CHAPTER 2:

THE EFFECT OF COMBINED RESISTANCE EXERCISE TRAINING AND VITAMIN D3 SUPPLEMENTATION ON MUSCULOSKELETAL HEALTH AND FUNCTION IN OLDER ADULTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

This chapter is taken verbatim (with the exception of the 2019 update) from the following publication in which I am principal author:

Antoniak, A.E. and Greig, C.A., 2017. The effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health and function in older adults: a systematic review and meta-analysis. *BMJ open*, 7(7), p.e014619.

2.1. ABSTRACT

Objectives

In older adults there is a blunted responsiveness to resistance training and reduced muscle hypertrophy compared with younger adults. There is evidence that both exercise training and vitamin D supplementation may benefit musculoskeletal health in older adults, and it is plausible that in combination their effects may be additive. The aim of this systematic review was to evaluate the effectiveness of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health in older adults.

Data sources

A comprehensive search of electronic databases, including Science Direct, MedLine, PubMed, Google Scholar and Cochrane Central Register of Controlled Trials (Cochrane CENTRAL accessed by Wiley Science). Eligible studies were randomized controlled trials including male and/or female participants (aged ≥ 65 years or mean age ≥ 65 years); enlisting resistance exercise training (RET) and vitamin D3 supplementation; including outcomes of muscle strength, function, muscle power, body composition, serum vitamin D/calcium status or quality of life (4) comparing results with a control group. The review was informed by a pre-registered protocol (Available from http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42015020157).

Results

7 studies were included, with a total of 792 participants aged 65 years or over (or mean age ≥ 65 years). Studies were categorized into two groups; group 1 compared vitamin D3 supplementation and exercise training versus exercise alone (describing the additive effect of vitamin D3 supplementation when combined with resistance exercise training) and group 2 compared vitamin D3 supplementation and exercise training versus vitamin D3 supplementation alone (describing the additive effect of resistance exercise training when combined with vitamin D3 supplementation).

Meta-analyses for group 1 found muscle strength of the lower limb to be significantly improved within the intervention group (0.98, 95% CI 0.73, 1.24, $p < 0.001$); all other outcomes showed small but non-significant positive effects for the intervention group. The Short Physical Performance Battery (SPPB), Timed Up and Go (TUG), muscle strength of the lower limb and femoral neck Bone Mineral Density (BMD) all showed significantly greater improvements in the intervention group for group 2 comparisons.

Conclusions

This review provides tentative support for the additive effect of resistance exercise and vitamin D3 supplementation for the improvement of muscle strength in older adults. For other aspects of musculoskeletal function, such as SPPB and TUG, no additional benefit beyond exercise was shown. Further evidence is required to draw firm conclusions or make explicit recommendations regarding combined exercise and vitamin D3 supplementation.

Strengths and Limitations of this study

- To the best of our knowledge this study represents the first review evaluating the combined effects of vitamin D3 supplementation and exercise in older adults
- Generally, outcome measure data could be graded as representing moderate quality
- Only seven studies were found to be eligible for inclusion, highlighting the lack of literature available on the topic
- The inclusion of one high risk study (as determined during the risk of bias analysis due to handling of missing data) was deemed necessary due to the lack of eligible studies

2.2. INTRODUCTION

Sarcopenia, originally defined as the age related loss of muscle mass (Rosenberg, 1989), now also encompasses low muscle strength and/or muscle function (Cruz-Jentoft et al., 2010b). The efficacy of resistance training in preventing or alleviating age-related musculoskeletal loss is well established; cited as the most promising intervention for improving symptoms of sarcopenia (Kosek et al., 2006).

Clear evidence exists demonstrating an association between resistance exercise training (RET) and muscle hypertrophy, which is maintained in older age (Henwood and Taaffe, 2005, Kosek et al., 2006, Stewart et al., 2014). However, in older adults there is a blunted responsiveness to RET in comparison with younger adults; a blunted muscle protein synthetic rate in response to a single bout of resistance exercise has been reported (Kumar et al., 2009), and others demonstrate a reduction in muscle hypertrophy in comparison to younger adults (Welle et al., 1996, Häkkinen et al., 1998, Raue et al., 2009, Greig et al., 2011b). This 'anabolic resistance' may be due to changes in gene expression and anabolic signalling; an attenuated anabolic hormone response to resistance exercise is observed in comparison to younger adults (Hameed et al., 2003).

Losses in muscle strength are associated with losses in functional ability, independence and increases in frailty, falls, and disability in older adults (Roubenoff, 1999, Janssen et al., 2002, Visser et al., 2002, Landi et al., 2012); therefore, there may be merit associated with a combination of interventions to boost responsiveness of older muscle to resistance exercise and combat anabolic resistance.

Vitamin D3 supplementation in humans has been shown to positively influence musculoskeletal health in older adults: increases in relative number and cross-sectional area (CSA) of muscle fibres (type II in particular) has been reported (Sørensen et al., 1979, Sato et al., 2005b, Ceglia et al., 2013), and muscle strength increased and fall rates decreased after treatment with vitamin D3 (Sato et al., 2005b). Vitamin D receptor (VDR) concentration significantly increased with vitamin D3 supplementation (Ceglia et al., 2013); conversely, supplementation conferred no benefits on strength, functioning and balance (Latham et al., 2003a, Lips et al., 2010, Uusi-Rasi et al., 2015). Moreover, a systematic review examining the effects of vitamin D3 supplementation in vitamin D replete adults aged over 18 years found no significant effect on grip or proximal lower limb muscle strength; however, pooled data including vitamin D deficient participants (serum 25(OH)D <25 nmol.l⁻¹) demonstrated a large effect on hip muscle strength (Stockton et al., 2011).

There is conflicting evidence surrounding the efficacy of vitamin D3 supplementation alone or in combination with exercise on musculoskeletal health, with no clear consensus regarding the management or prevention of sarcopenia. Although epidemiological data suggest a relationship between vitamin D3 and muscle weakness (Visser et al., 2003a), this association is not well understood, and evidence in published literature is lacking and contradictory. Considering the beneficial effects of both RET and vitamin D3 on muscle tissue, it is plausible an additive effect would exist if combined, optimizing the potential for healthy ageing muscle (Robinson et al., 2012b). Thus, the aim of this study was to assess the combined effect of RET and vitamin D3 supplementation on musculoskeletal health in older adults.

2.3. MATERIALS AND METHODS

A systematic review of peer-reviewed literature relating to the effect of RET and vitamin D3 supplementation on musculoskeletal health in older adults was conducted in accordance with a study protocol registered on the PROSPERO database (record number CRD42015020157). The protocol was informed by the Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green, 2011), and reporting conformed to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement (Moher et al., 2009).

2.3.1. Eligibility Criteria

Randomized controlled trials were sought for this study. Journal studies included: (1) male and/or female participants (aged ≥ 65 years or mean age ≥ 65 years) (2) enlisted RET and vitamin D3 supplementation (studies utilising vitamin D3 and calcium supplementation were included) (3) included measures of muscle strength, function, muscle power, body composition, serum vitamin D/calcium status or quality of life (4) compared results with a control group (no exercise/usual care/no vitamin D3 supplementation). Articles were excluded if participants were supplemented with additional protein or any supplement/medication with a known anabolic effect on muscle tissue.

2.3.2. Search methods for identification of studies

Articles published before March 2016 were included. A computerised search of Science Direct, MedLine, PubMed, Google Scholar and Cochrane Central Register of

Controlled Trials (Cochrane CENTRAL accessed by Wiley Science) databases was conducted. Table 1 shows the Medline search strategy, devised by AEA and LH.

2.3.3. Data items and collection

Data were extracted independently by 2 reviewers (AEA and ASA) using a standardised data extraction sheet; any disagreements were discussed and resolved with a third person (CAG). The inter-rater reliability assessed using Cohen's Kappa, was found to be excellent (86% agreement) (Hsu and Field, 2003). Data items including general information, participant characteristics and details of the intervention were extracted. For key outcomes the definition used by the authors, methodology, results, mean differences and the presence/absence of statistical significance were reported.

Table 2.1: Example Ovid MEDLINE search, to be adapted for other databases	
1	Aging/
2	Exp aged/
3	(65 adj2 (years or age* or old*))
4	(old* adj (adult* or people or person* or population* or men or women))
5	(elder* or senior* or geriatric* or ?enarian or ag?ing)
6	((age* or aging or old* or elder*) adj1 (musc*))
7	1 or 2 or 3 or 4 or 5 or 6
8	Vitamin D/
9	(cholecalciferol* or calciferol* or ergocalciferol*)
10	(supplements or dietary supplements)
11	((vitamin D* or cholecalciferol or calciferol* OR ergocalciferol) adj supplementation)
12	8 or 9 or 10 or 11
13	Muscle Development/
14	Muscle, Skeletal/
15	(Skeletal muscle adj2 (atrophy or sarcopenia or wasting or loss or deterioration))
16	Muscle Strength/
17	(skeletal muscle mass or size or fibres or fibers or area)
18	(musc* adj2 (function* or power or strength))
19	(musc* adj2 (grow* or hypertrophy or size or mass or csa or cross sectional area or volume))
20	Body Composition/
21	(lean adj3 mass)
22	(protein adj2 (turnover or synthesis or breakdown))
23	(nitrogen adj2 (balance or turnover or synthesis or breakdown or retention or loss or retain*))
24	Sarcopenia/
25	13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24
26	Exp exercise/
27	(resistance exercise or resistance exercise training)
28	((resistance or strength or weight or cardio or aerobic) adj3 (train* or condition* or exercise* or lift*))
29	(physical adj3 (activit* or exercise* or train* or exertion* or endurance* or therap* or conditioning or fitness))
30	(exercise adj3 (train* or intervention* or protocol* or program* or therap* or regim* or activit*))
31	26 or 27 or 28 or 29 or 30
32	7 and 12 and 25 and 31
33	Limit 32 to humans
34	Remove duplicates from 33

2.3.4. Risk of bias analysis

2 reviewers (AEA and CAG) independently assessed the validity of included studies, with provisions for moderation from a third reviewer. The Cochrane Collaboration's tool for assessing risk of bias was utilised, as described in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green, 2011); the use of scales for assessment is explicitly discouraged (Moher and Olkin, 1995, Moher et al., 1996). Pre-specified consensus points were devised and agreed by reviewers to ensure consistency. It was acknowledged that by nature of design, blinding of participants and personnel would be difficult in certain studies; therefore grading was based on the likelihood that outcome measures were influenced by the potential lack of blinding (Higgins and Green, 2011).

2.3.5. Grading the quality of evidence

The Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) handbook (Schünemann et al., 2009) was used to evaluate the quality of evidence of outcomes assessed within the meta-analyses. The GRADE approach utilises systematically produced questions to reach conclusions on degree of confidence in the estimate of the effect, with explicit and comprehensive criteria for up- or downgrading quality and multiple reviewers minimising individual grader bias. GRADE assesses patient important outcomes across five areas; risk of bias, inconsistency, indirectness, imprecision and publication bias, and grades outcomes as demonstrating high, moderate, low or very low quality of evidence.

2.4. RESULTS

2.4.1. Study selection:

7 studies were included within the review; Agergaard et al., 2015 (Agergaard et al., 2015b), Bunout et al., 2006 (Bunout et al., 2006b), Drey et al., 2011 (Drey et al., 2011), Gianoudis et al., 2014 (Gianoudis et al., 2014), Jessup et al., 2003 (Jessup et al., 2003), Uusi-Rasi et al., 2015 (Uusi-Rasi et al., 2015), and Verschueren et al., 2011 (Verschueren et al., 2011); the study flow diagram is presented in Figure 2.1.

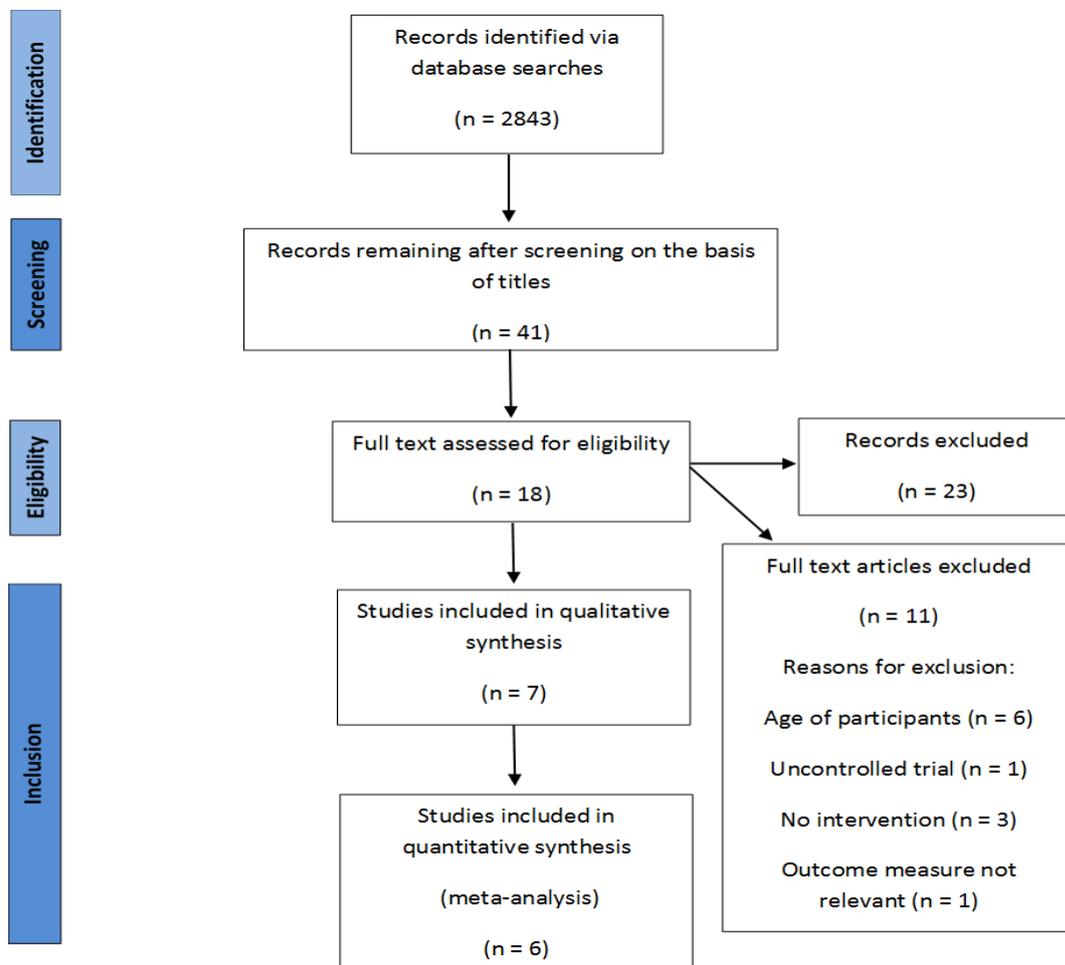


Figure 2.1: Flow of studies through the eligibility screening process and reasons for exclusion

Upon reading full text articles, it became clear that there were 2 separate groups of interventions; group 1, in which all participants took part in RET and the intervention arm was supplemented with vitamin D3 (describing the additive effect of vitamin D3 supplementation when combined with resistance exercise training), group 2 in which all participants were supplemented with vitamin D3 and the intervention arm took part in RET (describing the additive effect of resistance exercise training when combined with vitamin D3 supplementation); and studies using a combination of the 2 interventions (Table 2.2).

Table 2.2: Demographics of included studies								
	N	Mean age (y)	Sex (M:F)	Study design	Intervention group protocol	Control group protocol	Duration	Exercise intensity
Group 1: All participants exercised; intervention group received vitamin D supplementation								
Agergaard et al., 2015 (Agergaard et al., 2015b)	17	66.9	17:0	RCT	RET 3x per week & 1920 IU D3 + 800mg Ca/day	RET 3x per week & 800mg Ca/day	16 weeks	Progressed to 6 – 8 repetitions of 80 – 85% 1 RM
Group 2: All participants received vitamin D supplementation; intervention group exercised								
Drey et al., 2011 (Drey et al., 2011)	45	77	13:32	RCT	RET 2x 60 mins per week & >20 ng/ml = 1000 IU D3/day <20 ng/ml = 2000 IU D3/day	No exercise & >20 ng/ml = 1000 IU D3/day <20 ng/ml = 2000 IU D3/day	12 weeks	6 – 15 repetitions. Intensity ranged 10 – 16 on Borg scale
Gianoudis et al., 2014 (Gianoudis et al., 2014)	162	67	119:43	RCT	HV-PRT 3x per week & 1000 IU D3 + 700mg Ca/day	No exercise & 1000 IU D3 + 700mg Ca/day	12 months	5 – 12 repetitions. Intensity ranged 5 - 8 on Borg scale
Jessup et al., 2003 (Jessup et al., 2003)	18	69	0:18	RCT Parallel	RET 3x 60-90 mins per week & 400 IU D3 + 1000 mg Ca/day	No exercise & 400 IU D3 + 1000 mg Ca/day	32 weeks	1 RM progressed by 1 – 2kg increments through study
Verschueren et al., 2011 (Verschueren et al., 2011)	111	79	0:111	RCT	WBV 3x per week & High-dose = 1600 IU Or Conventional dose = 800 IU D3/day + 1000mg Ca/day	No exercise & High-dose = 1600 IU Or Conventional dose = 800 IU D3/day + 1000mg Ca/day	6 months	Baseline to 6m: Vibration frequency: 30-40 Hz Rest period 60 – 5s Vibration duration: 1 – 12 mins
Assigned to Group 1 & 2: Participants took part in a combination of exercise and vitamin D interventions								
Bunout et al., 2006 (Bunout et al., 2006b)	92	77	9:83	RCT	RET 2x 1.5h per week Or No exercise & 400 IU D3 + 800mg Ca/day	RET 2x 1.5h per week Or No exercise & 800mg Ca/day	9 months	Initially 5 – 10 repetitions. Intensity assessed using Borg scale, score of ≤4 = increase reps or theraband colour
Uusi-Rasi et al., 2015 (Uusi-Rasi et al., 2015)	409	74	0:409	RCT	RET 2x/week for 12 months, 1x/week for next 12 months Or No exercise & 800 IU D3/day	RET 2x/week for 12 months, 1x/week for next 12 months Or No exercise & Placebo/day	2 years	Supervised classes ranged from 1.6 to 5.6 METS. Weights: 60 – 75% 1 RM target
RCT: Randomized Controlled Trial, RET: Resistance Exercise Training, IU: International Units, Ca: Calcium, HV-PRT: High-Velocity Progressive Resistance Training; RM: Repetition maximum; Hz: Hertz; m: month; s: seconds								

2.4.2. Study demographics

7 eligible studies included a total of 792 participants of mean age 72.8 years (Table 2.2). Of these, 1 included only males (Agergaard et al., 2015b) and 3 included only females (Jessup et al., 2003, Uusi-Rasi et al., 2015, Verschueren et al., 2011). All studies included healthy participants living independently, except for 2 studies; (Jessup et al., 2003) included participants living within a retirement community and (Verschueren et al., 2011) included institutionalized participants living in nursing homes, service flats or cloistered communities.

2.4.3. Interventions

Studies assigned to group 1 included Agergaard et al., 2015 (Agergaard et al., 2015b); Bunout et al., 2006 (Bunout et al., 2006b) and Uusi-Rasi et al., 2015 (Uusi-Rasi et al., 2015). In group 1, all participants took part in RET; incorporating a warm-up and strengthening exercises utilising commercial weight machines (Agergaard et al., 2015b, Uusi-Rasi et al., 2015) or Thera-bands (Agergaard et al., 2015b). 2 studies included balance challenging aspects (Bunout et al., 2006b, Uusi-Rasi et al., 2015). All studies included supervised, progressive exercise sessions; progression was monitored by a 5 rep max (RM) test (Agergaard et al., 2015b), Borg scale (Bunout et al., 2006b) or metabolic equivalents (METs) (Uusi-Rasi et al., 2015). Total number of sessions delivered ranged from 36 (Agergaard et al., 2015b) to 156 (Uusi-Rasi et al., 2015), over a duration of 16 weeks (Agergaard et al., 2015b) to 24 months (Uusi-Rasi et al., 2015). All administered a vitamin D3 supplement, orally in tablet form; doses ranged from 400IU (Bunout et al., 2006b) to 1920 IU (Agergaard et al., 2015b) per day; in 2 studies participants were supplemented with 800mg calcium

per day (Agergaard et al., 2015b, Bunout et al., 2006b) and 1 study supplemented the control group with a placebo (Uusi-Rasi et al., 2015).

6 studies assigned to group 2 included; Bunout et al., 2006 (Bunout et al., 2006b), Drey et al., 2011 (Drey et al., 2011) Gianoudis et al., 2014 (Gianoudis et al., 2014), Jessup et al., 2003 (Jessup et al., 2003), Uusi-Rasi et al., 2015 (Uusi-Rasi et al., 2015) and Verschueren et al., 2011 (Verschueren et al., 2011). Within group 2, all participants took a vitamin D3 supplement, orally in tablet form. Doses ranged from 400 IU (Bunout et al., 2006b, Jessup et al., 2003) to 2000 IU (Drey et al., 2011) per day; 1 study monitored serum 25(OH)D at baseline to determine supplement dosage (Drey et al., 2011). In 4 studies (Bunout et al., 2006b, Gianoudis et al., 2014, Jessup et al., 2003, Verschueren et al., 2011) all participants were supplemented with calcium; doses ranged from 700mg (Gianoudis et al., 2014) to 1000mg (Jessup et al., 2003, Verschueren et al., 2011) per day. The intervention group took part in RET. Studies utilised machine weights and pulleys (Drey et al., 2011, Gianoudis et al., 2014, Jessup et al., 2003, Uusi-Rasi et al., 2015), Thera-bands (Bunout et al., 2006b), weighted vests (Jessup et al., 2003) and Whole Body Vibration (WBV) machines (Verschueren et al., 2011) for resistance. 5 studies included balance challenging aspects (Bunout et al., 2006b, Drey et al., 2011, Gianoudis et al., 2014, Jessup et al., 2003, Uusi-Rasi et al., 2015). All studies employed supervised, progressive exercise sessions monitored via a Borg scale (Bunout et al., 2006b, Drey et al., 2011, Gianoudis et al., 2014), addition of weights to weighted vests (Jessup et al., 2003), estimation of METs or individual ability (Verschueren et al., 2011). Total number of sessions delivered ranged from 24 (Drey et al., 2011) to 156 (Uusi-Rasi et al., 2015), over a duration of 12 weeks (Drey et al., 2011) to 24 months (Uusi-Rasi et

al., 2015). Note that 2 studies included comparators which allowed allocation to both groups (Bunout et al., 2006b, Uusi-Rasi et al., 2015).

2.4.4. Outcome measures

All outcomes are listed in Table 2.3. Group 1 studies had few outcomes in common; however, all measured muscle strength (Agergaard et al., 2015b, Bunout et al., 2006b, Uusi-Rasi et al., 2015); isometric knee extensor strength was measured using a strain gauge (Agergaard et al., 2015b, Uusi-Rasi et al., 2015) and isometric quadriceps strength was measured using a quadriceps table (Bunout et al., 2006b). Hand grip strength was measured using a hand grip dynamometer (Bunout et al., 2006b). Magnetic resonance imaging (MRI) was used to measure the CSA of the quadriceps (Agergaard et al., 2015b), whilst (Bunout et al., 2006b) analysed fat and lean mass using dual-energy X-ray absorptiometry (DXA). 2 studies measured timed-up and go (TUG), femoral neck and spine bone mineral density (BMD) (Bunout et al., 2006b, Uusi-Rasi et al., 2015). 1 study analysed fibre type and muscle quality (Agergaard et al., 2015b).

Of group 2 studies, (Bunout et al., 2006b, Gianoudis et al., 2014, Uusi-Rasi et al., 2015, Verschueren et al., 2011) assessed lower limb strength, and (Bunout et al., 2006b, Jessup et al., 2003) measured grip strength. Muscle power was measured as sit-to-stand transfer power (Drey et al., 2011) and the stair climb test (Gianoudis et al., 2014). The short physical performance battery (SPPB) was assessed by (Bunout et al., 2006b, Gianoudis et al., 2014), and the TUG by (Bunout et al., 2006b, Gianoudis et al., 2014, Uusi-Rasi et al., 2015). BMD of the femoral neck (Bunout et al., 2006b, Gianoudis et al., 2014, Jessup et al., 2003, Uusi-Rasi et al., 2015, Verschueren et al., 2011) and spine (Bunout et al., 2006b, Gianoudis et al., 2014,

Jessup et al., 2003, Uusi-Rasi et al., 2015) were measured using DXA. Lean mass was measured using DXA (Bunout et al., 2006b, Drey et al., 2011, Gianoudis et al., 2014) and X-ray computed tomography (CT) (Verschueren et al., 2011). Balance was assessed via the Romberg ratio (Bunout et al., 2006b), four square step test (Gianoudis et al., 2014), an AccuSway platform (Jessup et al., 2003) and backwards walking (Uusi-Rasi et al., 2015). Other outcomes included endurance (12-minute walk (Bunout et al., 2006b)), the 30 second sit-to-stand test (Gianoudis et al., 2014), normal walking speed and the 5-time chair stand test (Uusi-Rasi et al., 2015).

Table 2.3: Summary of included study outcome measures and significant results

		Outcome measures	Significant results (% change per week in intervention Vs placebo group)
Agergaard et al., 2015 (Agergaard et al., 2015b)	Muscle strength	Isometric knee extensor (strain gauge)	Muscle strength – Increased (p<0.0001) but no between-group difference (1.24% Vs 0.70% per week)
	Muscle CSA	MRI of quadriceps muscle (6mm thick)	Muscle CSA – Increased (p=0.001) but no between-group difference (0.41% Vs 0.71% per week)
	Muscle quality	Muscle strength/CSA	Muscle quality – N/S
Bunout et al., 2006 (Bunout et al., 2006b)	Muscle strength	Quadriceps (table) & hand grip strength (dynamometer)	Muscle strength – Increased with exercise (p<0.001), no effect of vit D (0.46% Vs 0.59% per week)
	Muscle function	SPPB, TUG	Muscle function – SPPB (p=0.002) no effect of vit D (0.41% Vs 0.29% per week) TUG: Increased in both groups (p=0.004) (0.07% Vs 0.18% per week)
	BMD	Femoral neck & spine (DXA)	BMD – Femoral neck increased with vit D, decreased without (p=0.006) (+0.03% Vs -0.03% per week). Spine was N/S
	Body sway	Romberg ratio	Body sway – Lower with vit D than without (p=0.05) (-0.08% Vs +0.48% per week)
	Endurance	Distance walked in 12 minutes	Endurance – N/S
Drey et al., 2011 (Drey et al., 2011)	Muscle power	Lower limb sit-to-stand transfer power (force plate)	Muscle power - Increased with vit D intake (p=0.017). (+0.01% Vs 0.002%) (0.69% Vs 0.21% per week)
	Muscle function	SPPB, SF-LLFDI	Muscle function – SPPB increased with exercise (p=0.009) (0.86% Vs -0.05%) (+0.85% Vs -0.43% per week), SF-LLFDI was N/S
	BC	aLM (DXA)	Body composition – aLM was N/S
Gianoudis et al., 2014 (Gianoudis et al., 2014)	Muscle strength	Lower limbs (bilateral leg press) and back (seated row)	Muscle strength- Exercise increased strength by +3% (p<0.05) (0.47% Vs 0.29% per week)
	Muscle power	Timed stair climb test	Muscle power – Exercise increased power by +5% (p<0.05) (0.21% Vs 0.14% per week)
	Muscle function	30 second sit-to-stand test, TUG	Muscle function – Exercise improved Sit-to-stand by +16% (p<0.001) (0.35% Vs 0.05% per week). TUG -N/S
	BMD	Femoral neck & spine (DXA)	BMD – Exercise increased femoral neck & spine BMD by +0.1% (p<0.05) (FN: +0.01% Vs -0.01%; spine: 0.03% Vs 0.01% per week)
	BC	Total body lean & fat mass (DXA)	Body composition – Lean & fat mass – N/S
	Dynamic balance	Four Square Step Test	Dynamic balance – Exercise improved by +6% (p<0.01) (-0.24% Vs -0.12% per week)

Table 2.3: Summary of included study outcome measures and significant results continued

		Outcome measures	Significant results
Jessup et al., 2003 (Jessup et al., 2003)	Muscle strength BMD Body sway	Hand grip (dynamometer), mean of 8 tests (stack machine) Femoral neck & spine (DXA) AccuSway force platform	Muscle strength – increased with exercise (p=0.0156). No effect of vit D (0.57% Vs 0.19% per week) BMD femoral neck – increase with exercise (p=0.00001), increase with vit D (p=0.016) (0.30% Vs -0.17% per week) Spine – increase with exercise (p=0.0094), vit D supplementation N/S (0.39% Vs -0.03% per week) Body sway – N/S
Uusi-Rasi et al., 2015 (Uusi-Rasi et al., 2015)	Muscle strength Muscle function BMD Dynamic balance	Max isometric leg extensor strength at a knee angle of 110° SPPB, TUG Femoral neck & spine (BMD) Backwards walking	Muscle strength – increased with exercise (p<0.001). Vit D supplementation N/S (0.15% Vs 0.13% per week) Muscle function – SPPB = N/S. TUG improved in vitamin D and exercise group (p=0.01) (-0.02% Vs +0.01% per week) BMD – Femoral neck – Vit D maintained BMD (p=0.02) as did exercise (p=0.01) (-0.01% Vs -0.01% per week). Spine – N/S Dynamic balance – Improved with exercise (placebo: p=0.001, vit D: p=0.03). No additive effect of vit D (0.25% Vs 0.25% per week)
Verschueren et al., 2011 (Verschueren et al., 2011)	Muscle strength BMD Muscle mass	Isometric & dynamic knee extensor strength Femoral neck (DXA) Mass of upper leg (Multi-slice CT)	Muscle strength – Isometric: N/S. Dynamic: improved in all groups. Vit D=no effect (0.48% Vs 0.05% per week) BMD – Improved in all groups. No difference between training of vit D groups (0.03% Vs 0.03% per week) Muscle mass – N/S
CSA: Cross-sectional Area, MRI: Magnetic Resonance Imaging, ELISA: Enzyme-linked Immunosorbent Assay, BMD: Bone Mineral Density, SPPB: Short Physical Performance Battery, TUG: Timed Up and Go, DXA: Dual-energy X-ray Absorptiometry, SF-LLFDI: Short Form of the Late Life Function and Disability Instrument, aLM: appendicular Lean Mass, QoL: Quality of Life, Multi-slice CT: Multi-slice X-ray Computed Tomography			

2.4.5. Risk of bias within studies

For all studies, a high proportion of components were assigned an unclear risk of bias due to insufficient information and the unknown effect on study outcome measures. Many studies reported insufficient information on concealment and blinding procedures, or whether procedures were in place in the event of unblinding. In total, 6 studies were judged to have an unclear risk of bias (Agergaard et al., 2015b, Bunout et al., 2006b, Drey et al., 2011, Jessup et al., 2003, Uusi-Rasi et al., 2015, Verschueren et al., 2011). Component 1 was assessed as having a low risk of bias for all studies. 1 study was assessed as having an overall high risk of bias (Gianoudis et al., 2014) due to component 5, as no data were entered into the analyses for participants with missing data.

Author, year	Components of risk of bias							Summary	Comments on high risk components
	1	2	3	4	5	6	7		
								High (0)	
Agergaard et al., (2015)	L	U	L	L	U	L	L	Unclear (2) Low (5)	N/A
								High (0)	
Bunout et al., (2006)	L	U	U	U	U	U	U	Unclear (6) Low (1)	N/A
								High (0)	
Drey et al., (2011)	L	L	U	U	L	L	U	Unclear (3) Low (4)	N/A
								High (1)	One high risk component, 5
Gianoudis et al., (2014)	L	U	U	U	H	L	L	Unclear (3) Low (3)	ITT analysis utilised, but no data entered for participants with missing data
								High (0)	
Jessup et al., (2003)	L	U	U	U	U	U	L	Unclear (5) Low (2)	N/A
								High (0)	
Uusi-Rasi et al., (2015)	L	U	U	U	U	L	L	Unclear (4) Low (3)	N/A
								High (0)	
Verschueren et al., (2011)	L	U	U	U	U	L	L	Unclear (4) Low (3)	N/A

Figure 2.2: Summary of risk of bias analysis for included studies

Risk of bias domains of assessment. 1: Random sequence generation, 2: Allocation concealment, 3: Blinding of participants and personnel, 4: Blinding of outcome assessment, 5: Incomplete outcome data, 6: Selective reporting, 7: Other sources of bias. Judgements possible: H – High risk of bias, U – Unclear risk of bias, L – Low risk of bias

2.4.6. GRADE analysis

The GRADE summary of findings table for groups 1 and 2 are shown in Tables 2.4 and 2.5.

Table 2.4: GRADE analysis of Group 1 measurement outcomes included in the quantitative synthesis											
Quality Assessment							Summary of Findings				
Outcome	Included studies	ROB	Inconsistency	No serious Indirectness	Imprecision	Publication bias	Groups (Intervention/control)	Effect size (direction)	Significance	95% CI	Quality
Muscle strength (lower limb)	[1,2,6]	No serious ROB	Serious inconsistency substantial heterogeneity	No serious indirectness	No serious imprecision	Undetected [^]	131/135	0.98 (Intervention)	$p < 0.0001$	(0.73, 1.24)	⊕⊕⊕○ Moderate
TUG	[2,6]	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CIs cross line of no effect/ OIS not reached)	Undetected [^]	124/125	0.37 (Intervention)	$p = 0.37$	(-0.68, 0.26)	⊕⊕⊕○ Moderate
BMD (hip)	[2,6]	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CIs cross line of no effect/ OIS not reached)	Undetected [^]	124/125	0.02 (Intervention)	$p = 0.15$	(-0.01, 0.05)	⊕⊕⊕○ Moderate
BMD (spine)	[2,6]	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CIs cross line of no effect/ OIS not reached)	Undetected [^]	124/125	0.02 (Intervention)	$p = 0.41$	(-0.03, 0.07)	⊕⊕⊕○ Moderate

ROB: Risk of Bias; TUG: Timed Up and Go; RCT: Randomized Controlled Trial; CI: Confidence Interval; BMD: Bone Mineral Density; OIS: Optimum Information Size.
[^]Insufficient data to produce funnel plots. GRADE scoring: ⊕⊕⊕⊕ High; ⊕⊕⊕○ Moderate; ⊕⊕○○ Low; ⊕○○○ Very low

Table 2.5: GRADE analysis of Group 2 measurement outcomes included in the quantitative synthesis

Quality Assessment							Summary of Findings				
Outcome	Included studies	ROB	Inconsistency	Indirectness	Imprecision	Publication bias	Groups (intervention/control)	Effect size (direction)	Significance	95% CI	Quality
SPPB	[2,3]	No serious ROB	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected [^]	45/46	1.09 (Intervention)	$p = 0.02$	(0.15,2.03)	⊕⊕⊕⊕ High
TUG	[2,6]	No serious ROB	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected [^]	124/126	-1.57 (Intervention)	$p = 0.001$	(-2.50, -0.64)	⊕⊕⊕⊕ High
Muscle strength (lower limb)	[2,6]	No serious ROB	Serious inconsistency (substantial heterogeneity)	No serious indirectness	No serious imprecision	Undetected [^]	124/126	2.69 (Intervention)	$p = 0.002$	(0.96,4.42)	⊕⊕⊕○ Moderate
Hand grip strength	[2,5]	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected [^]	31/33	0.85 (Intervention)	$p = 0.55$	(-1.93,3.63)	⊕⊕⊕○ Moderate
Weight	[2,4,5]	Serious ROB*	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected [^]	112/114	-0.12 (Intervention)	$p = 0.37$	(-0.38,0.14)	⊕⊕○○ Low
Lean mass	[2,4]	Serious ROB*	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected [^]	103/105	0.02 (Intervention)	$p = 0.98$	(-1.31,1.35)	⊕⊕○○ Low
Fat mass	[2,4]	Serious ROB*	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected [^]	103/105	-0.39 (Intervention)	$p = 0.76$	(-2.82, 2.05)	⊕⊕○○ Low
BMD (hip)	[2,4,5,6]	Serious ROB*	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected [^]	124/126	0.04 (Intervention)	$p = 0.002$	(0.01,0.06)	⊕⊕⊕○ Moderate
BMD (spine)	[2,4,5,6]	Serious ROB*	Serious inconsistency (substantial heterogeneity)	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected [^]	124/126	0.02 (Intervention)	$p = 0.24$	(-0.001,0.05)	⊕○○○ Very low

* ([4] was evaluated as high risk for incomplete outcome data); [^]Insufficient data to produce funnel plots. GRADE scoring: ⊕⊕⊕⊕ High; ⊕⊕⊕○ Moderate; ⊕⊕○○ Low; ⊕○○○ Very low

Within group 1, all studies were evaluated as moderate quality of evidence; no serious risk of bias was detected. Due to the nature of the studies included within this review, no serious indirectness was detected; all outcomes were measured directly without the use of a surrogate. Publication bias was not detected, and due to the number of studies included, it was not possible to produce funnel plots for any outcomes. Although publication bias was “not detected”, it is difficult to conclude that there was a complete absence of bias since studies with significant results are more likely to be published than those reporting null or non-significant results (Higgins and Green, 2011) Published, peer-reviewed articles were included in this review, since the Cochrane Handbook for Systematic Reviews of Interventions further suggests that the inclusion of unpublished studies may introduce additional bias, as these studies have not been strengthened by the peer-review process and may be of lower methodological quality (Higgins and Green, 2011). Reasons for downgrading the quality of evidence included serious inconsistency due to substantial heterogeneity, and serious imprecision due to confidence intervals crossing the line of no effect.

Within group 2 studies, 5 outcomes were graded as high to moderate quality of evidence (SPPB, TUG, muscle strength of the lower limb, hand grip strength and BMD of the femoral neck). Remaining outcomes were graded as low or very low quality, meaning that one could have little or very little confidence in the effect estimate. Common reasons for downgrading outcomes included a combination of serious risk of bias (due to the inclusion of study (Gianoudis et al., 2014)), serious imprecision or serious inconsistency.

2.4.7. Results of individual studies and synthesis of results

Results of the 2 groups of studies are reported separately. Qualitative syntheses were conducted for studies with similar interventions and outcomes measures using RevMan 5.3 software. Study outcomes reporting results in the same units were pooled using a fixed-effect meta-analysis. Effect sizes are expressed as percentage mean differences or standardized mean differences (when outcomes were measured utilising different methods), with 95% confidence intervals. Higher weighting was assigned to studies with smaller standard deviations and a larger sample size (Higgins and Green, 2011). Analyses were completed from extracted data; where necessary data were estimated from statistics or figures, or requested from the authors of the article. Heterogeneity was assessed via the chi squared test (Figures 2.3 – 2.15 and Tables 2.4 and 2.5). Article (Verschueren et al., 2011) was not included in any of the quantitative analyses since the exercise intervention modality was considered to be too dissimilar to compare to the other included articles. Within each group, there were outcomes unsuitable for quantitative synthesis, due to a lack of studies with common outcomes or aspects of studies too dissimilar for comparison; therefore, a narrative analysis was utilised.

2.4.8. Quantitative synthesis

Outcomes compared for group 1 included muscle strength of the lower limb, TUG and BMD of the femoral neck and spine (Figures 2.3-2.6). Only muscle strength of the lower limb was found to be significant, with a large effect size in favour of the intervention group (Figure 2.3: 0.98, 95% CI 0.73, 1.24, $p < 0.001$).

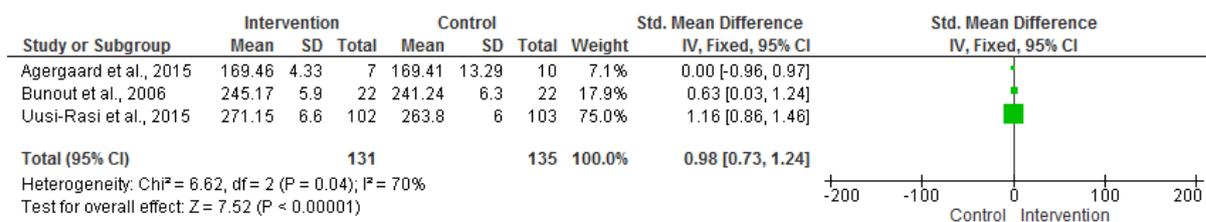


Figure 2.3: Group 1 analysis of muscle strength of the lower limb

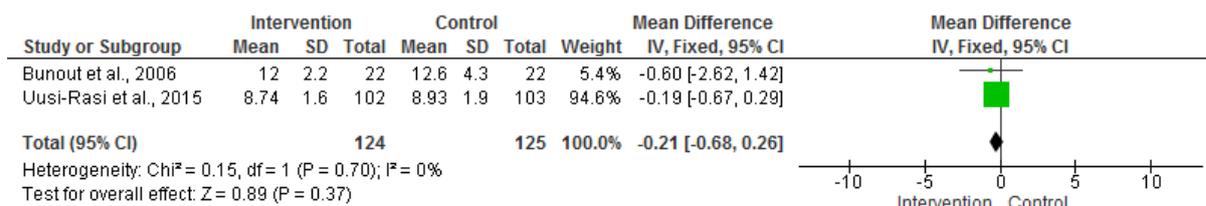


Figure 2.4: Group 1 analysis of the TUG test

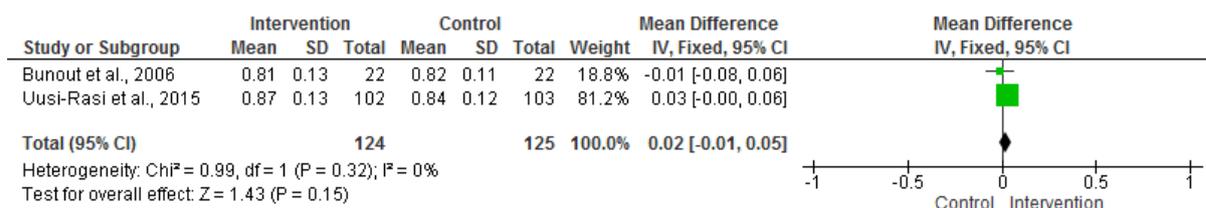


Figure 2.5: Group 1 analysis of BMD of the hip

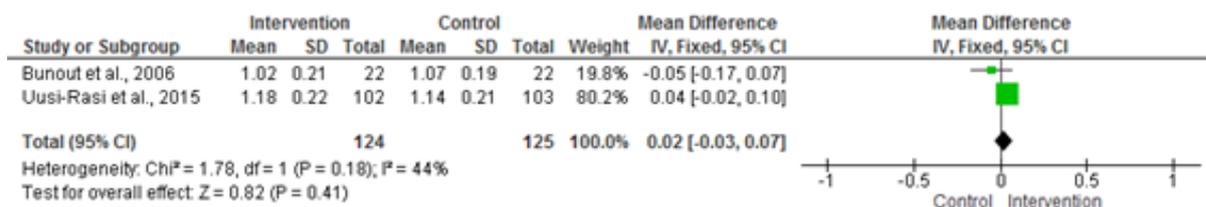


Figure 2.6: Group 1 analysis of BMD of the spine

Group 2 comparisons included the SPPB (Figure 2.7), TUG (Figure 2.8), muscle strength of the lower limb (Figure 2.9), hand grip strength (Figure 2.10), weight (Figure 2.11), lean mass (Figure 2.12), fat mass (Figure 2.13), BMD of the femoral neck (Figure 2.14) and spine (Figure 2.15). Of these outcomes, hand grip strength, weight, lean mass, fat mass and the BMD of the spine were found to be non-significant. However, SPPB score was more improved in the intervention group (1.09,

95% CI 0.15, 2.03. $p = 0.02$), with a significant and large effect. Similarly, TUG was significantly reduced within the intervention group (-1.57, 95% CI -2.50, -0.64. $p = 0.0010$). The results of the quantitative analysis also supported the combined intervention for muscle strength of the lower limb (2.69, 95% CI 0.95, 4.42). $p = 0.002$), and BMD of the femoral neck (0.04, 95% CI 0.01, 0.06. $p = 0.002$).

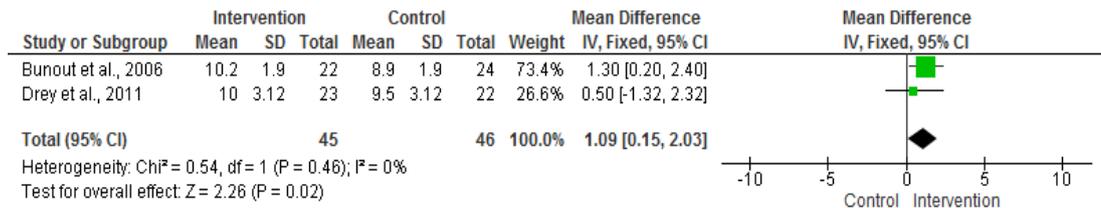


Figure 2.7: Group 2 analysis of the SPPB test

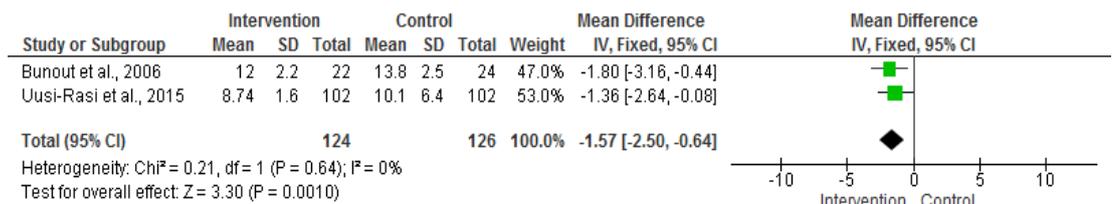


Figure 2.8: Group 2 analysis of the TUG test

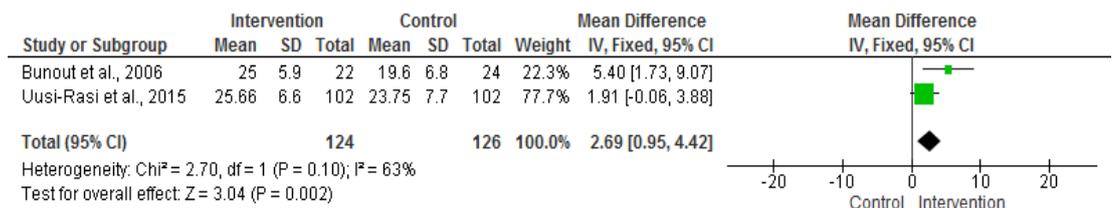


Figure 2.9: Group 2 analysis of muscle strength of the lower limb

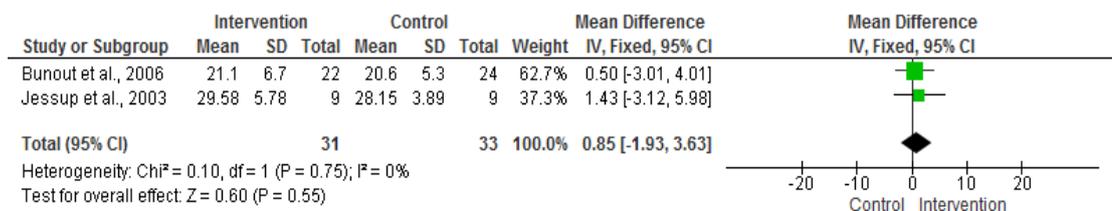


Figure 2.10: Group 2 analysis of hand grip strength

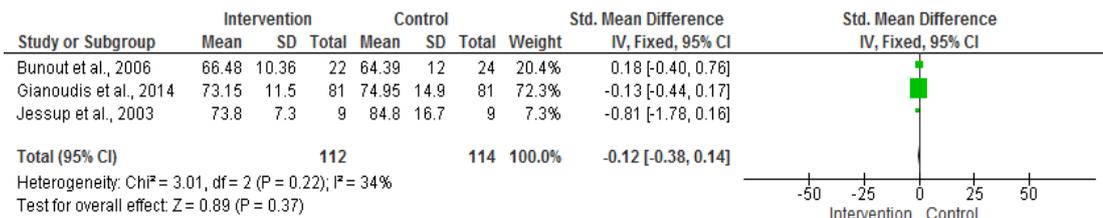


Figure 2.11: Group 2 analysis of total body weight

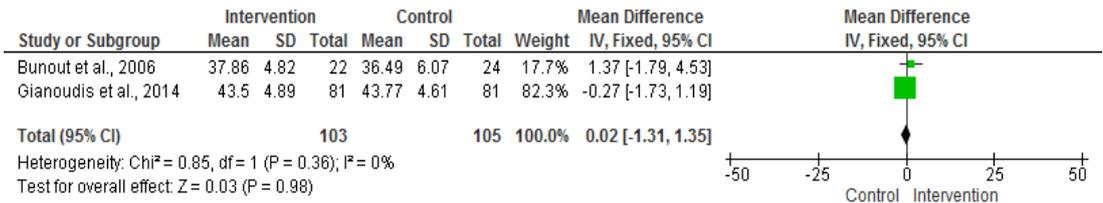


Figure 2.12: Group 2 analysis of lean mass

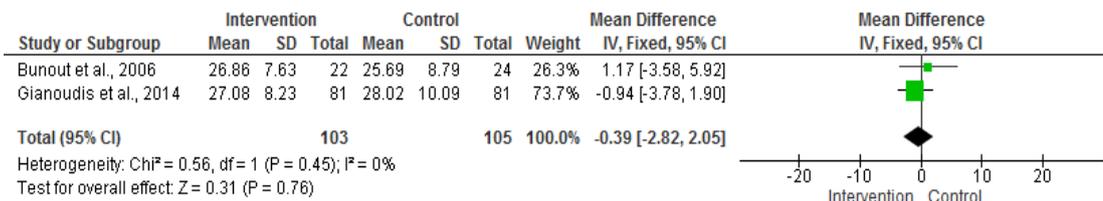


Figure 2.13: Group 2 analysis of fat mass

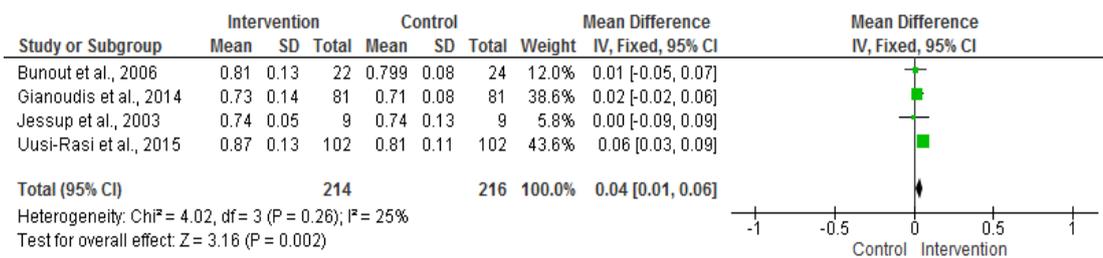


Figure 2.14: Group 2 analysis of BMD of the hip

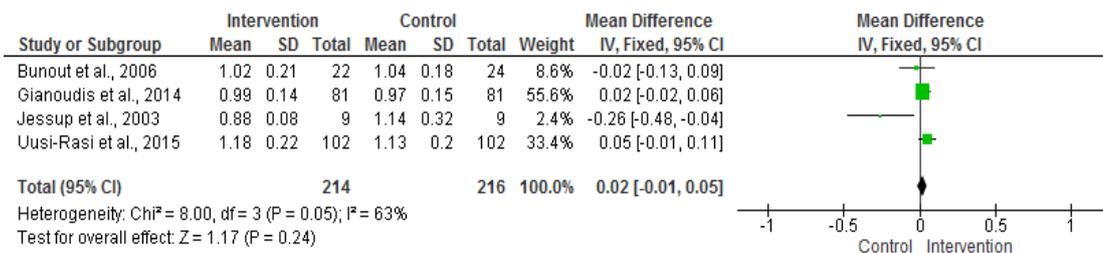


Figure 2.15: Group 2 analysis of BMD of the spine

2.4.9. Qualitative synthesis

Referring to the narrative synthesis guidelines provided by the Cochrane Consumers and Communication Review Group (Ryan.R, 2013), it was appropriate to apply 2 steps listed; developing a preliminary synthesis and exploring the relationships within and between studies. To develop a primary synthesis, results were systematically tabulated to identify patterns across studies (Appendices A-C). Exploring the relationships between and within studies for group 1, the control group in study (Agergaard et al., 2015b) demonstrated a significant percentage increase in CSA of the quadriceps from baseline in comparison to the intervention group (+8.46% versus +4.94%, $p < 0.05$).

Comparing primary outcomes for group 2, the percentage increase in isometric knee extensor strength for study (Verschueren et al., 2011) was greater in the intervention group (+3.01% versus +0.11%), although not statistically significant. Muscle power was compared in studies (Drey et al., 2011) and (Gianoudis et al., 2014), expressed as sit-to-stand transfer power and functional stair climbing muscle power respectively. Both studies reported a significant percentage increase in muscle power within the intervention groups, and smaller, non-significant increases within the control groups (sit-to-stand transfer power intervention group +8.00% versus +2.61%, $p = 0.017$; functional stair climbing muscle power intervention group +10.51% versus +7.32%, $p < 0.05$).

The 30 second sit-to-stand test showed significant favourable results for the combined intervention of exercise and vitamin D3 (+10.40% versus +6.20%, $p < 0.05$). Within study (Uusi-Rasi et al., 2015), normal walking speed and the 5-time chair stand time deteriorated non-significantly in both groups. The 12-minute walk test in

study (Bunout et al., 2006b) was further improved within the control group, although this did not achieve statistical significance. The four square step test, body sway and backwards walking were significantly more improved in the intervention groups. Only Romberg ratio showed the greatest improvement within the control group; Romberg ratio was decreased in comparison with the intervention group, although the results were non-significant (+2.8% versus -0.60%).

For group 2 secondary outcomes, small and non-significant gains in appendicular lean mass were demonstrated in the intervention group of study (Drey et al., 2011). In study (Verschueren et al., 2011), muscle mass of the upper limb decreased non-significantly in both the intervention and control groups, although to a lesser extent in the intervention group. BMD of the femoral neck was gained in both groups, although by a higher percentage in the control group; both trends were non-significant.

In summary, meta-analyses for group 1 found muscle strength of the lower limb to be significantly improved within the intervention group (0.98, 95% CI 0.73, 1.24, $p < 0.001$). All other outcomes showed small but non-significant positive effects for the intervention group. The SPPB, TUG, muscle strength of the lower limb and femoral neck BMD all showed significantly greater improvements in the intervention group for group 2 comparisons.

The narrative analysis revealed significant differences in body composition, muscle power, muscle function and balance. A significant percentage increase in quadriceps CSA was observed in the control group of study (Agergaard et al., 2015b). The combined intervention of RET and vitamin D3 supplementation resulted in a greater percentage increase in muscle strength and power, and a greater improvement in the 30 second sit-to-stand test, the four-square step test, body sway and backwards

walking. However, vitamin D3 supplementation alone resulted in a greater improvement in the 12-minute walk test and Romberg ratio.

2.5. Discussion

The aim of this systematic review was to assess the combined effect of RET and vitamin D3 supplementation on musculoskeletal health in older adults. Only 7 studies were eligible for inclusion, with a total of 792 participants, highlighting the lack of available literature on the topic. Studies were categorised into 2 groups; studies in which all participants took part in RET and the intervention group was supplemented with vitamin D3, or studies in which all participants were supplemented with vitamin D3 and the intervention group took part in RET. 2 studies were categorized into both group 1 and group 2.

2.5.1. Quantitative analysis

Data analysis conducted for this review included meta-analyses and narrative reviews. Meta-analyses for group 1 included muscle strength of the lower limb, TUG and BMD of both the femoral neck and spine. Evidence of additional benefit was shown for all outcomes within the intervention group; however, the effect size was small and non-significant for TUG and BMD of the femoral neck and spine. Muscle strength of the lower limb was the only significant outcome of group 1, with a large effect size observed within the intervention group (0.98, 95% CI 0.73, 1.24.

$p < 0.00001$). Although numerous studies have demonstrated the beneficial effect of RET on muscle strength in older adults (Henwood and Taaffe, 2005, Kosek et al., 2006, Stewart et al., 2014), this result provides evidence that vitamin D3

supplementation may enhance these effects in older adults. Skeletal muscle myopathies associated with vitamin D deficiency are well documented (Smith and Stern, 1967), and symptoms of significant muscle weakness are reversed with treatment of the deficiency (Glerup et al., 2000c). A systematic review and meta-analysis reported a gain in lower extremity strength with vitamin D supplementation only in vitamin D deficient older adults; no effect was observed in replete adults (Stockton et al., 2011). Similarly, no effect of vitamin D₃ supplementation on isometric quadriceps strength was demonstrated after 6 months in vitamin D replete older adults (Grady et al., 1991). Interestingly, although the studies included within group 1 (Agergaard et al., 2015b, Bunout et al., 2006b, Uusi-Rasi et al., 2015) did not specify serum 25(OH)D concentration as inclusion/exclusion criteria, baseline and post-intervention serum 25(OH)D were within the 'sufficient' range (>30 nmol.L⁻¹). A greater increase of muscle strength in replete older adults represents a novel finding of this review. Preliminary support for combined vitamin D supplementation and RET was demonstrated in a 3-month longitudinal study examining the effect of serum 25(OH)D and exercise training on functional performance in older men and women aged 65 years and over. No significant improvements in function were reported in participants with lower serum 25(OH)D (<47.5 nmol.L⁻¹), however higher serum 25(OH)D (>67.5 nmol.L⁻¹) was associated with greatest improvements in functionality and muscle strength (Okuno et al., 2010a).

This finding must be considered within the context of the risk of bias and GRADE analyses. The risk of bias analysis showed an overall unclear risk of bias for the included studies, and the GRADE analysis concluded that the evidence was of moderate quality; however, serious inconsistency due to moderate heterogeneity ($I^2 = 70\%$) was detected. This heterogeneity may have been due to the differing duration

of interventions (12 weeks to 24 months), differences between measurement methodologies, differences between exercise regimens (although all adopted progressive RET), doses of vitamin D3 (400 IU to 1920 IU per day), or may indicate that these studies were unsuitable for comparison.

Significant effects for the SPPB, TUG, muscle strength of the lower limb, and the BMD of the femoral neck were observed within the intervention groups of group 2 studies; unsurprisingly, RET was found to have a positive influence. In a recent systematic review and meta-analysis, exercise significantly increased SPPB score and decreased TUG time, with large effect sizes (1.87 and -2.47 respectively (Giné-Garriga et al., 2014b)); similar results are reported within this review. Vitamin D is a regulator of BMD, proliferating calcium and phosphate absorption in the intestine and acting directly on bone cells (Lips et al., 2006). Vitamin D has previously been shown to influence BMD, fracture rate and risk (Brinca et al., 2015); studies of patients who have sustained a hip fracture typically demonstrated low serum vitamin D (≤ 30.0 nmol.L⁻¹; (LeBoff et al., 1999)). Supplementation of vitamin D and calcium has been shown to significantly decrease the rate of bone loss in the hip and spine (Tang et al., 2007). GRADE analyses for these outcomes concluded the quality of evidence to be high (SPPB and TUG) or moderate (muscle strength of the lower limb and BMD of the femoral neck).

Closer examination of the control groups within significant outcomes for group 2 was undertaken to evaluate the effect of vitamin D3 supplementation alone. Intriguingly, although the intervention groups (RET and vitamin D3 supplementation) showed evidence of benefit in number of outcomes, the control groups (vitamin D3 supplementation alone) showed mixed, or even negative impacts on the same

outcomes. SPPB score was decreased post-intervention compared with baseline by 0.30% and 0.50% in the control groups of studies (Bunout et al., 2006b) and (Drey et al., 2011) respectively. Muscle strength of the lower limb and BMD of the femoral neck showed mixed results for the intervention groups, with some studies reporting small increases and others reporting small losses (non-significant). Previous reports of the effect of vitamin D supplementation on muscle strength and physical functioning are mixed; the InCHIANTI study of people aged 65 years or over reported a significant association between serum 25(OH)D $<25 \text{ nmol.L}^{-1}$ and SPPB score (Houston et al., 2007a). Similarly, a large prospective cohort of older adults aged 65 years or over found those with low ($<25 \text{ nmol.L}^{-1}$) 25(OH)D were significantly more likely to experience losses in grip strength and higher rates of appendicular lean mass loss compared to those with higher ($>50 \text{ nmol.L}^{-1}$) 25(OH)D (Visser et al., 2003a). Conversely, another large, prospective study found no association between serum 25(OH)D, walking speed and time for repeated chair stands (Verreault et al., 2002). The TUG test time was actually significantly increased within the control group of study (Bunout et al., 2006b), and increased by a smaller, non-significant amount in study (Uusi-Rasi et al., 2015). Again, participants included in studies (Bunout et al., 2006b) and (Uusi-Rasi et al., 2015) had sufficient serum 25(OH)D levels, indicating that supplementation in replete older adults may not confer additional benefits to neuromuscular function unless combined with exercise.

2.5.2. Narrative analysis

Studies in group 1 (Agergaard et al., 2015b, Bunout et al., 2006b, Uusi-Rasi et al., 2015) had few body composition outcomes in common, therefore a narrative analysis was conducted. The CSA of the quadriceps was analysed within study (Agergaard et

al., 2015b), and results showed that although the intervention group did experience a +4.94% increase from baseline, the control group (not supplemented with vitamin D3) actually showed a significantly higher increase in quadriceps CSA (+8.46%, $p < 0.05$).

These results do not provide evidence for the additive effects of combined exercise training and vitamin D3. Other study groups have reported changes in muscle CSA consequent to RET which are both smaller (Häkkinen et al., 1998, Trappe et al., 2002) and comparable (Ferri et al., 2003) to those reported in study (Agergaard et al., 2015b). Interestingly, a study (Agergaard et al., 2015b) also assessed “muscle quality” (muscle strength/CSA); although non-significant, the intervention group improved their muscle quality to a greater degree than the control group (+9.61% versus +0.66% change from baseline). The intervention and control groups both increased their muscle strength to a similar degree, and there was no significant difference between these changes; however, the control group (as previously mentioned) demonstrated a larger increase in their muscle CSA. This shows that the gains in muscle strength in the intervention group surpassed the improvements made in muscle CSA, indicative of an increased functionality of the muscle to produce force; conceptually more relevant in combatting the effects of sarcopenia than muscle size and strength alone (Fragala et al., 2015).

Results of the narrative analysis for group 2 showed that the combined intervention of RET and vitamin D3 supplementation was significantly more beneficial than vitamin D3 supplementation alone for sit-to-stand transfer power, functional stair climbing muscle power, 30 second sit-to-stand, 5-time chair stand, the four square step test, body sway and backwards walking. Only body sway was negatively

affected by vitamin D3 supplementation, although the within group change was non-significant. Other outcomes of interest included normal walking speed, which deteriorated in both groups, the distance walked in 12 minutes and Romberg ratio, in which the control groups made the most improvement, although not significantly.

2.5.3. Limitations

Few published studies were eligible for inclusion within this review, although this serves to highlight the knowledge gap with respect to this topic. The inclusion of a high risk study was deemed necessary due to the lack of available literature, although this had a negative effect on the perceived quality of evidence for the outcomes in which it was reported. Generally, outcome measure data could be graded as representing moderate quality, although there were several outcome measures graded as low or very low quality, due to the high variability of participant numbers, duration of interventions, exercise methodologies or differing vitamin D3 doses and period of supplementation employed within the studies. Furthermore, data produced from meta-analyses including study (Uusi-Rasi et al., 2015) may have been skewed due to the high weighting assigned for this study as a result of the large number of participants recruited.

Of the individual studies included within this review, none reported inclusion/exclusion criterion for vitamin D status, and although at baseline serum vitamin D was not significantly different between the groups in 5 studies (Uusi-Rasi et al., 2015, Agergaard et al., 2015b, Bunout et al., 2006b, Drey et al., 2011, Verschueren et al., 2011), 2 studies reported no data for serum vitamin D pre or post-intervention (Gianoudis et al., 2014, Jessup et al., 2003). Additionally, analysis methods used within 5 studies included did not account for confounding factors

(Agergaard et al., 2015b, Bunout et al., 2006b, Drey et al., 2011, Gianoudis et al., 2014, Verschueren et al., 2011), and participants were not stratified on the basis of any characteristics in 3 studies (Agergaard et al., 2015b, Jessup et al., 2003, Uusi-Rasi et al., 2015), although these were single-sex studies. Unfortunately, several outcome measures were unsuitable for inclusion within the qualitative analysis due to differing measurement methodologies utilised or too few outcome measures in common.

2.6. Conclusion

This review provides tentative support for the additive effect of combined RET and vitamin D3 supplementation for the improvement of muscle strength in older adults. For other aspects of musculoskeletal function, such as SPPB and TUG, no additional benefit beyond that gained from exercise training was found. This review showed no evidence of benefit of vitamin D3 supplementation alone, however, few studies were identified during the literature search, highlighting that further evidence is required to draw any firm conclusions or make explicit recommendations regarding vitamin D3 supplementation for musculoskeletal health and function in older adults

2.7. 2019 Update

The systematic review search strategy was re-applied to electronic databases on 02/09/19; the search identified several studies of interest, the majority of these studies included participants <65 years of age (Carrillo et al., 2013, Ebid et al., 2017, Jung et al., 2018, Lithgow et al., 2018) and one study lacked a true control group (Mieszkowski et al., 2018), therefore making them ineligible for inclusion. However, one study would be eligible for inclusion (Aoki et al., 2018).

Only one study reported an association between vitamin D3 supplementation and muscle mass; 4000 IU/d vitamin D3 significantly increased total muscle mass and leg lean mass in comparison to 800 IU/d vitamin D3 supplementation and exercise training in older adults who were vitamin D insufficient at baseline (Mieszkowski et al., 2018). Finally, vitamin D3 supplementation significantly improved postural and anteroposterior steadiness (Aoki et al., 2018) in comparison to exercise training or vitamin D3 supplementation alone.

Table 2.6: Characteristics and results of ineligible and relevant studies to the systemic review 2019 update, continued

	Baseline characteristics					Groups		Results and comment
	N	Mean age (y)	Sex (% female)	25(OH)D (nmol/L)	Study period	Intervention	Control	
Mieszkowski et al, 2018	42	69.0	100	47.6	Oct-Jan	1) 4000 IU D3/d + high-intensity Nordic walking (high-dose) 2) 4000 IU D3/d + moderate-intensity Nordic walking (high-dose) 3) 800 IU D3/d + high-intensity Nordic walking (low-dose) 4) 800 IU D3/d + moderate-intensity Nordic walking (low-dose) All for 2 hours per session, 3x/wk for 12 wks		- Skeletal muscle mass (+4% vs +2%) and leg lean mass (+4% vs +0%) significantly increased in the high-dose vitamin D groups in comparison to the low-dose groups. - There was a significant effect of exercise intensity (MI-NW) and vitamin D dose (high-dose) on peak knee torque flexion ($p = 0.0327$). - Elbow extension ($p = 0.0001$) and flexion ($p = 0.0231$) significantly increased by 11% in the HI-NW group regardless of vitamin D dose.
Aoki et al, 2018	148	70.5	78.5	29.0	Nov '14 – March '16	1) Exercise + 1000 IU D3/d for 24wks 2) Exercise alone for 24 wks 3) 1000 IU D3/d for 24 wks Exercise was single-leg stand (3 x/d for 1 minute) and single-leg squat (18 reps) daily		- Lower limb muscle mass (bioelectrical impedance), knee extensor and flexion strength (dynamometer) and muscle power (sit-to-stand test) increased significantly in all groups with no between-group differences. - Postural steadiness (single-leg stance) was significantly improved only in the exercise and vitamin D group. - Anteroposterior stability (functional reach test) improved significantly only in the vitamin D group

IU: International units; RET: Resistance exercise training; Wk: week; d: day; DXA: Dual-energy X-ray absorptiometry; 1-RM: 1 repetition maximum

CHAPTER 3:

IS SERUM 25(OH)D CONCENTRATION ASSOCIATED WITH LEAN MASS AND STRENGTH AND IS SARCOPENIC STATUS SEASONAL-DEPENDENT IN SUBGROUP OF POSTMENOPAUSAL WOMEN FROM THE D-FINES COHORT?

3.1. ABSTRACT

Introduction

Vitamin D deficiency is known to be associated with myopathy and myalgia. Older adults are at an increased risk for both vitamin D deficiency and sarcopenia (loss of muscle mass and function). The role of vitamin D in regulating muscle mass and strength is not well established, with previous studies reporting conflicting and inconclusive results. Since 25(OH)D status is known to be season-dependent, there is a rationale to suggest that aspects of muscle health, including sarcopenia, may also vary by season. The aim of the current study was to assess the relationship between serum 25(OH)D concentration, lean mass and muscle strength. The secondary aim was to establish whether sarcopenic status was season-dependent.

Methods

This was a secondary data analysis of a subgroup of 188 postmenopausal women participating in the 2006-2007 D-FINES (Vitamin D, Food Intake, Nutrition and Exposure to Sunlight in Southern England) study. The cohort was assessed as a whole group and 2 subgroups; postmenopausal women <65 years (n = 139) and postmenopausal women ≥ 65 years (n = 49). Outcome measures included lean mass (DXA), muscle strength (handgrip dynamometry) and serum 25(OH)D concentration (enzymeimmunoassay). Derived outcomes included appendicular skeletal muscle mass and appendicular skeletal muscle index. Sarcopenic status was assessed using the European Working Group on Sarcopenia in Older People 2018 criteria.

Non-parametric partial correlation using BMI as a covariate was used to evaluate the study aims.

Results

There was no association between total lean mass and ASM in any group at any seasonal timepoint. Following adjustment for BMI, relative appendicular skeletal muscle index was positively and significantly associated with 25(OH)D concentration for the whole cohort in autumn ($r_s = 0.253$, 95% CI 0.08, 0.42), $p = 0.002$), however, this relationship was not observed in either subgroup nor in the spring months. A small but significant negative relationship between seasonal change in total lean mass and change in 25(OH)D ($r_s = -0.211$, 95% CI -0.39, -0.03), $p = 0.018$) was observed for the whole cohort. Muscle strength was positively associated with 25(OH)D concentration at all seasonal timepoints for all groups, with significance observed during the summer and autumn months for the whole cohort and postmenopausal women <65 years after adjustment for seasonal BMI. Sarcopenia prevalence was highest in women ≥ 65 years and varied by season in both postmenopausal groups. Serum 25(OH)D concentration was lower in sarcopenic women.

Conclusions

Sarcopenic status appeared transient and season-dependent, with the highest percentage of sarcopenic women observed in the spring. As the sample of sarcopenic participants was small, further research investigating year-round sarcopenic status is recommended.

3.2. INTRODUCTION

Vitamin D deficiency is highly prevalent and has been recently described as a “global pandemic” (Pludowski et al., 2018), with older adults being a group particularly at risk (van Schoor and Lips, 2018) due to factors including a decreased cutaneous capacity to synthesise vitamin D (MacLaughlin and Holick, 1985) and limited sun exposure due to physical inactivity and reduced time spent outdoors (Kühn et al., 2018). Additionally, it is well established that serum 25(OH)D concentration is known to vary by season (Christensen et al., 2010, Bird et al., 2012, Darling et al., 2014a, Elizondo-Montemayor et al., 2017), although it has been suggested that this variation is minimal (Lagunova et al., 2009) or not present (Pourhassan and Wirth, 2018, Vallejo et al., 2018) in older adults.

There is evidence for a plausible link between vitamin D and skeletal muscle function; the discovery of the vitamin D receptor (VDR) and the reduction in its expression in skeletal muscle tissue with age suggests a direct effect of vitamin D on muscle (Bischoff-Ferrari et al., 2004). In an animal study, murine C2C12 cells treated with 25(OH)D and 1,25(OH)₂D exhibited an increase in VDR and CYP24AA1 expression and a downregulation of the skeletal muscle growth and development inhibitor myostatin, providing support for the theory that vitamin D has a direct effect on proliferation, differentiation and a potential anabolic effect on myotube size (Girgis et al., 2014). Additionally, case-report and observational studies have shown that vitamin D deficiency is associated with myopathy and myalgia (Ziambaras and Dagogo-Jack, 1997) which are reversible upon correction of the deficiency (Ziambaras and Dagogo-Jack, 1997, Glerup et al., 2000a).

Age related losses in muscle mass, strength and function (sarcopenia) (Cruz-Jentoft et al., 2018) are associated with poor quality of life (Beaudart et al., 2015a) and are prevalent in fallers and those at risk of falling (Barnes et al., 2018). A recent study concluded that individuals with a combination of low muscle mass and function were predicted to be 12.28 times (95% CI: 7.95,18.96) more likely to lose their independence at 90 years of age (dos Santos et al., 2017). Although vitamin D may play a role in the regulation of muscle mass and/or muscle strength, this relationship is not currently well understood, and the role of serum 25(OH)D concentration in relation to muscle mass and strength in adults remains inconclusive. While some studies report a positive association between 25(OH)D and muscle mass (Szulc et al., 2004, Tieland et al., 2013, Ito et al., 2014) and strength (Visser et al., 2003b, Gerdhem et al., 2005, Mastaglia et al., 2011b, Grimaldi et al., 2013, Beaudart et al., 2014a, Carson et al., 2018), others report no effect on muscle mass (Marantes et al., 2011, Beaudart et al., 2014a, Carson et al., 2018) or strength (Marantes et al., 2011). Additionally, to the best of our knowledge, there has been no investigation to date to determine if sarcopenia status is season-dependent; should muscle mass and strength, components of sarcopenia classification, be associated with serum 25(OH)D concentration, which are known to be affected by season (Darling et al., 2013, Serdar et al., 2017, Klingberg et al., 2015), it is plausible to suggest that sarcopenic status, calculated at different seasonal timepoints throughout the year, may vary. This may have important implications for the treatment and diagnosis of sarcopenia.

Therefore, the primary aim of the present study was to cross-sectionally analyse data obtained from a large cohort of postmenopausal women and investigate associations between serum 25(OH)D concentration and lean mass and strength and whether

these associations are influenced by season. The secondary aim was to investigate whether there are seasonal differences in sarcopenia status.

3.3. METHODS

3.3.1. Participants

Data were obtained and analysed from a total of $n = 188$ postmenopausal women, comprising $n = 139$ postmenopausal women <65 years and $n = 49$ women ≥ 65 years who participated in the 2006-2007 D-FINES, a single-centre cohort study investigating the vitamin D status and bone health of pre- and postmenopausal Caucasian and Asian women (Vitamin D, Food Intake, Nutrition and Exposure to Sunlight in Southern England) study (Darling et al., 2013). The study was conducted in accordance with the 1964 Declaration of Helsinki and received ethical approval from the relevant Research Ethics Committees (National Health Service NHS REC 06/Q1909/1 and University of Surrey EC/2006/19/SBMS). Written informed consent was obtained from all participants.

Recruitment and data collection methodologies are presented in detail elsewhere (Darling et al., 2013). Briefly, the D-FINES study recruited 365 women from General Practitioner (GP) surgeries in the southeast of England (Surrey, Hampshire, Berkshire and outer London). Exclusion criteria included any conditions resulting in a disorder of calcium homeostasis, currently taking medications affecting bone, calcium or vitamin D metabolism, supplemental use of vitamin D or cod liver oil and abnormal liver, thyroid or renal function (assessed via blood test) or use of hormone replacement therapy within the previous year. Participants were invited to attend the University of Surrey Clinical Investigation Unit on 4 separate occasions; during

the summer, autumn and winter of 2006 and in the spring of 2007. Summer was defined as falling between the dates of 21/06/2006 and 20/09/2006, autumn between the dates of 21/09/2006 and 20/12/2006, winter as falling between the dates of 21/12/2006 and 20/03/2007 and spring as falling between the dates of 21/04/2007 and 20/06/2007. Postmenopausal status was defined as not menstruating for over 3 months. During each of these visits, anthropometric data including height, weight and grip strength were collected, along with venepuncture to measure serum 25(OH)D concentration. Additionally, during the autumn of 2006 and the spring of 2007 visits, body composition was estimated using Dual X-ray Absorptiometry (DXA).

3.3.2. Serum 25(OH)D concentration measurement

Fasting venous blood samples were taken from consenting participants during each of the 4 seasonal assessment points by phlebotomists at the Clinical Investigation Unit at the University of Surrey. Samples were analysed by the Vitamin D Research Group laboratory based at the Manchester Royal Infirmary, which is certified by the International Organization for Standardization (ISO 9001:2000 and ISO 13485:2003) and participates in the Vitamin D quality assurance scheme (DEQAS). Serum 25(OH)D concentration was measured using a manual enzymeimmunoassay (Immunodiagnostic Systems Ltd, Boldon, Tyne and Wear, UK) following manufacturer guidelines. Manufacturer reference ranges for 25(OH)D were 19-58 ng/mL (48-144 nmol/L, with some seasonal variability), sensitivity was 2 ng/ml (5 nmol/L), intra- and inter-assay coefficients of variation were 6% and 7%, respectively. Although seasonal variation in serum 25(OH)D concentration has previously been reported within the D-FINES cohort (Darling et al., 2013, Darling et al., 2014b), subgroups of postmenopausal women younger and older than 65 years have not

previously been studied. The D-FINES cohort and its longitudinal nature provided a unique opportunity to explore seasonal changes in both muscle function and serum 25(OH)D and whether they were associated.

3.3.3. Muscle strength measurement

Handgrip strength was measured at all 4 time points (autumn, winter, spring and summer) using a JAMAR® hydraulic hand dynamometer (J. A. Preston Corporation, Clifton, NJ, USA), which has previously been shown to demonstrate excellent reliability for isometric grip strength (ICC = 0.947) (Hogrel, 2015). Measurements were taken while the participant was seated, forearms resting on the arms of the chair with their wrists just over the end of the chair's arm and their feet flat on the floor. Comfort was assessed and the size of the handle adjusted if necessary. Mean maximal grip strength, expressed in kilograms, was calculated from a set of 3 measurements on each hand (left and right) from which a mean value for each hand was calculated.

3.3.4. Lean mass assessment

Total body lean mass was assessed at the Guildford Nuffield Hospital via Dual X-ray Absorptiometry (DXA) using a Hologic QDR 4500 (Hologic Inc, USA) machine in autumn 2006 and spring 2007. Mean difference in lean mass estimates between operators of +0.58 (0.47) kg and an interrater coefficient of variation of 1.9% have been reported previously in 22 human cadavers (22% female, mean age = 79.6 years, mean weight = 69.59 kg), repositioned and scanned 3 times (Scafoglieri et al., 2011). DXA, computerised tomography (Levine et al., 2000) and magnetic resonance imaging (Maden-Wilkinson et al., 2013) estimates of lean mass correlate highly. DXA is considered a reference standard for lean mass estimation (Buckinx et al., 2018b)

and is recommended by the European Working Group on Sarcopenia in Older People, 2018 (EWGSOP2) (Cruz-Jentoft et al., 2018).

3.3.5. Derived muscle outcomes

Appendicular skeletal muscle mass (ASM) was calculated as the sum of the total lean mass of the arms and legs in kilograms. ASM was divided by height squared (relative appendicular skeletal muscle index); this variable is suggested by multiple consensus groups as a method to quantify relative muscle mass by controlling for body size (Cruz-Jentoft et al., 2010a, Fielding et al., 2011, Chen et al., 2014, Cruz-Jentoft et al., 2018). Sarcopenia was defined according to the EWGSOP2 2018 criteria for women (Cruz-Jentoft et al., 2018); grip strength of the right hand <16kg and low muscle mass, categorised as ASM <15kg, and sarcopenic status were assessed in autumn and spring. Sarcopenic status and subsequent analyses involving sarcopenic participants were calculated only for participants with available ASM and grip strength data for both the autumn and spring timepoints to accurately reflect any seasonal changes in status.

3.3.6. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0 with statistical significance set at an α -level of $p = 0.05$. Normality checks revealed significant deviation from the normal distribution for some outcomes and therefore log transformation was attempted. With log transformation unable to normalise all outcome variables, all data were maintained in their original non-transformed form.

The relationship between seasonal total lean mass and ASM and 25(OH)D concentration was assessed using Spearman correlation. The Spearman's Rho

correlation coefficient was interpreted in the following way; <0.4 as weak, 0.4 – 0.69 as moderate and 0.7 to 1.0 as strong (Schober et al., 2018).

Non-parametric partial correlation using BMI as a covariate (confirmed before analyses) was used to assess cross-sectional associations between serum 25(OH)D concentration and relative appendicular skeletal muscle index (since both ASM and BMI variables account for height) and muscle strength outcomes throughout the seasons. BMI has previously been utilised as a covariate in similar studies due to the known influence of BMI/body size on serum 25(OH)D concentration and strength (Bean et al., 2003, Visser et al., 2003b, Cruz-Jentoft et al., 2018). Differences between sarcopenic and non-sarcopenic participants were assessed using the Mann-Whitney U test. Chi-squared tests were used to analyse differences between groups in categorical variables. The cohort of postmenopausal women was analysed as three groups: the whole group plus two sub-groups; postmenopausal women <65 years of age and postmenopausal women ≥65 years of age, as age is a major risk factor for sarcopenia (Tournadre et al., 2019) and contributes to aspects of its aetiology, such as declining anabolic hormone concentration (Brook et al., 2016), increasing physical inactivity (Sparling et al., 2015) and malnutrition (Landi et al., 2018).

3.4. RESULTS

3.4.1. Participant characteristics

Data from 188 postmenopausal women were analysed, including 139 postmenopausal women aged <65 years (mean age 58.68 (standard deviation (SD) 3.23 years; age range = 48 to 64 years) and 49 postmenopausal women aged ≥65

years (mean age 76.59 (2.51) years; age range = 65 to 79); participant characteristics are shown in Table 3.1. Mean serum 25(OH)D concentration and percentage of participants considered vitamin D adequate, insufficient and deficient for all groups by season are presented in Table 3.2. Briefly, mean serum 25(OH)D concentration ranged from 38.79 – 60.22 nmol/L and the percentage of the cohort considered to be vitamin D deficient ranged from 10.0 – 35.6%, dependent on season; as expected, serum 25(OH)D concentration was highest in the summer and thus this was the season with the lowest percentage of participants considered to be vitamin D deficient. Surprisingly, the group of postmenopausal women < 65 years exhibited the lowest mean serum 25(OH)D concentration (in the winter) and the group of postmenopausal women > 65 years exhibited the highest mean serum 25(OH)D concentration (in the summer).

On average, the cohort of women were overweight based on BMI (25.0 to 29.9 kg.m⁻²) and there were no significant differences in height, weight, BMI, total lean mass and seasonal serum 25(OH)D concentration between postmenopausal women <65 years and postmenopausal women aged ≥65 years. Postmenopausal women aged ≥65 years exhibited significantly lower mean grip strength at all seasonal time points compared with postmenopausal women aged <65 years; this difference was most marked during the winter period (21.80 (5.20) kg vs 18.90 (4.73) kg, $p = 0.001$), during which the grip strength was 15.34% lower in postmenopausal women aged ≥65 years.

Table 3.1: D-FINES study participant characteristics							
	All postmenopausal women		Postmenopausal women aged <65 years		Postmenopausal women aged ≥65 years		P
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	
Age (years)	188	61.00 (4.97)	139	58.68 (3.23)	49	76.59 (2.51)	<0.001
Height (cm)	173	160.77 (6.56)	130	160.86 (6.89)	43	160.50 (5.52)	0.731
Ethnicity	188	77% white, 19% South Asian, 4% other	134	74% white, 21% South Asian, 5% other	49	84% white, 9% South Asian, 2% other	0.088
Autumn weight (kg)	173	69.80 (12.94)	130	70.47 (13.71)	43	67.77 (10.16)	0.236
Autumn BMI (kg.m⁻²)	173	27.10 (5.35)	130	27.34 (5.66)	43	26.37 (4.23)	0.306
Autumn total lean mass (kg)	171	39.78 (5.19)	129	39.93(5.39)	42	39.33 (4.52)	0.512
Spring weight (kg)	166	70.21 (13.12)	125	70.77 (14.04)	41	68.51 (9.74)	0.341
Spring BMI (kg.m⁻²)	166	27.30 (5.50)	125	27.53 (5.89)	41	26.59 (4.06)	0.341
Spring total lean mass (kg)	166	40.12 (5.29)	125	40.24 (5.56)	41	39.75 (4.39)	0.609
Summer HGS (kg)	179	20.90 (5.27)	134	21.38 (5.38)	45	19.45 (4.69)	0.033
Autumn HGS (kg)	174	21.18 (4.90)	130	21.84 (4.86)	44	19.25 (4.54)	0.002
Winter HGS (kg)	168	21.03 (5.23)	124	21.80 (5.20)	44	18.90 (4.73)	0.001
Spring HGS (kg)	169	20.95 (5.15)	126	21.50 (5.33)	43	19.34 (4.26)	0.017

BMI: Body mass index; HGS: Handgrip strength
p relates to postmenopausal women aged <65 years and postmenopausal women aged ≥65 years between-group difference

Table 3.2: Seasonal 25(OH)D concentrations and corresponding vitamin D status of all postmenopausal women and women <65 years and ≥65 years subgroup

	All postmenopausal women					Postmenopausal women aged <65 years					Postmenopausal women aged ≥65 years				
	N	Mean 25(OH)D (SD)	Adequate (%)	Insufficient (%)	Deficient (%)	N	Mean 25(OH)D (SD)	Adequate (%)	Insufficient (%)	Deficient (%)	N	Mean 25(OH)D (SD)	Adequate (%)	Insufficient (%)	Deficient (%)
Summer	176	56.29 (23.03)	60.8	23.9	15.3	128	54.81 (22.36)	60.15	21.88	17.97	48	60.22 (24.56)	62.5	27.1	10.4
Autumn	162	50.40 (21.65)	26.8	37.6	35.6	122	49.75 (21.61)	48.36	29.51	22.13	40	52.38 (21.94)	50.0	40.0	10.0
Winter	149	38.79 (16.98)	26.8	37.6	35.6	112	37.84 (16.95)	25.00	37.50	37.50	37	41.68 (16.97)	32.4	43.2	24.3
Spring	147	40.64 (19.22)	27.2	41.5	31.1	113	40.44 (19.74)	29.20	38.05	32.75	34	41.31 (17.62)	20.6	52.9	26.5

Vitamin D adequacy defined as serum 25(OH)D concentrations ≥50.0 nmol/L, inadequacy defined as 30.0 – 49.9 nmol/L and deficiency defines as ≤29.9 nmol/L

3.4.2. Serum 25(OH)D concentration and lean mass and strength

There were no statistically significant associations between 25(OH)D concentration and total lean mass or ASM during the autumn or spring in any of the 3 groups (Table 3.3). Relative appendicular skeletal muscle index was negatively associated with 25(OH)D concentration in all groups and at all timepoints, significantly so in postmenopausal women <65 years in the autumn ($r_s = -.221$. 95% CI -0.42, -0.04, $p = 0.018$) and spring ($r_s = -.218$ 95% CI -0.39, -0.05, $p = 0.026$). Following adjustment for corresponding seasonal BMI, significance was only observed in the whole cohort ($r_s = .253$. 95% CI 0.08, 0.42, $p = 0.002$). Change in serum 25(OH)D between autumn and spring was significantly and negatively associated with the change in total lean mass in the whole cohort, with an increase in lean mass associated with a decrease in 25(OH)D.

Handgrip strength was significantly associated with 25(OH)D concentration within the whole cohort and in postmenopausal women <65 years and in the summer within postmenopausal women ≥ 65 years. Following adjustment for corresponding seasonal BMI, muscle strength was significantly associated with 25(OH)D concentrations during the summer and autumn months for the whole cohort and the postmenopausal women <65 years, whilst a weak non-significant relationship was observed in the winter and spring months for these groups. Postmenopausal women ≥ 65 years demonstrated a positive association between 25(OH)D concentration and muscle strength at all seasonal timepoints, although statistical significance was not achieved.

Table 3.3: Associations between serum 25(OH)D concentration and lean mass and muscle strength									
	All postmenopausal women			Postmenopausal women aged <65 years			Postmenopausal women aged ≥65 years		
	N	r _s (95% CI)	p	N	r _s (95% CI)	p	N	r _s (95% CI)	p
<i>25(OH)D x Total lean mass Autumn</i>	154	.111 (-0.08, 0.28)	0.170	117	.115 (-0.06, 0.29)	0.219	37	.083 (-0.22, 0.38)	0.079
<i>25(OH)D x Total lean mass Spring</i>	140	.050 (-0.11, 0.22)	0.559	108	.040 (-0.14, 0.21)	0.684	32	.193 (-0.18, 0.49)	0.290
<i>25(OH)D x ASM Autumn</i>	148	.037 (-0.12, 0.19)	0.655	114	.032 (-0.13, 0.21)	0.731	34	.050 (-0.32, 0.42)	0.778
<i>25(OH)D x ASM Spring</i>	139	-.061 (0.09, -0.02)	0.474	108	-.073 (0.05, 0.35)	0.455	31	.049 (-0.32, 0.43)	0.491
<i>25(OH)D x relative appendicular skeletal muscle index Autumn*</i>	145	.253 (0.08, 0.42)	0.002	111	-.019 (-0.22, 0.19)	0.839	31	.263 (-0.11, 0.59)	0.139
<i>25(OH)D x relative appendicular skeletal muscle index Spring*</i>	134	.117 (-0.05, 0.29)	0.173	103	-.083 (0.28, 0.11)	0.402	28	-.145 (-0.61, 0.39)	0.443
<i>Change in total lean mass x change in serum 25(OH)D Autumn to Spring</i>	125	-.211 (-0.39, -0.03)	0.018	101	-.032 (-0.90, -0.04)	0.051	24	-.362 (-0.69, 0.07)	0.082
<i>25(OH)D x HGS Summer*</i>	164	.240 (0.10, 0.37)	0.002	120	.272 (0.12, 0.41)	0.002	40	.278 (-0.03, 0.52)	0.075
<i>25(OH)D x HGS Autumn*</i>	148	.182 (0.01, 0.35)	0.026	111	.226 (0.05, 0.39)	0.016	34	.128 (-0.25, 0.49)	0.458
<i>25(OH)D x HGS Winter*</i>	142	.157 (-0.01, 0.31)	0.059	105	.175 (-0.01, 0.35)	0.072	33	.196 (-0.15, 0.51)	0.259
<i>25(OH)D x HGS Spring*</i>	135	.116 (-0.01, 0.29)	0.176	103	.129 (-0.01, 0.33)	0.191	29	.065 (-0.35, 0.43)	0.726

*Model is adjusted for BMI; all other variables are unadjusted.
ASM: Appendicular skeletal muscle mass; Relative appendicular skeletal muscle index is ASM/h² HGS: Handgrip strength.
p relates to the Spearman Correlation analysis

3.4.3. Seasonal sarcopenia classification

Seasonal sarcopenia classification is shown in Table 3.4. Only the women with ASM and handgrip strength data available for both the autumn and spring timepoints were included in this analysis. The percentage of women classified as sarcopenic was higher in the spring when compared with autumn in all 3 groups. Sarcopenia status appeared transient for 5 women in total, which meant that their status may be season-dependent.

Table 3.4: Seasonal sarcopenia classification						
	N participants	N classified as sarcopenic in autumn (%)	N classified as sarcopenic in spring (%)	Sarcopenic in both autumn and spring	Sarcopenic only in autumn	Sarcopenic only in spring
All postmenopausal women	154	7 (4.55)	10 (6.49)	6	1	4
Postmenopausal women aged <65 years	118	6 (5.08)	7 (5.93)	5	1	2
Postmenopausal women aged ≥65 years	36	1 (2.78)	3 (8.33)	1	0	2
Sarcopenia classification based on the EWGSOP2 criteria of combined low muscle mass and strength, which for women is handgrip strength <16kg and ASM <15kg (Writing Group for the European Working Group on Sarcopenia in Older People 2 et al., 2018). Number of included participants are those with all required data for the autumn and spring time points						

3.5. DISCUSSION

The scale and longitudinal nature of the D-FINES study provided a unique opportunity to retrospectively assess aspects of skeletal muscle health and function and their association with serum 25(OH)D status. Serum 25(OH)D concentration followed the anticipated seasonally-dependent pattern, with peak concentration observed during the summer period and lowest concentration observed during the winter and spring months for both the whole cohort and the postmenopausal subgroups. Serum 25(OH)D concentration was higher within postmenopausal women ≥ 65 years compared with postmenopausal women < 65 years at all seasonal timepoints, although these differences were not statistically significant. One explanation for this finding is that the postmenopausal women < 65 years included a higher percentage of Asian women and a lower percentage of Caucasian women than the postmenopausal women ≥ 65 years (21% vs 9% and 74% vs 84%, respectively). As with premenopausal women in the D-FINES cohort (Darling et al., 2013), postmenopausal Asian women in the present study presented with significantly lower mean 25(OH)D concentration than postmenopausal Caucasian women and could be classified as vitamin D deficient at all seasonal time points (summer: 63.83 nmol/L vs 27.22 nmol/L, autumn: 57.51 nmol/L vs 22.06 nmol/L, winter: 43.35 nmol/L vs 22.06 nmol/L, spring: 45.43 nmol/L vs 23.11 nmol/L, all $p < 0.001$).

Overall, the cohort had sufficient serum 25(OH)D concentration during the summer and autumn months, but were classified as insufficient during the winter and spring months. Mean 25(OH)D concentration during the summer months was higher than

previously reported in community-dwelling older women in a UK population study; age-matched women participating in the Health Survey for England, 2000 presented with serum 25(OH)D concentration that were 17.97% lower than those observed in postmenopausal women ≥ 65 years within the D-FINES cohort, which did not vary seasonally (Hirani and Primatesta, 2005).

The prevalence of vitamin D deficiency, defined as serum 25(OH)D concentration < 30 nmol/L, was higher within this cohort than previously reported at all seasonal timepoints. A study of worldwide vitamin D status in adults aged 50 to 105 years found that 10% of adults in England had a serum 25(OH)D concentration of < 30 nmol/L (Palacios and Gonzalez, 2014). However, the percentage of postmenopausal women within the D-FINES cohort considered to be vitamin D insufficient (< 50 nmol/L) was lower than previously reported in the above studies (Hirani and Primatesta, 2005, Palacios and Gonzalez, 2014), meaning that women in the D-FINES cohort were more likely to be categorized into one of the 2 extremes of 25(OH)D concentrations (sufficiency or deficiency), again, perhaps due to the high prevalence of deficiency observed in the Asian participants.

Postmenopausal women ≥ 65 years were significantly weaker than postmenopausal women < 65 years at all seasonal timepoints, which is consistent with longitudinal reports of an age related decline in muscle strength (Hughes et al., 2001). Although women ≥ 65 years were 9.92% weaker than women < 65 years during the summer period, this difference is smaller than expected based on previously demonstrated losses of handgrip strength per decade of 16.67% in a cohort of 1314 men and 1315 women aged ≥ 20 years who participated in the North West Adelaide Health study (Massy-Westropp et al., 2011), particularly as the mean ages of the 2 postmenopausal groups were almost 2 decades apart (handgrip strength assessed in

both hand using a Jamar Analogue Hand Dynamometer from a seated position, with the elbow at the participant's side, flexed at 90°). More modest losses in grip strength have been reported in a recent Australian study of 378 health adults aged 20 to 95 years without musculoskeletal deficits; an average loss of 12.78% grip strength was observed between the age ranges of 40 – 59 years and 60 – 69 years and a loss of grip strength of 10.83% observed between 60 – 69 years and 70 – 79 years (Ingram et al., 2019).

Comparing muscle strength of the D-FINES cohort to British handgrip strength normative data taken from 130 men and 130 women (age range = 15 to 92 years), postmenopausal women <65 years were 28.16% weaker and the muscle strength of the postmenopausal women ≥65 years was comparable to women of a similar mean age during the summer months. Dominant hand handgrip strength during this study was assessed using a B+L hydraulic pinch gauge, with the participant seated and the arm in the 90° flexion position (Gilbertson and Barber-Lomax, 1994). Again, the handgrip strength of the Caucasian participants was significantly higher than observed in the Asian participants in both subgroup and at all seasonal timepoints with the exception of the summer months in postmenopausal women ≥65 years; the previous British study did not provide details of participant ethnicity, making a direct comparison difficult.

However, the muscle strength of both postmenopausal subgroups was below the average expected for age-matched women in a more recent study; handgrip strength data collated from 12 British population studies which enrolled a total of 49,964 participants (26,687 females) showed that the mean handgrip strength of the postmenopausal women <65 years fell between the 10th and 25th centiles and the postmenopausal women ≥65 years fell between the 25th and 50th centiles of the

corresponding age group normative data measuring dominant hand handgrip strength using a number of brands of dynamometer from a seated position (Dodds et al., 2014). Relating the D-FINES cohort to the handgrip strength reported in a further study, all 3 of the D-FINES groups (whole postmenopausal cohort and both postmenopausal subgroups) displayed grip strength values that were below the 10th centile of age-matched females in the previous Australian study measuring dominant hand grip strength with a Jamar+ Digital dynamometer from a seated position (arm at the participant's side, bent at 90°) (Ingram et al., 2019).

Neither the whole cohort nor the postmenopausal subgroups could be defined as having clinical muscle weakness at any seasonal timepoint, based on group mean values, as defined as handgrip strength <16kg (McLean et al., 2014, Cruz-Jentoft et al., 2018). However, further analysis of individual data revealed that 12.23% to 16.55% of postmenopausal women <65 years and 16.33% to 22.45% of postmenopausal women ≥65 years did have muscle weakness, which varied with season (lowest percentage in the summer and highest in the autumn). Observing individual participant data, postmenopausal women ≥65 years consistently demonstrated a higher percentage of participants with clinical muscle weakness, most apparent during the autumn months.

Neither the whole D-FINES cohort nor the 2 subgroups could be described as having low muscle quantity, based on group mean values, defined using either the Foundation for the National Institutes of Health (FNIH) (ASM <15.02 kg) (McLean et al., 2014) or the EWGSOP2 (ASM <15.00 kg) (Cruz-Jentoft et al., 2018) criteria. However, assessing individual participant data, 30.22% to 30.94% of postmenopausal women <65 years and 20.41% to 24.49% of postmenopausal women ≥65 years were found to have low muscle quantity, which varied seasonally,

with postmenopausal women <65 years demonstrating lower ASM compared with postmenopausal women ≥65 years, most apparent in the autumn.

There was no significant association between serum 25(OH)D concentration and total lean body mass or ASM in the autumn and spring in any of the groups. Although the unadjusted relative appendicular skeletal muscle index in the D-FINES cohort was significantly and negatively associated with serum 25(OH)D concentrations in the autumn and spring in postmenopausal women <65 years (Appendix D); following adjustment for corresponding seasonal BMI, this relationship was no longer significant, suggesting that the significance observed was due to variation in BMI in this group. After adjustment for corresponding seasonal BMI, only the relative appendicular skeletal muscle index of the whole cohort in the autumn was significantly associated with 25(OH)D concentration ($r_s = .253$, 95% CI 0.08, 0.42, $p = 0.002$). Serum 25(OH)D and muscle mass is a contentious subject, with no clear consensus on the role of vitamin D in muscle hypertrophy, although the findings here do not support previous findings of an association (Szulc et al., 2004, Tieland et al., 2013, Ito et al., 2014). Of these studies, one reported a significant association in men only (Szulc et al., 2004), one recruited only frail older adults (mean age = 79 years) based on the Fried criteria (Tieland et al., 2013) and the final study observed a significant relationship between serum 25(OH)D and skeletal muscle index only in participants with low muscle mass at baseline (average ASM at baseline could be classified as low muscle quantity by the FNIH and EWGSOP2 criteria listed above) (Ito et al., 2014).

The current finding of no association between appendicular lean mass and serum 25(OH)D concentration is in agreement with conclusions drawn from a previous study of 356 women (mean age = 57 years), in which no association was found between

any quartile of 25(OH)D concentration and ASM assessed via DXA ($p = 0.511$); this persisted after adjustment for age, height physical activity, season and fat mass ($p = 0.237$) (Marantes et al., 2011). However, these conclusions were drawn from a US population of 667 adults, including 356 women (mean age = 57 years); the main difference with the D-FINES cohort was that although reflective of the community enrolled, 98% of participants were Caucasian, meaning that black and Asian participants were substantially underrepresented (Marantes et al., 2011). A more recent study in 51 Northern Irish COPD patients (including 23 females, mean age = 68.7 years) with serum 25(OH)D concentrations comparable with the D-FINES cohort found no association between fat-free mass (assessed by bioelectrical impedance) and 25(OH)D concentrations during the winter ($R = 0.089$, $p = 0.588$) or summer ($R = 0.270$, $p = 0.092$) months (Carson et al., 2018).

Surprisingly, between the autumn and spring seasons a small but significant negative association between change in total lean mass and serum 25(OH)D concentration was observed within the whole cohort of postmenopausal women ($r_s = -.211$. 95% CI -0.39, -0.03), $p = 0.018$). Lean mass estimated in the spring was significantly higher than that estimated in the autumn ($Z = -3.25$, $p = 0.001$), with the reverse true for serum 25(OH)D concentration ($Z = -6.78$, $p < 0.001$). A longitudinal study of 125 healthy postmenopausal women (mean age = 62.5 years) found that total and regional lean body mass estimated by DXA varied seasonally, with a larger percentage change than observed within the D-FINES cohort; whole body lean mass was significantly lower in the period of December to June than throughout June to December (-2.04 (0.24)%, $p < 0.001$) of the previous year (Dawson-Hughes and Harris, 1992). A previous study has shown that serum 25(OH)D concentrations in women were significantly and negatively associated with ASM/height² in a group of

195 older women (mean age = 75 years) who were considered vitamin D sufficient (25(OH)D = 57.67 nmol/L) with adequate muscle quantity (ASM = 15.20 kg) (Iannuzzi-Sucich et al., 2002). Although total body lean mass has been shown previously to vary seasonally, it is also important to consider that the very small change in lean mass (+0.34 kg) for the D-FINES cohort was within the variability reported for the DXA model utilised (Scafoglieri et al., 2011), and therefore although statistically significant, may not be a reliably meaningful change.

In the initial, unadjusted analyses (Appendix D), muscle strength assessed via grip strength was significantly associated with serum 25(OH)D concentration at all seasonal timepoints for the whole cohort and postmenopausal women <65 years; significant associations were observed in the summer months for the postmenopausal women ≥65 years. After adjusting for the BMI of each corresponding season, the association between handgrip strength and seasonal 25(OH)D concentration remained significant for the summer ($r_s = .240$. 95% CI 0.01, 0.37, $p = 0.002$), autumn ($r_s = .182$. 95% CI 0.01, 0.35, $p = 0.026$) and borderline for the winter months ($r_s = .157$. 95% CI -0.01, 0.31, $p = 0.059$) in all postmenopausal women. For postmenopausal women <65 years, a significant association remained during the summer ($r_s = .272$. 95% CI 0.12, 0.41, $p = 0.002$) and autumn months ($r_s = .226$. 95% CI 0.05, 0.39, $p = 0.016$), however for postmenopausal women ≥65 years, no associations remained significant. Within the D-FINES cohort, BMI was negatively and significantly associated with both serum 25(OH)D concentration and handgrip strength at all seasonal timepoints, as has been demonstrated in previous studies (Parikh et al., 2004, Kull et al., 2009, Massy-Westropp et al., 2011). Controlling for BMI accounted for some of the variation in the range of observable BMIs (range in the whole cohort = 16.87 to 52.73 kg.m⁻² across the 4 seasons), therefore reducing

the strength of the relationship between BMI and muscle strength and resulting in some significant associations becoming non-significant.

The positive association observed between serum 25(OH)D concentration and muscle strength is consistent with a large evidence base of previous studies to the same effect (Visser et al., 2003b, Gerdhem et al., 2005, Mastaglia et al., 2011b, Grimaldi et al., 2013, Beaudart et al., 2014a, Carson et al., 2018). Muscle strength has also previously been shown to vary seasonally; in a group of 88 community-dwelling older adults (mean age = 69.2 years), ankle dorsiflexion strength, measured 5 times over 1 year, was shown to significantly vary by season, with the highest ankle strength observable in the summer months, although was not significantly associated with 25(OH)D concentration (Bird et al., 2012). The relationship between 25(OH)D concentration and handgrip strength has also been shown to vary seasonally; a significant association between 25(OH)D and handgrip strength was observable in the summer months ($R = 0.334$, $p = 0.024$) but not the winter months ($R = 0.191$, $p = 0.243$) in a group of 51 COPD patients (mean age = 68.7 years) living in Northern Ireland (Carson et al., 2018).

The lack of association between serum 25(OH)D concentration and parameters of skeletal muscle mass but positive association, albeit small, with muscle strength is suggestive that vitamin D may play a role in muscle strength and/or functioning rather than hypertrophy. In a longitudinal analysis of the relationship between changes in muscle mass and strength, although positively related to knee extensor and flexor strength, muscle mass explained only 5% of the decline in strength and was significant only for knee flexor strength in women (Hughes et al., 2001).

Regarding sarcopenia prevalence, the rates within the total D-FINES cohort, which ranged from 4.55% to 6.49%, dependent on season, were within or below the ranges previously reported; a systematic review of 252 studies of sarcopenia prevalence based on the EWGSOP criteria (Cruz-Jentoft et al., 2010a) reported prevalence rates of 1% to 29% in community-dwelling adults aged >50 years (Cruz-Jentoft et al., 2014b). Another study reported a prevalence rate of 14% to 27.6% assessed using DXA to estimate ASM in 250 older adults (62.8% women, mean age = 74.1 years) using the EWGSOP criteria to diagnose sarcopenia (Beaudart et al., 2015b). Within the D-FINES cohort, 2.78% to 8.33% of postmenopausal women ≥ 65 years were considered to be sarcopenic in the autumn and spring months, respectively. During both the autumn and spring, 100% of sarcopenic participants identified in this group were Caucasian. 5.08% to 5.93% of postmenopausal women <65 years were considered to be sarcopenic in the autumn and spring months, respectively. In this group, 50% of identified sarcopenic participants were Caucasian and 50% were Asian during the autumn. During the spring, 57.14% of postmenopausal women <65 years were Caucasian and 42.86% were Asian. A higher prevalence of sarcopenia in postmenopausal women ≥ 65 years compared with postmenopausal women <65 years was expected since sarcopenia prevalence has been shown to increase with age (Volpato et al., 2013).

Comparing non-sarcopenic participants with participants identified as sarcopenic during either the autumn, spring or both seasonal timepoints, ASM, total lean mass and handgrip strength were significantly lower in sarcopenic participants than non-sarcopenic participants (all $p < 0.005$), as would be expected. Serum 25(OH)D concentration was lower and less variable in sarcopenic participants than in non-sarcopenic participants at each seasonal timepoint, although no statistical

significance was observed at any timepoint. Serum 25(OH)D concentrations at each seasonal timepoint in non-sarcopenic vs sarcopenic participants were as follows; summer: 56.70 (23.13) vs 49.35 (21.27) nmol/L, autumn: 50.95 (21.90) vs 42.76 (16.85) nmol/L, winter: 38.86 (16.88) vs 37.85 (19.25) nmol/L and spring 40.70 (19.38) vs 39.86 (17.73) nmol/L. This finding is in agreement with a previous study comparing 66 sarcopenic with 66 non-sarcopenic older adults (mean age = 71 years); 25(OH)D concentration was significantly lower during the “summer” period of June to November and lower, albeit not significantly, throughout the “winter” period of December to May ($p = 0.017$) (Verlaan et al., 2017).

A finding novel to the current study was evidence that sarcopenia classification may be transient and season-dependent, although it is important to note that this was concluded from a relatively small number of participants (7 in the autumn and 10 in the spring). The percentage of participants classified as sarcopenic based on the EWGSOP2 criteria (Cruz-Jentoft et al., 2018) differed from autumn to spring, with the highest percentage of sarcopenic women identified in the spring for both postmenopausal groups. A total of 5 women from the available cohort were found to present a transient sarcopenic status, with 4 of the 5 women (80%) found to be sarcopenic only in the spring. More women found to be sarcopenic only in the spring than only sarcopenic in the autumn in all 3 groups compared; 2 of the 3 postmenopausal women <65 years (66.67%) were sarcopenic only in spring and all postmenopausal women ≥ 65 years (2 women) with transient sarcopenic status were observed in spring.

Of participants with ASM and handgrip data available for both the autumn and spring months, ASM in the spring was significantly higher than in the autumn (16.28 kg vs 16.15 kg, $Z = -2.55$, $p = 0.011$); however, both handgrip strength (21.20 kg vs 21.52

kg, $Z = 1.07$, $p = 0.284$) and serum 25(OH)D concentrations (42.83 nmol/L vs 51.18 nmol/L, $Z = 6.41$, $p < 0.001$) were lower in the spring. Although ASM, muscle strength and serum 25(OH)D concentrations have been found to vary seasonally in this study, other factors including parathyroid hormone concentration have been associated with sarcopenia (Visser et al., 2003b) and have been found to vary with season (Dawson-Hughes et al., 2014). Furthermore, physical activity is known both to be associated with sarcopenia and vary seasonally (Scott et al., 2010) with outdoor physical activity positively influencing cutaneous production of vitamin D and therefore 25(OH)D concentrations (Orces, 2019). In the autumn, non-sarcopenic participants spent more time per day walking than sarcopenic participants (98.52 minutes vs 76.09 minutes, $p = 0.504$), although the reverse was true in spring (92.90 minutes vs 106.88 minutes, $p = 0.716$).

The D-FINES cohort presents a large and rich data set; however, findings of the current study should be interpreted within the context of some limitations. Firstly, the use of correlation to assess association means that causality cannot be inferred. The number of sarcopenic participants was small, relative to the whole cohort, meaning that the subgroup of sarcopenic participants within the current study may not be representative of the general sarcopenic population within the UK. Dietary intake of vitamin D was not assessed or included within any analyses within the current study, which, although the relative dietary contribution to overall vitamin D status is lower than cutaneous production (<20% total annual intake; (Macdonald et al., 2011a)) does represent a limitation of the current study.

One further limitation of the study is the definition of menopausal status; women were determined to be postmenopausal following 3 months of amenorrhoea, although it is

widely accepted that postmenopausal status be defined as 12 months of amenorrhea following the final menstrual period (Soules et al., 2001).

Unfortunately, appropriate measures of muscle function were not available at timepoints corresponding with lean mass and strength assessments, therefore it was not possible to categorise the severity of sarcopenia observed in this cohort. Thus, the seasonal variability of sarcopenia severity was not investigated in the current study, but would be of future interest alongside a potential transient sarcopenia diagnosis throughout the year, as it was only possible to assess sarcopenic status in the autumn and spring seasons.

Handgrip strength may not present a true reflection of total body strength, since upper body strength loss is slower than lower body strength loss; in 68 women of ages ranging from 46 to 78 years followed up after an average of 9.7 years, knee extensor strength was shown to decline by an additional 5.6% per decade than elbow flexion strength ($p < 0.001$) (Hughes et al., 2001). Furthermore, handgrip strength is vulnerable to the methodology selected, particularly elbow positioning. Although the current study followed the American Society of Hand Therapists recommended positioning (seated participant with elbow flexed at 90°) (Fess, 1981), grip strength has previously been shown to be significantly greater when assessed from a standing position with the elbow fully extended (Liao et al., 2014)

Additionally, lower extremity muscle power may be a more appropriate outcome when assessing sarcopenic status, since muscle power is a superior predictor of functional status than muscle strength (Suzuki et al., 2001, Byrne et al., 2016) and more relevant to activities of daily living such as stair climbing, walking and standing from a seated position (Foldvari et al., 2000). In the Longitudinal Aging Study

Amsterdam cohort of 449 older men and women (mean age = 75 years), lower extremity performance, assessed as the sum of a 3-meter timed walk test and a 5-time chair rise test, was significantly associated with handgrip strength in men ($\beta = 0.079$, $p < 0.001$) but not in women ($\beta = 0.046$, $p = 0.100$) following adjustments for BMI, number of self-reported diseases, physical activity and cognitive function (MMSE) (Visser et al., 2000).

3.6. CONCLUSIONS

This study provides evidence suggesting that there is no association between serum 25(OH)D concentration and total lean mass, ASM or relative ASM; this finding is supported by some previous work but contended by other groups. Although change in 25(OH)D concentration from autumn to spring was negatively associated with the change total body lean mass, the change in lean mass was within the measurement variability for DXA and thus should be interpreted with caution. Muscle strength was shown to be positively associated with 25(OH)D at all seasonal timepoints, suggesting that serum 25(OH)D concentration is associated with muscle strength and functional outcomes rather than muscle hypertrophy. Sarcopenic status was shown to be transient and seasonal in a small sample of postmenopausal women; confirmation of this finding in a larger and therefore more representative sample including men would instil more confidence in this outcome.

CHAPTER 4

METHODS AND PROTOCOL DEVELOPMENT

THE INFLUENCE OF COMBINED VITAMIN D3 SUPPLEMENTATION AND RESISTANCE EXERCISE TRAINING ON MUSCULOSKELETAL HEALTH IN OLDER MEN AND WOMEN (EXVITD): PROTOCOL FOR A RANDOMIZED CONTROLLED TRIAL

This chapter is taken verbatim (with the exception of Table 4.5) from the following article published in the *BMJ Open* for which I am principal author:

Welford, A.E., Lanham-New, S., Lord, J., Doyle, A., Robinson, J., Nightingale, P., Gittoes, N. and Greig, C.A., 2020. Influence of combined vitamin D3 supplementation and resistance exercise training on musculoskeletal health in older men and women (EXVITD): protocol for a randomised controlled trial. *BMJ open*, 10(3), p.e033824.

4.1. Abstract

Introduction

Sarcopenia is a progressive loss in muscle mass, strength and function, the adverse consequences of which are severe, affecting quality of life and placing an increasing burden on social and healthcare systems. Vitamin D status is known to be associated with markers of sarcopenia, namely muscle mass, strength and function. Also, resistance exercise training (RET) is currently the only proven intervention to treat sarcopenia. However, very little data exist on the influence of combining the two interventions of vitamin D supplementation and resistance exercise training.

The aim of the present study was to determine whether vitamin D3 supplementation is any more effective in improving musculoskeletal function when combined with resistance exercise training (RET) compared with exercise training alone in older adults.

Methods and analysis

This was a **double-blinded randomized placebo-controlled trial** that aimed to recruit a target of 127 eligible men and women aged ≥ 65 years living independently or in sheltered housing within the Birmingham area and randomized into to 2 groups: i) 6 months RET and placebo, or ii) 6 months RET and 800IU/d vitamin D3. Measures of body composition (DXA), muscle function (SPPB, TUG), falls and fractures as events were assessed. Assessments took place at baseline and post-intervention, with intermittent monitoring of bone turnover, calcium and vitamin D. The primary

outcome was lower limb extensor power output. Analyses of within-group changes and between-group differences in outcome measures were planned.

Ethics and dissemination

Ethical approval for the EXVITD study was granted by the Black Country NHS Research Ethics Committee (14/WM/1220). The study was conducted according to the principles of the Declaration of Helsinki. Trial registration number: NCT02467153. Trial sponsor: University of Birmingham. Protocol version 10.0.

4.2. Introduction

The UK has an ageing population; life expectancy has increased rapidly in the previous 2 decades, with life expectancy at birth between 2010 and 2012 reaching 82.72 for females and 78.85 years for males (Office for National Statistics, 2015). Importantly, healthy life expectancy is not keeping pace; adults over 65 years are expected to spend approximately 7.9 years in poor health (Office for National Statistics, 2014a). Sarcopenia refers to the age-related loss of muscle mass, strength and function (Cruz-Jentoft et al., 2018). In the face of an increasing proportion of older adults and the dramatic increase in pressure on health and social care (Janssen et al., 2004), many are likely to suffer the adverse consequences of sarcopenia, namely impaired functional ability, increased frequency of falls and fractures, with attendant morbidity and mortality; a higher incidence of hospitalizations and longer length of hospital stay (Beudart et al., 2017). Sarcopenia therefore represents a serious and increasing public health problem. The causes of sarcopenia are unclear, however, there are numerous factors associated aetiology (Bano et al., 2017, Steffl et al., 2017, Robinson et al., 2017, De Spiegeleer et al., 2018). One such example is vitamin D deficiency; older adults are considered an “at risk group”, with prevalence rates of hypovitaminosis d reaching 89% in residential care (Schilling, 2012). Consequences of vitamin D deficiency include muscle weakness and an increased risk of falls and fractures (Holick and Chen, 2008). While it is known that vitamin D is essential for calcium and phosphorous homeostasis and bone health (Holick and Chen, 2008), we know relatively little about the direct effects of vitamin d on muscle mass and function in humans. The majority of evidence for an effect on muscle is based upon animal models, which have reported increases in

muscle protein synthesis in response to vitamin d supplementation but which may not mimic the human condition (Garcia et al., 2011, Srikuea et al., 2012), and cross-sectional epidemiology (Wicherts et al., 2007, Visser et al., 2003b, Houston et al., 2007b) which cannot establish causality. Meta-analyses examining the effect of vitamin D supplementation on musculoskeletal parameters report conflicting evidence, although supplementation was shown to improve muscle strength, with effects more pronounced in older and vitamin d deficient participants (Beudart et al., 2014a).

Another factor associated with sarcopenia is physical activity. Resistance exercise training (RET) is the most promising intervention for improving sarcopenia (Kosek et al., 2006). We and others have shown that even in very old adults (>75 years), RET improves muscle strength and functional outcomes although the hypertrophic ability of older muscle is blunted compared with younger adults (Greig et al., 2011a, Raue et al., 2009). Therefore, in order to help older adults maintain good musculoskeletal health, interventions to optimise responsiveness to physical activity are likely to be most effective if they are multimodal and include resistance exercise. One such example is the combination of RET with vitamin D supplementation.

Very few data exist that test the combined effects of vitamin D₃ supplementation and RET on the musculoskeletal health of older adults, as highlighted by a recent systematic review and meta-analysis (Antoniak and Greig, 2017). The review concluded vitamin D supplementation and exercise training significantly improved muscle strength within the lower limb compared with to exercise training alone (0.98, 95% CI 0.73 to 1.24, $p < 0.001$) (Antoniak and Greig, 2017). However, the limitations of the review serve to highlight the lack of knowledge within this area; only 3 studies

were included in this meta-analysis, and the high weighting of one particular study meant that only tentative conclusions could be drawn.

Therefore, the principal aim of the EXVITD study was to determine whether vitamin D3 supplementation was any more effective in improving musculoskeletal function when combined with RET compared with RET alone. Secondary objectives included; (i) determining the seasonal variation of 25(OH)D in a population of frailer older adults both free-living and those living in supported housing. (ii) Investigating the association between baseline 25(OH)D and physical activity measured using accelerometry and responsiveness to RET (iii) Determining between-group differences with respect to changes in falls as events and quality of life (iv) Determining between-group differences with respect to changes in fractures as events (v) Determining the influence of RET on serum inflammatory markers (e.g. IL-6, TNF- α) (vi) Investigating the influence of RET on serum stress markers (cortisol, DHEAS).

4.3. Methods

4.3.1. Study design

This study protocol is presented in line with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines. The EXVITD study was a two-arm exploratory double-blinded randomized placebo-controlled trial. A target total of 127 participants were sought to be identified, recruited and randomized 1:1 into 2 groups: i) RET and 800IU vitamin D3 per day or, ii) RET and a daily placebo for 6 months. Eligibility was assessed at screening and all outcome

measures were collected at baseline and 6 months, with venepuncture outcomes additionally assessed at months 1 and 3.

The study was registered as a randomized controlled trial on clinicaltrials.gov (NCT02467153). The EXVITD study took place within Birmingham, at both the University of Birmingham and the Wellcome Trust Clinical Research Facility at the Queen Elizabeth Hospital. Data collection began in July 2017 and collection/analysis was completed in October 2019 31/12/2019.

4.3.2. Study population

We aimed to recruit 127 men and women aged ≥ 65 years, who were ambulatory (with/without aids) and lived independently or within sheltered housing accommodation in Birmingham, West Midlands, UK. We selected this population as they are more susceptible to sarcopenia and subsequent functional deficits and therefore stand to benefit from interventions aimed to improve musculoskeletal health. Eligibility criteria are summarised in Table 4.1. Since confirmation of eligibility in this low-risk study did not require the interpretation of medical notes/ history or a physical examination, the study Chief Investigator (CI) was suitably qualified to confirm eligibility. However, any queries about eligibility were raised with the medical expert before a decision was made.

Table 4.1: EXVITD study eligibility criteria¹

Inclusion criteria	Aged ≥65 years
	Ambulatory (with or without walking aids)
	Living independently or within sheltered housing accommodation
Exclusion criteria	History of myocardial infarction within previous 2 years
	Cardiac illness: moderate/ severe aortic stenosis, acute pericarditis, acute myocarditis, aneurysm, severe angina, clinically significant valvular disease, uncontrolled dysrhythmia, claudication within last 10 years; thrombophlebitis or pulmonary embolus within last 2 years
	History of cerebrovascular disease (cerebrovascular accident (CVA), transient ischemic attack (TIA)) within last 2 years
	Acute febrile illness within the previous 3 months
	Severe airflow obstruction; uncontrolled metabolic disease (e.g., thyroid disease or cancer)
	Significant emotional distress, psychotic illness or depression within the previous 2 years
	Lower limb fracture sustained within the previous 2 years/ upper limb fracture within the previous 6 months
	Non-arthroscopic lower limb joint surgery within the previous 2 years
	Any reason for loss of mobility for > 1 week in the previous 2 months or > 2 weeks in the previous 6 months
	Resting systolic pressure >200 mmHg or resting diastolic pressure >100mmHg
	Poorly controlled atrial fibrillation; poor (chronic) pain control
	Moderate/ severe cognitive impairment (mini mental state examination (MMSE) score <23)
	Vitamin D deficiency (serum 25(OH)D3 <30nmol/l); current antiresorptive/anabolic treatment for osteoporosis
	Treatment with bisphosphonates for osteoporosis in the past two years
	Current use of glucocorticoids; known primary hyperparathyroidism
	Renal impairment (Stage 4 or 5)

¹Based on previously published criteria of exercise studies with older adults (Greig et al., 1994)

4.3.3. Recruitment

Recruitment strategies were three-fold and are detailed below.

i) Primary Care approach

We worked with the West Midlands Clinical Research Network who assisted with recruitment via primary care and helped to identify additional supported housing facilities under their aegis. Eligible participants from surrounding areas were identified via an electronic practice-based search of registers using the criteria described in Table 1. The General Practitioner (GP) reviewed and excluded anyone they deemed unsuitable for reasons other than those identified in the protocol (e.g. already taking part in another study).

A Patient Approach letter was sent to patients identified from the GP together with a Participant Information Sheet (PIS, see Appendix E), reply form and FREEPOST envelope addressed to the study team. Alternatively, the research team were contacted directly by phone or email. Patients not responding to the first invitation received one reminder after 3 weeks, including an acknowledgement that the letter was to be ignored by those responding to the initial letter. When the study team received a signed patient contact agreement consent form, they contacted the potential participant via telephone to discuss the study in more detail, go through the questionnaire responses and if eligible, obtain written consent.

ii) Sheltered housing approach

Sheltered housing managers and head offices were contacted directly, and where possible, study information was presented at residents' meetings, coffee mornings and on communal notice boards. Residents could contact the study team directly or

through the housing manager. When a response was received, the participant was contacted as detailed above.

iii) Independent living approach

We recruited older adults living independently via several methods. Study information was displayed at appropriate locations, for example seniors' groups, community centres and libraries. Additionally, advertisement was via appropriate and relevant websites, print media (e.g. magazines, leaflets, newsletters) and social media. Members of the Birmingham 1000 Elders cohort managed by the University of Birmingham were also recruited. Study information was presented at seniors' group meetings and direct contact to a member of the study team was answered as detailed above.

4.3.4. Eligibility screening

The assessments completed during eligibility screening are documented in Table 4.2, and were conducted via one of following methods:

- 1) For participants identified via the primary care or independent living approach, the health questionnaire (see Appendix F) was conducted via telephone, with subsequent tests completed at the University of Birmingham.
- 2) For participants identified via the sheltered housing approach, all eligibility screening tests were conducted within the participant's own home, provided health and safety requirements for the blood draw are met (i.e. hand washing facilities available nearby and impermeable surfaces). If requirements were not satisfied, transport was arranged for the participant and the visit took place at the University of Birmingham.

Table 4.2: Eligibility screening assessments

	Location of assessment	Exclusion criterion
Informed consent	At participant's home or the University of Birmingham	-
General health questionnaire	Telephone call, participant's home or the University of Birmingham	Answering "yes" to any question outlined in the exclusion criteria (Table 3.1)
Mini mental state exam (MMSE)	At participant's home or at the University of Birmingham	Score < 23
Blood draw assessing 25(OH)D status	At participant's home or at the University of Birmingham	Serum 25(OH)D < 30 nmol/L
Physical activity monitoring (ActivPAL accelerometry)	At participant's home or at the University of Birmingham	-

4.3.5. Consent

It was the responsibility of the investigator to obtain written informed consent for each participant prior to performing any trial related procedure. A PIS was provided to facilitate this process and the participant was given at least one week to read the PIS and discuss participation with others outside the research team. For participants recruited via the primary care network approach, a signed patient contact agreement consent form was received before any contact was made.

Investigators ensured they adequately explained the aim, trial treatment, anticipated benefits and potential hazards, and the participant was given the opportunity to ask questions. Investigators also stressed that participation was voluntary and that the participant was free to refuse to take part and could withdraw from the trial at any

time without it affecting their future care. If interest in participation in the trial is confirmed they were asked to sign and date the consent form (see Appendix G).

At each visit the participant's willingness to continue in the trial was ascertained. Throughout the trial the participant had the opportunity to ask questions about the trial. Any new information that was relevant to the participant's continued participation was to be provided to the study team. Where new information became available which may have affected the participants' decision to continue, participants were given time to consider and if happy to continue were re-consented. Re-consent was documented in the medical notes and the participant's right to withdraw remained. With the participant's consent, their GP was informed of their participation.

4.3.6. Randomization, allocation and blinding procedures

Participants were randomised by a University Hospitals Birmingham statistician to 1 of 2 arms: 1) RET + 800 IU vitamin D3 daily (intervention) or 2) RET + placebo daily for six months (control). Participants were stratified on the basis of vitamin D status (high ≥ 50 nmol/L, low = 30-50 nmol/L), physical activity (measured pre-randomisation using accelerometry, achieving the minimum recommended step count per day, not achieving the recommended step count per day), and sex. "High" vitamin D status was defined as ≥ 50 nmol/L as this cut-off point is widely accepted as describing vitamin D adequacy; "low" vitamin D status was considered to be between 30 – 50 nmol/L, widely accepted as describing inadequacy (van Schoor and Lips, 2018). We declined to include participants with hypovitaminosis D as we felt it unethical to withhold treatment from those participants randomized to the placebo group.

The physical activity cut-off point of 7000 steps per day (averaged from data collected using ActivPAL accelerometers worn for 7 days) was chosen based on

review evidence recommending older adults achieve between 7000 – 10,000 steps per day, or 7100 if averaged over 1 week (Tudor-Locke et al., 2011). This recommendation was calculated based on an assumed average adult cadence per minute and applied to the Canadian physical activity guidelines recommending that older adults achieve 150 minutes of moderate-to-vigorous activity per week (Paterson and Warburton, 2010); a variation of both the WHO guidelines (World Health Organization, 2010) and the recent UK Chief Medical Officers' report on physical activity guidelines for older adults (Department of Health and Social Care, 2019).

Randomisation was computer-generated using the random number function in Microsoft Excel with a mixture of block sizes of four and six. Allocation of participants into study group and labelling and allocation of the tablets into supplement bottles and adherence monitoring was completed within the University of Birmingham, by an individual with no other involvement in the study.

The study team and participants were blinded to group allocation. Randomisation codes were kept in individual sealed envelopes to avoid unblinding the whole cohort if an individual participant allocation was requested by the study team's medical expert. In order to avoid assessor unblinding, all blood results were viewed by the study team's medical expert who may have been unblinded as a consequence. Unblinding decisions were to be taken by the study medical expert, in consultation with the CI.

Arrangements for emergency unblinding if both the medical expert and CI were unavailable were according to established UHB practice embedded within its governance structure. The Clinical Research Facility (CRF) does not open 24 hours a

day, however the CRF Clinical Manager had 24-hour access to the relevant information. The study timeline schematic is shown in Figure 4.1.

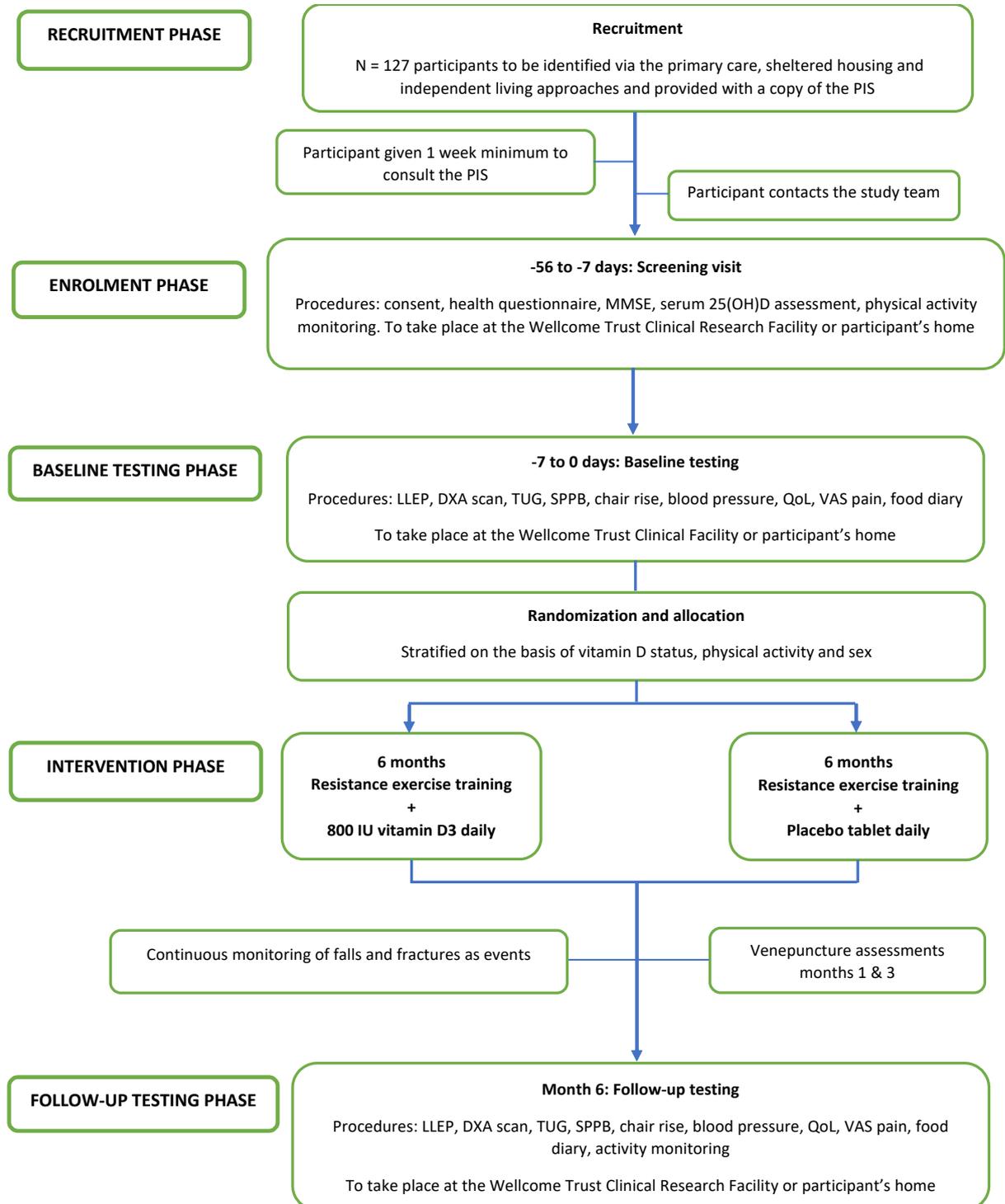


Fig 4.1: Study timeline schematic

PIS: participant information sheet, MMSE: mini mental state exam, LLEP: lower limb extensor power output, DXA: dual energy X-ray absorptiometry, TUG: timed up and go, SPPB: short physical performance battery, VAS: visual analogue scale pain questionnaire

4.3.7. Study intervention: Intervention arm

i) Resistance Exercise Training

A rolling group exercise programme with a maximum of 12 participants per group and no more than 2 groups running concurrently was established. Group 1 alone ran for 3 months to check for practical issues. The exercise training intervention was delivered by the study team in partnership with a specialist exercise instructor who delivered initial training, supervision and regular quality assessment. The specialist exercise instructor provided a copy of their Public, Personal Trainer and Coaches liability Insurance certificate to the CI.

The RET programme was developed by our falls prevention nurse specialist and approved by the Falls Lead Physiotherapist for UHB and includes; a dynamic warm-up and aerobic section (approximately 15 minutes), balance and co-ordination section (approximately 10 minutes), resistance training section (approximately 20-25 minutes) and a cool down with stretches (approximately 10-15 minutes). Each session lasted for approximately 60 minutes, with 2 sessions per week for 6 months. The sessions included elements of current established programmes for falls prevention/ core stability (e.g., OTAGO (Robertson et al., 2001)) and was tailored and progressed individually to a range of abilities within the target group. The resistance training section utilised physiotherapy bands and ankle weights, of which a range of colours and weights was supplied.

Attendance at group sessions was monitored via a register, and participants were asked to report any non-attendances to a member of the study team. Progression was assessed via the 30 second chair rise test (also a policy of the specialist exercise instructor), band colour/ ankle weight utilised and repetitions of resistance

exercises. Groups ran throughout the year to mitigate for seasonal variation in vitamin D status. Group 1 ran from July to January (summer to winter), Group 2 ran from April to December (spring to winter) and Group 3 ran from December to August (winter to summer).

Exercise sessions were held in communal living spaces for participants recruited via the sheltered housing route or within the Morris Club Centre, an NHS health, fitness and wellbeing centre located on the Queen Elizabeth Hospital campus, for participants recruited via the independent living or primary care approach. Pre-paid transport to and from the exercise sessions was offered to all participants in order to remove a potential barrier to participation and promote participant retention. During the study participants were asked not to undertake any additional classes/ supplements; after the study was completed a book or DVD of the training exercises was given to encourage continued physical activity as a lifestyle choice.

ii) Vitamin D3 supplementation

In line with the current Institute Of Medicine (Ross et al., 2011b) and Royal Osteoporosis Society (The Royal Osteoporosis Society, 2019b) guidelines, the daily dose of vitamin D3 was 800 IU. This was below the UK Food Standards Agency publicly stated safe limit for daily vitamin D intake at 1000 IU (Food Standards Agency, 2003), although this has been criticised as overly conservative (Vieth, 2006). The Institute of Medicine set a Tolerable Upper Intake Level (UL) for vitamin D of 4000 IU per day (Ross et al., 2011b) and the European Food Safety Agency (EFSA) Panel on Dietetic Products, Nutrition and Allergies have set a no observed adverse effect level (NOAEL) of 10,000 IU per day (EFSA Panel on Dietetic Products and Allergies, 2012). Additionally, hypercalcemia, the hallmark of vitamin D intoxication,

has only been consistently observed in anecdotal evidence when 25(OH)D concentrations are between 375–500 nmol/L (Jones, 2008). Therefore, assessment and monitoring of serum 25(OH)D and calcium occurred throughout the study (baseline, months 1, 3 and 6) although we did not anticipate any reason for early termination of the study.

Individual supplies of over the counter vitamin D supplements provided by IVC Brunel Healthcare were given to participants in tablet form packed in pots; pots contained a 4-week supply of supplements (28 tablets) and participants were instructed to take one 800 IU tablet per day. Tablets were stored at ambient temperature by the study team within the University of Birmingham, and distributed to participants every 4 weeks. Tablet manufacture was undertaken by Brunel Healthcare in a Medicines and Healthcare products Regulatory Agency (MHRA) licensed facility, labelling and allocation of tablets into pots took place within the University of Birmingham by an individual with no other involvement in the study. Labels contained the following information: number of tablets (28); dose (20 µg/800 IU); schedule and directions of use; storage information; date; participant ID; batch information; trial name (EXVITD) and study team contact number. Compliance was monitored by returning used tablet pots, with gentle compliance reminders given to participants during the exercise sessions.

Study intervention: Control arm

Participants randomized to the control arm of the trial received the RET intervention alongside the intervention group and a daily placebo supplement distributed and taken in the same way as the vitamin D3 supplements. The composition of the

placebo tablet was as follows; 73% Sorbitol P60W, 25% sieved Xylitab 300, 2% Magnesium Stearate Veg EP.

4.3.8. Outcome assessments

A list of scheduled outcome assessments is presented in Table 4.3. Outcome measures were assessed by the study team, with the exception of the DXA scan which was performed by a radiographer.

Table 4.3: EXVITD study outcome measures							
	Trial period						
	Enrolment	Allocation	Post-allocation			Follow-up	
	-56 to -7 days (Screening)	-7 to 0 days (Baseline)	Month 1	Month 3	Month 6	+1 to +7 days	
Enrolment							
Informed consent ¹	X	X	X	X	X	X	
Health questionnaire	X						
MMSE	X						
Venepuncture	X	X	X	X	X	X	
Physical activity monitoring (accelerometry – ActivPAL)	X					X	
Allocation		X					
Interventions							
Resistance exercise training							
Vitamin D or placebo							
Assessments							
Lower limb extensor power (Nottingham power rig)		X				X	
Body composition & BMD (DXA)		X				X	
Chair rise (Leonardo force plates)		X				X	
Functional ability (SPPB & TUG)		X				X	
Blood pressure		X				X	
Falls as events	X	X	X	X	X	X	
Fractures as events	X	X	X	X	X	X	
Quality of life (SF-36)		X					
Musculoskeletal pain questionnaire		X				X	
Food diary		X				X	
¹ Informed consent form will be signed during the screening visit and will be re-confirmed verbally at each following time point. BMD: bone mineral density, DXA: dual energy X-ray absorptiometry, SPPB: short physical performance battery, TUG: timed up and go, SF-36: 36 item short form survey.							

4.3.9. Reliability, validity and appropriateness of outcome measure variables to be implemented in the EXVITD study

Table 4.4: Reliability, validity and appropriateness of outcome measure variables to be implemented in the EXVITD study		
Outcome variable	Measurement variable	Reliability/validity/appropriateness in an older population
Muscle power	LLEP (Nottingham power rig)	<p>The Nottingham Power Rig, manufactured by the Medical Engineering Unit of The University of Nottingham, serial number LEPR/08/052 using LegRig Version 2.0 (2003) software edition was used to measure explosive power in the dominant leg of participants. The test is completed in a seated position, with the participant extending their dominant leg in order to depress the foot press as quickly and forcefully as possible. The depression of the foot press rotates the flywheel and an optoswitch is used to record angular velocity and calculate LEP (Bassegy and Short, 1990). This action incorporates hip extension, knee extension, and ankle plantar flexion and is considered the “gold standard” of power measurement in older adults (Gray and Paulson, 2014). Since the test is performed seated, it is considered a safe alternative measure of muscle power to jump tests in frail older adults (Lindemann et al., 2003). The measurement of muscle power using the Nottingham leg rig has been found to be significantly associated with measurement of power using an isokinetic dynamometer (Spearman’s rho = 0.73, $p < 0.001$) and two-legged jumps on a force plate (Spearman’s rho = 0.86, $p < 0.001$) (Bassegy and Short, 1990). The test-retest coefficient of variation has been reported as 8% in a population of 419 women aged 63-75 years (Portegijs et al., 2005). Muscle power was selected as the primary outcome measure since muscle power is a superior predictor of functional status than muscle strength (Suzuki et al., 2001, Byrne et al., 2016) and power of the lower limbs has been found to be an independent predictor of self-reported functional status in community-dwelling older women (Foldvari et al., 2000). Muscle power declines more rapidly with ageing than muscle strength (Skelton et al., 1994) and low leg muscle power is associated with a 2-3 fold greater risk of mobility limitations compared with low muscle strength (Bean et al., 2003); mobility limitation is an independent risk factor for disability, hospitalization and mortality and reduces quality of life and independence in older adults (Pahor et al., 2014).</p>



Figure 4.2: The Nottingham Power Rig, reproduced with permission from (Barker et al., 2012), copyright Elsevier. License number 4662990754731

Outcome variable	Measurement variable	Reliability/validity/appropriateness in an older population
Body composition	DXA	<p>Total body composition and BMD was assessed using a GE Lunar iDXA encore 2011 running software version 13.60.033. Quality assessment checks are completed 3 times per week with the machine switched on and water and encapsulated phantoms provided by the manufacturer are used to calibrate the machine 3 times per week.</p> <p>DXA differentiates and quantifies different materials in the body using 2 beam energies which are attenuated or absorbed in relation to tissue type and amount. DXA discriminates between lean and bone materials, and provides estimates of 3 body compartments; lean, bone and fat. Since lean and fat masses comprise more water than bone, these tissues will attenuate the beam energy to a lesser degree than bone (Buckinx et al., 2018a).</p> <p>DXA is widely available, produces lower radiation doses and measurements of muscle mass and quality are highly correlated with both CT (multi-slice thigh fat-free mass, $r^2 = 0.96$)(Levine et al., 2000) and MRI (whole body lean mass $r = 0.94$) (Chen et al., 2007). Although DXA does have a number of limitations, such as the inability to measure intramuscular fat and differences in results between devices (Buckinx et al., 2018a), it has been cited as a reliable method of indirectly estimating muscle mass in older adults (Chen et al., 2007). Indeed, DXA is the current “reference technique of choice for estimating muscle mass and body composition in research and clinical practice”, and is widely used in RCTs to estimate skeletal muscle mass (Buckinx et al., 2018a).</p>
BMD	DXA	<p>BMD is calculated by converting the radiation energy per pixel into areal density, which is the number of pixels in the area and the amount of bone in each pixel (Berger, 2002).</p> <p>The assessment of BMD to predict fracture risk has limitations, namely, BMD does not fully describe bone strength nor quality, does not account for bone size nor architecture, is not a true three dimensional measure and cross-comparison between different DXA machines is difficult (Pors Nielsen, 2000). However, from BMD, T and Z scores can be calculated, which are used to diagnose osteoporosis(World Health Organization, 1994); BMD estimation via DXA has become “universally adopted as a standard to define osteoporosis” (Link, 2012). In the EXVITD study, additional images of the hip and spine were chosen since these site-specific scans are preferred to diagnose osteoporosis and predict fracture risk (Sözen et al., 2017). DXA used to estimate BMD has been described as highly precise (with a maximum acceptable precision error of 2-2.5% (Link, 2012)) and BMD of the hip is a strong predictor of hip fracture risk in men and women (Johnell et al., 2005).</p>

Outcome variable	Measurement variable	Reliability/validity/appropriateness in an older population
Muscle function	SPPB	<p>The Short Physical Performance Battery test (SPPB) was designed to assess lower extremity function in older adults and consists of 3 domains; balance, gait speed and chair rise tests (Guralnik et al., 1994). The test has a maximum score of 12 points, with a score ≤ 8 points indicative of poor physical performance (Cruz-Jentoft et al., 2010c). SPP score is associated with mobility disability, hospitalization and mortality (Guralnik et al., 2000, Beaudart et al., 2016b). The SPPB test has been found to be a highly valid and reliable measure of function in a variety of older populations (Freire et al., 2012, Gómez et al., 2013, Olsen and Bergland, 2017) and also sensitive to change in the event of medical events including myocardial infarction, stroke and hip fracture; participants experiencing such medical events were significantly more likely to record poorer summary performance change scores (Ostir et al., 2002). Therefore, the SPPB test is a standard measure of physical performance in older adults in a research and clinical setting (Cruz-Jentoft et al., 2010c) and is also a validated test for sarcopenia severity diagnosis (Writing Group for the European Working Group on Sarcopenia in Older People 2 et al., 2018).</p>
Muscle function	TUG	<p>The Timed Up and Go test (TUG) is a measure of muscle function developed specifically for older adults and is the time (in seconds) taken to stand up from a chair, walk 3 meters, turn around, return and sit down (Podsiadlo and Richardson, 1991). Aspects of the TUG test are characteristic of activities of daily living important for the maintenance of physical independence, i.e. the muscle strength to rise from a chair, gait speed, turning, and the co-ordination to sit safely from a standing position (Vervoort et al., 2016); poor TUG test performance has been associated with mortality (HR = 1.79; 95% CI, 1.33, 2.42, $p < 0.001$) (Bergland et al., 2017). The TUG test is sensitive and specific when identifying older people with sarcopenia (sensitivity = 67%, specificity = 88.7%) (Martinez et al., 2015) and those who are prone to falls (sensitivity = 87%, specificity = 87%) (Shumway-Cook et al., 2000). Additionally, a high inter-rater reliability has been reported amongst community-dwelling older adults (ICC = 0.98) (Shumway-Cook et al., 2000) and the TUG test is recommended by both the American and British Geriatrics societies to assess gait speed, balance and fall risk in older adults (Panel on Prevention of Falls in Older Persons and Society, 2011). The TUG test is recommended by EWGSOP2 to assess low physical performance (Writing Group for the European Working Group on Sarcopenia in Older People 2 et al., 2018) and a cut-off point of ≥ 20 seconds for low performance is suggested (Bischoff et al., 2003).</p>

Outcome variable	Measurement variable	Reliability/validity/appropriateness in an older population
Physical activity	Accelerometry (ActivPAL™)	<p>Accelerometry was used to estimate average physical activity over 7 days. The activPAL™ monitor (PAL Technologies Ltd, Glasgow, Scotland) is a small accelerometer device affixed to the anterior midline of the thigh where it is able to determine static and dynamic acceleration, classify activities into 3 categories (sitting/lying, standing, stepping) and provides an estimation of energy expenditure (EE) expressed in metabolic equivalents (METs). Step count, time spent sitting/lying, estimated energy expenditure and postural transitions were recorded and a daily average was estimated from the 7-day data. PAL Technologies use Intelligent Activity Classification algorithms to translate the positional and acceleration data received from the monitor into information about posture (i.e, sitting/lying, standing and postural transitions), step count and stepping speed (Edwardson et al., 2017). Energy expenditure is indirectly measured using these categories of movement, and intensity of exercise inferred using METs (≥ 3.0 METs assumed as moderate-vigorous activity) (Montoye et al., 2017).</p> <p>Although subjective measures of activity estimation such as self-report questionnaires and activity diaries are more practical for large-scale cohorts (Aguilar-Farías et al., 2015), they may overestimate sedentary time in older adults (Van Cauwenberg et al., 2014), they rely on participant recall and they can place a high level of burden on the participant (Atkin et al., 2012). Conversely, activPAL monitoring has reported excellent inter-device reliability (ICC = 0.99) and an overall 95.9% agreement between a second-by-second observed analysis and the monitor (Grant et al., 2006). Additionally, activPAL has been reported to record step count and cadence under 3 treadmill conditions (fast, normal and slow speeds) and during a 500 metre outdoor walk to a high degree of accuracy ($< 1\%$ absolute percentage error) in a group of 20 older adults of mean age 71.9 years (Grant et al., 2008).</p>

Outcome variable	Measurement variable	Reliability/validity/appropriateness in an older population
Musculoskeletal pain	VAS pain	<p>The visual analogue scale (VAS) for pain is a generic questionnaire for evaluating pain. It consists of a coloured scale from 0 to 10 (0 = “no pain”, 10 = “worst imaginable pain”); a score <4 indicative of good pain management (Burckhardt and Jones, 2003) and a score of ≤ 2 a cut-off point for “acceptable” pain (Wolfe and Michaud, 2007). The minimum change of clinical relevance in a cohort of patients with rheumatoid arthritis was shown to be 1.1 points on the 10 point scale, which was the calculated standard error of the mean (Wolfe and Michaud, 2007). The questionnaire is completed by the participant, with a line drawn perpendicular to their experience of pain both in a seated and standing position for the previous 24 hours. The participant burden of completing the questionnaire is low, since it can be completed in under 1 minute and the VAS is reported to be sensitive to change in clinical trials (Burckhardt and Jones, 2003). Test-retest reliability has been reported as $r = 0.937$ for literate and $r = 0.712$ for illiterate patients with rheumatoid arthritis (Ferraz et al., 1990). Chronic pain assessed by the VAS has been shown to be significantly associated with poor self-reported health status, sleep disorders, depression and malnutrition in 105 nursing home residents of mean age 82.2 years (Zanocchi et al., 2008).</p>
Quality of life	SF-36	<p>The general population health questionnaire the Short Form (SF)-36 is a quick and comprehensive version of the 149-item health questionnaire developed as part of the Medical Outcomes study (Ware Jr and Sherbourne, 1992). The SF-36 questions encompass 3 domains of health; functional status, wellbeing and an overall evaluation of health. A 2005 review of quality of life assessment in older adults found that the SF-36 was the most extensively used assessment, with good evidence supporting the questionnaire reliability, validity and responsiveness to change, particularly in community-dwelling older adults with lower levels of morbidity (Haywood et al., 2005). The SF-36 was completed quickly (median time of 8 minutes) by 195 patients aged 65 years and over and the authors remarked questions regarding functional ability made the SF-36 particularly relevant for use in older adults (Hayes et al., 1995).</p>

Outcome variable	Measurement variable	Reliability/validity/appropriateness in an older population
Ground reaction force & peak mechanical power of the lower limbs	Leonardo Mechanograph® Ground Reaction Force Plate	<p>The Leonardo Mechanograph® Ground Reaction Force Plate (Novotec Medical, Pforzheim, Germany) running the Leonardo Mechanograph® GRFP Research Edition Software version 4.2 was used to assess ground reaction force and peak mechanical power of the lower limbs. The Leonardo jump plates are calibrated at set up with no ongoing checks.</p> <p>The Leonardo Force Plate comprises a bench (45cm high) and a force platform containing 4 strain gauge force sensors to measure vertical ground reaction force (Matheson et al., 2013). The equipment records the maximal total relative power per body weight (CRTP_{rel}) and the maximal velocity (CRT_v) during standing throughout the 5 time chair rise test (also performed as part of the SPPB test (Guralnik et al., 1994)). The chair rise test was chosen as the single two-legged jump may be contraindicated in frail older adults. The chair rise test evaluates an action which is relevant in everyday life (Buehring et al., 2015) by assessing the muscle power required to co-ordinate the movement of standing from a seated position (Dietzel et al., 2015).</p> <p>The Leonardo Force Plate has been found to have excellent test-retest reliability (ICC of up to 0.99) in a range of different age groups, including older adults (Rittweger et al., 2004, Veilleux and Rauch, 2010, Buehring et al., 2015), good inter and intra-rater reliability (Matheson et al., 2013) and measurements of lower limb power correlate well with the Nottingham power rig ($r = 0.6$) and isokintetic dynamometry ($r = 0.68$) (Lindemann et al., 2003).</p>

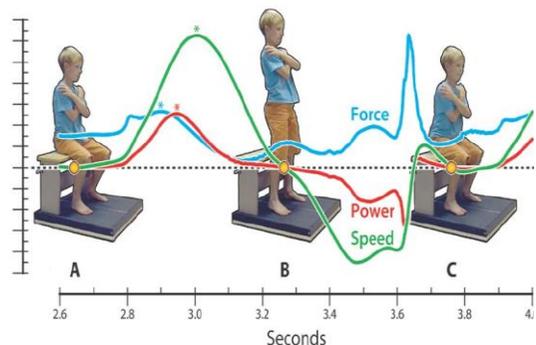


Figure 4.3: The chair rise test using the Leonardo Mechanograph® Ground Reaction Force Plate. Reproduced with permission from (Veilleux and Rauch, 2010)

Outcome variable	Measurement variable	Reliability/validity/appropriateness in an older population
Blood pressure	Systolic and diastolic blood pressure	<p>Blood pressure is assessed as an exclusion criterion at baseline and also measured at 6 months using a Welch Allyn vital sign monitor 300 series (WelchAllyn, Inc., Skaneateles Falls, NY). The monitor is calibrated and serviced once annually by a medical engineer. Blood pressure was measured in accordance with University Hospitals Birmingham clinical guidelines; briefly, the procedure was explained to the participant and consent was ascertained. The participant was given a minimum of 5 minutes to relax whilst seated before the first measurement was taken. After ensuring that no tight items of clothing were constricting the arm, the blood pressure cuff was then placed on the arm, with the centre directly over the brachial artery. The arm was supported at the level of the arm with a pillow before the first seated reading was taken. Directly after this measurement was recorded, the participant was asked to stand, and their standing blood pressure measurement was recorded. Each sitting and standing measurement was repeated 3 times, with a 5 minute break in between. High blood pressure was an exclusion criterion for the EXVITD study, with high blood pressure categorised as a resting systolic pressure >200 mmHg or resting diastolic pressure >100mmHg. High systolic and diastolic blood pressures have been shown to be positively associated with cardiovascular health outcomes such as stroke (MacMahon et al., 1990), coronary heart disease and an increase in long-term mortality (van den Hoogen et al., 2000). The Welch Allyn vital sign monitor has been shown to give highly accurate measures of systolic and diastolic blood pressures across a range of different pulse rates (40 – 200 beats per minute) (Davis et al., 2005). The accuracy of 5 units of Welch Allyn vital sign monitors were assessed using an oscillometric non-invasive blood pressure stimulator (Biotek BP pump); 93% of the 150 measurements taken on the Welch Allyn vital sign monitor showed an absolute mean difference between the Biotek simulator below 5mmHg, with an absolute mean bias of 0.7±2.4 mmHg and no clinically relevant precision error between monitors; this resulted in the Welch Allyn vital signs monitor being assigned an A/A rating according to the European Society of Hypertension criteria (O'Brien et al., 2002).</p>
<p>LLEP: Lower limb extensor power; LEP: Leg extensor power; DXA: Dual-energy X-ray absorptiometry; BMD: Bone mineral density; CT: Computerised tomography; MRI: Magnetic resonance imaging; RCT: Randomized controlled trial; HR: Hazard ration; CI: Confidence interval; ICC: Intraclass correlation</p>		

4.3.10. Sample size calculation

We estimated $n=114$ participants would give 80% power at the 5% level (two-sided test) to detect an additional 19% improvement in the primary outcome measure (lower limb power output) above that expected as a result of RET alone (i.e., 29%, assuming standard deviations of 26% and 43% in the two groups). This represented a gain of over 10 'mobility years' assuming an annual loss of power of 1.5%. This sample size also allowed detection of a 1.0-point increase on the SPPB score over and above an assumed 1.5-point increase due to exercise training alone. These calculations are based on a combination of our own pilot data and previously published data (Greig et al., 2011a, Skelton et al., 1995, Young et al., 1983, Perera et al., 2006). Informed by our experience of exercise interventions with older adults and patient groups we added 13 participants to cover an anticipated 10% drop-out rate.

4.3.11. Statistical analysis

All data was entered into a database and analysed using IBM SPSS Statistics for Windows, version 25.0; data analysis was conducted on both an intention to treat and per protocol basis. Normality was assessed by visual inspection of Q-Q plots and the characteristics of participants at baseline were summarised using means and standard deviations, medians and quartiles or counts and percentages, as appropriate. The comparison of primary interest was the difference at baseline and 6-month follow-up between the intervention and control groups. A small number of variables were found to be non-normally distributed; they were significantly skewed and kurtotic, demonstrated significant Kolmogorov-Smirnov/Shapiro-Wilk results and deviations on the normal Q-Q plots. A number of log transformations were attempted,

however, no one transformation was able to normalise all outcome variables and so data was maintained in its non-transformed form. When assessing within-group changes, the Wilcoxon Signed Ranks Test was utilised for these variables and paired T-tests were implemented for normally distributed variables.

Between-group comparisons, adjusted for baseline results, were assessed by analysis of covariance (ANCOVA) for primary and secondary outcome measures, using the Bonferroni correction to control for type I errors arising from multiple comparisons. Covariates applied to the analysis included sex, physical activity and baseline serum 25(OH)D; variables used to stratify participants. Biomarkers at baseline, months 1, 3 and 6 were compared using a repeated-measures analysis of variance (ANOVA). All statistical tests conducted were two-sided with an alpha level of $p = 0.05$. Little's Missing Completely At Random (MCAR) test was non-significant and thus missing data was addressed using multiple imputation as suggested by the study statistician; this was due to the risk of bias, potential loss of precision and power of excluding individuals with missing data from analyses by using exclusion methods such as pairwise or listwise (Sterne et al., 2009). 96.77% of variables at baseline were complete, with 85.48% of all values found to be complete. 5 imputations were calculated using the automated method with minimum/maximum constraints set for variables; linear regression modelling was utilised by SPSS.

4.3.12. Data collection, management and monitoring

All personal data was handled and stored with the strictest confidence and in accordance with the Data Protection Act 2018. Personal details such as home address, name, date of birth and contact number were provided by the participant in the signed participant contact agreement form or in person during an introductory

meeting, with the purpose of future contact. Potential participants were made aware that personal data collected during the study would be kept confidential and stored securely at the University of Birmingham in a locked cabinet accessible only to members of the study team. Additionally, participants were aware that relevant sections of medical notes and data collected during the study may be looked at by responsible individuals from the University Hospitals Birmingham NHS Foundation Trust.

Once allocated to one of the study groups, participants were assigned a unique personal identification code, which was used from that point onwards to identify them in all documentation, correspondence between the participating sites and the case report form (see Appendix H). In the case of specific issues and/or queries from regulatory authorities, it would be necessary to have access to the complete trial records, provided that participant confidentiality was maintained. Personal data will be kept for 10 years after the last data capture in line with University of Birmingham policy to allow for verification.

4.3.13. Missing data

Data reported on each case report form was consistent with the source data and any discrepancies were explained. All missing and ambiguous data was queried.

Individual data sets were checked by the CI at regular intervals and discrepancies highlighted and listed. These were viewed and discussed by the Trial Management Group if and when they arose.

4.3.14. Storage and analysis of samples

In total, 5 blood samples (10mL) were taken from participants completing the study; timepoints are shown in Table 3.3. The samples were analysed at the Queen

Elizabeth clinical haematology laboratory (full blood count, blood biochemistry, liver, and renal function, serum 25(OH)D), the University of Birmingham Institute of Inflammation and Ageing (stress and inflammatory markers) under the supervision of a member of the study team and at the Royal Liverpool Hospital Clinical Laboratories (bone turnover markers) following a material transfer agreement. Blood analysed at the Queen Elizabeth hospital was sent for processing immediately, bloods analysed by the University of Birmingham and the Royal Liverpool Hospital were stored at the Wellcome Trust Clinical Research Facility at the Queen Elizabeth hospital at -80°C for analysis once data collection was complete. Collection, analysis, storage and destruction of residual blood samples was according to local policy and standard operating procedures aligned to the University of Birmingham Quality Management System.

Serum 25(OH)D concentrations were analysed at the Queen Elizabeth clinical haematology laboratory using a chemiluminescent microparticle immunoassay (Alinity i 25-OH Vitamin D Reagent Kit 08P45 by Abbott Laboratories, Illinois, United States). The manufacturer's reference ranges were 6.6 – 53.2 ng/mL (16.6 – 132.9 nmol/L) varying with season, with a lower limit of detection of 3.5 ng/mL (8.8 nmol/L) and a within-laboratory (includes within-run, between-run and between-day) coefficient of variation of 3.3 nmol/L (SD = 0.67). The laboratory is a member of the National External Quality Assurance Scheme (NEQAS) and is externally accredited by the United Kingdom Accreditation Service (UKAS), number 7860 against ISO15189.

Frozen serum samples taken at baseline, months 1, 3 and 6 were transported on dry ice to the University of Birmingham Institute of Inflammation and Ageing for analysis of inflammatory markers including IL-1 β , IL-6, IL-8, IL-10 and TNF- α . The Luminex®

MAGPIX® CCD Imager was used with Magnetic Luminex® High Performance Assay multiplex kits. Manufacturers report less than 0.5% cross-reactivity and interference was observed for all markers. Manufacturer minimum detectable dose (MDD) ranges, intra- and inter-assay precision, recorded as coefficient of variation (CV), for each of the markers measured are as follows; IL-1 β : MDD = 0.164 – 0.820 pg/mL, intra-assay precision = 1.9% and inter-assay precision = 12.5%; IL-6: MDD = 0.200 – 1.24 pg/mL, intra-assay precision = 2.5% and inter-assay precision = 13.9%; IL-8: MDD = 0.163 – 0.923 pg/mL, intra-assay precision = 1.3% and inter-assay precision = 13.7%; IL-10: MDD = 1.54 – 10.1 pg/mL, intra-assay precision = 2.5%, inter-assay precision = 13.4%; TNF- α : MDD = 0.302 – 2.24 pg/mL, intra-assay precision = 1.9%, inter-assay precision = 14.1%.

Frozen serum samples taken at baseline, months 1, 3 and 6 were transported on dry ice to The Royal Liverpool Hospital Clinical Laboratories, where carboxy-terminal collagen crosslinks (CTX), a degradation product of Type I collagen was analysed as a measure of bone resorption and overall bone health. Bone turnover markers, such as CTX have been shown to predict BMD change in response to intervention therapies for osteoporosis (Greenblatt et al., 2017), with decreases in CTX associated with a reduction in spinal fractures and an increase in BMD (Eastell et al., 2011). CTX was analysed using an electrochemiluminescence immunoassay (Elecsys Cobas® β -CrossLaps/serum by Roche Diagnostics, Roche Holding AG, Basel, Switzerland). The manufacturer's reference ranges were 0.299 – 1.008 ng/ml, with a lower limit of detection of 0.01 ng/ml and a repeatability and intermediate precision coefficient of variation of 2.2 and 3.8, respectively.

4.3.15. Monitoring and auditing

This was low-risk single centre trial and thus the study team did not consider the support of a Data Monitoring Committee to be necessary; however, monitoring of the study by the University of Birmingham Clinical Research Compliance Team, including access to source documents as requested, was permitted.

4.3.16. Ethical considerations

A favourable opinion was granted by the Black Country NHS Research Ethics Committee (REC) in December 2014 (14/WM/1220). All study team members received necessary training and conducted the study in accordance with Good Clinical Practice guidelines.

4.3.17. Safety monitoring

Serious adverse events (SAEs), defined as any event that could be related to the study that caused injury or hospitalisation were to be reported to the CI and the Clinical Research Facility's Clinical Manager for review within 24 hours after first becoming aware of the event. All SAEs were to be reviewed formally every 2 weeks during Clinical Research Facility operations meetings which include representation from the Trust Research, Development and Innovation office, however, no SAEs occurred during the study period.

4.3.18. Dissemination policy

Results of this trial will be submitted for publication in peer reviewed journals. The manuscript will be prepared by the study team led by the CI. Authors will acknowledge that the trial was performed with the support of the Royal Osteoporosis Society. Participants will be contacted and provided with a copy of the publication.

4.4. Discussion

Although exercise is currently the only proven mechanism to improve the symptoms of sarcopenia (Offord and Witham, 2017), the anabolic response to RET is blunted in older compared to younger adults (Greig et al., 2011a, Raue et al., 2009) and strategies to potentially overcome this effect are lacking. A recent systematic review highlighted the lack of data in this area (Antoniak and Greig, 2017) and provided tentative support for the combined intervention of vitamin D and RET (Antoniak and Greig, 2017). Very few studies to date have been appropriately designed to test the combined effects of vitamin D and exercise in older adults, and of the studies which have, poor exercise compliance (Bunout et al., 2006a) and small sample sizes have been reported (Agergaard et al., 2015a), limiting interpretation of the data.

Therefore, the EXVITD study aimed to address these issues and bridge the gap in knowledge regarding the potential enhancement of the effects of RET by vitamin D supplementation. A sample size of $n=127$ represents a substantial addition to the current data and as the study team delivered the intervention (both the supplements and the RET sessions), adherence was monitored closely (registers of attendees were taken at each session by the PhD student or exercise instructor, with weekly communications of this information at the end of each week) and encouraged in person. A finding that RET and vitamin D supplementation is effective compared with RET alone would support the development of future multimodal interventions to maintain bone and muscle health in old age and pave the way for further mechanistic and intervention studies examining the effects of RET/vitamin D in conjunction with other promising anabolic agents.

The strengths of this study include the design; a randomized double-blinded placebo-controlled trial is the most appropriate methodology to utilise to assess the primary and secondary outcomes. A range of outcome measures will provide a wealth of information regarding the musculoskeletal health of the participants. Additionally, the present study aimed to recruit older men and women, meaning that the results of the trial will be generalizable with respect to sex.

One limitation of the study is the lack of a precise and quantifiable measure of exertion, exercise progression or muscle loading. Exertion was not be assessed in the present study; the use of objective measures such as heart rate monitors or rate of perceived exertion scales were rejected due to additional participant burden. Exercise progression and muscle loading would be more objectively measured if gym-based resistance equipment were to be employed, however, the benefit of using body weight, ankle weights and physiotherapy bands is that these equipment will be more familiar and readily adapted to daily practice, so that new-found exercise habits may be maintained following the close of the study. Additionally, previous studies have emphasized benefit of vitamin D supplementation in deficient participants (Beudart et al., 2014a); since vitamin D deficiency is an exclusion criterion, we may be less likely to observe meaningful clinical effects of supplementation in our vitamin D insufficient or replete participants.

4.5. Personal development and lessons learned

Table 4.5. Personal reflections

This is a short reflective section detailing my personal development throughout the study and the lesson that I learnt during recruitment and through implementing the study.

The various amendments submitted throughout the EXVITD study are displayed in Table 4.6. It took 7 minor and 6 substantial amendments to arrive at a working protocol (Version 10.0). The addition of this short reflective section within the methods chapter was deemed necessary, as a substantial amount of time during the PhD was spent attempting to recruit. Originally, we aimed to recruit men and women aged ≥ 70 years and living in sheltered housing accommodation within Birmingham. It was expected that this group of older adults would be pre-frail or frail but still able to maintain independence and hence an appropriate population to benefit from an exercise intervention.

The reality of the experience was rather different from expected; older adults living within sheltered housing accommodation were older and much frailer, with many more co-morbidities (such as dementia) than expected. Many of the residents considered 3 sessions of exercise per week for 6 months was too big a time commitment for them; residents were also wary about taking any supplements in addition to their prescribed medications. Moreover, the

support we initially received from the Bournville Village Trust (a large housing association overseeing 8000 properties in Birmingham) ended when their manager left the organisation.

An additional advantage to targeting sheltered housing accommodation was that use of a communal living area for the exercise session would make attendance easy for the residents. In practice, it was extremely difficult to gain any access to the residents. Supported housing accommodation managers acted as “gatekeepers” and access to residents was only permitted if they agreed; approximately two thirds of opportunities to discuss the study with residents were denied or ignored, therefore, residents were unable to make an informed decision about participation in the study. Figure 4.4 displays the flowchart for recruitment via sheltered housing.

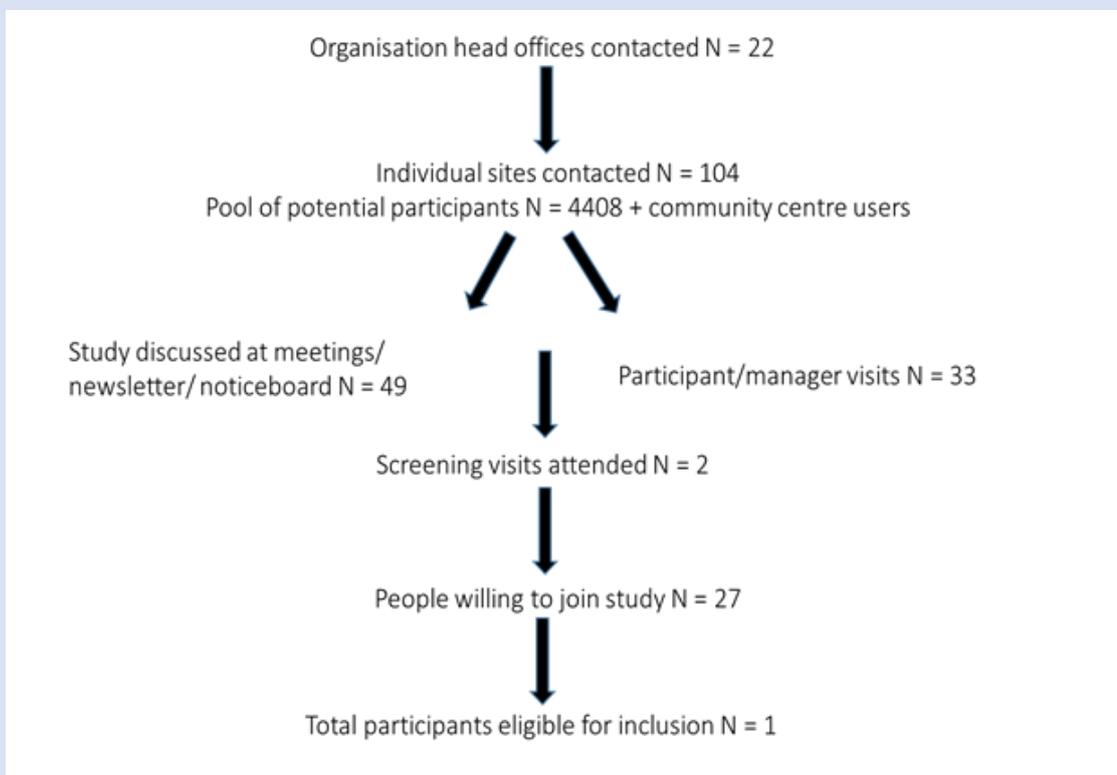


Figure 4.4: Sheltered housing accommodation recruitment flow diagram

Head offices of sheltered housing organisations were initially contacted, then sheltered housing managers. If they agreed, the study was presented at resident's meetings and coffee mornings or the study flyer was displayed on communal noticeboards or within newsletters. Of the interested residents, only 1 was found to be eligible; the main reason for lack of eligibility was that many of the residents were already prescribed or taking supplemental vitamin D, which at the time was an exclusion criterion.

The approval of substantial amendments 2 to 6 meant that we were able to recruit and form our first group of participants; via these amendments we were able to advertise and recruit more widely without the prescribed/supplemental vitamin D exclusion criterion, recruit from a wider pool of potential participants by lowering the age entry criterion from 70 years to 65 years and also recruiting community-dwelling participants to participate in exercise sessions held in a public space. The obvious downside to these amendments was that we recruited a slightly different group of older adults than we were originally aiming to include; our group of participants were more independent and functionally able than we planned. Additionally, we did not recruit our target number of participants as indicated by the sample size calculation, meaning that the study was underpowered.

Table 4.6: Protocol development			
Amendment type & number	Date	New documentation version	Reason for amendment
Initial ethics approval	08/12/14	Protocol V. 1.0 PIS V. 2.0	
Minor amendment 1	21/08/15	Protocol V.2.0	<ul style="list-style-type: none"> - Addition of falls & fractures as events - Use of CRF instead of health research bus - Storage, labelling & allocation of vitamin D at UoB instead of CRF - No urine samples to be taken
Minor amendment 2	14/12/15	PIS V.3.0	<ul style="list-style-type: none"> - PIS amended to reflect above changes
Minor amendment 3	05/01/16	Protocol V.3.0 PIS V.4.0	<ul style="list-style-type: none"> - Addition of CTX & PTH blood markers
Substantial amendment 1	25/01/16	Protocol V.4.0 PIS V.5.0	<ul style="list-style-type: none"> - Additional hip & spine DXA scans
Minor amendment 4	11/04/16	Protocol V.5.0	<ul style="list-style-type: none"> - Updates to protocol: update contact details/co-investigators/change “drug” to “supplement” - Blood pressure to be measured at baseline - Advertise study on Birmingham City Council website
Substantial amendment 2	22/07/16	Protocol V.6.0 PIS V.6.0	<ul style="list-style-type: none"> - Lowering of age criterion from 70 years to 65 years - Advertise study via the 1000 Elders database/ appropriate websites/ print media - Recruit community-dwelling (independent) older adults
Substantial amendment 3	01/11/16	Protocol V.7.0	<ul style="list-style-type: none"> - Removal of daily supplemental vitamin D (>400IU) and/or calcium (>500mg) use exclusion criterion - Advertisement of study displayed in GP practices
Substantial amendment 4	10/02/17	Protocol V.8.0	<ul style="list-style-type: none"> - Addition of Clinical Research Network and BUPA support for recruitment - Use of public spaces to hold exercise intervention
Minor amendment 5	12/04/17	Protocol V.9.0	<ul style="list-style-type: none"> - Additional 21 days between enrolment and intervention start to allow time to conduct multiple screening visits and merge participants into 1 exercise group
Minor amendment 6	31/05/17	GP letter V.2.0	<ul style="list-style-type: none"> - Amend GP letter to reflect the lowering of age criterion and removal of a urine sample request
Substantial amendment 5	06/07/17	GP summary V.1.0 Patient invitation letters V.1.0	<ul style="list-style-type: none"> - Submission of CRN supporting documentation
Substantial amendment 6	04/08/17	Patient contact agreement V.1.0 Patient reminder letter V.1.0	<ul style="list-style-type: none"> - Submission of further CRN supporting documentation
Minor amendment 7	28/08/18	Protocol V.10.0	<ul style="list-style-type: none"> - Health questionnaire completed during screening to be completed via the telephone

CHAPTER 5

RESULTS OF A RANDOMIZED CONTROLLED TRIAL INVESTIGATING THE INFLUENCE OF COMBINED VITAMIN D3 SUPPLEMENTATION AND RESISTANCE EXERCISE TRAINING ON MUSCULOSKELETAL HEALTH IN OLDER MEN AND WOMEN (EXVITD)

5.1. Baseline characteristics

Participant characteristics at baseline measurement are summarised in Table 5.1; median age and interquartile range of the participants in the placebo (n = 12) and vitamin D (n = 12) groups were 70.50 ± 5.75 and 68.00 ± 6.50 years, respectively. There was a similar distribution of males and females in each group and the mean BMI categorised the participants as overweight. Importantly, there were no significant differences in stratifying outcomes (sex, serum 25(OH)D and mean step count). Mean baseline daily step counts fell below the recommended 7000 – 10,000 steps per day for participants in both groups, although was achieved by 41.67% (n=10) of all participants. Mean serum 25(OH)D concentrations at baseline were within the adequate range for both groups; placebo group = 50.65 ± 14.85 nmol/L and vitamin D group = 51.31 ± 18.03 nmol/L. 11 (36.67%) and 19 (63.33%) participants at initial screening were considered vitamin D insufficient (<50 nmol/L) and adequate (>50 nmol/L), respectively. There were significant differences at baseline in height, weight, perceived general health assessed by the SF-36 questionnaire and total lean mass. The significant differences in total body weight were due to the inclusion of one participant with a BMI of over 45 in the placebo group. There were no between-group differences in baseline total body weight if this participant were excluded (placebo group = 76.97 (14.89) kg, vitamin D group = 72.54 (16.29) kg, $t(1,21) = 0.68$, $p = 0.505$). However, analyses were conducted both including and excluding this participant, and their inclusion did not significantly affect any of the results, therefore, to keep the sample as representative as possible participants were not removed due to outlying data points, alternatively, where appropriate, relative outcome measures were also calculated (e.g. relative LLEP, peak relative power).

5.2. EXVITD attrition and compliance

Of the 24 participants randomized to the intervention, 19 (79.17%) completed the study, resulting in an attrition rate of 20.83%. There were 2 dropouts from the placebo group and 3 from the vitamin D group; reasons given for withdrawal were ill health ($n = 3$), changing mind about participation ($n = 1$) and no explanation ($n = 1$). 89.08% of prescribed exercise sessions were attended by participants and supplement compliance was 92.95% (Appendices J and K). On average, participants significantly increased their 30 second chair rise test challenge score by 14 points ($t(17) = 9.46, p < 0.001$, calculated from Appendix I exercise progression log), with no significant difference between the groups. The 30 second chair rise test was used as a method of assessing progression and was also a policy implemented by Move It Or Lose It (company that trained the seated exercise specialist). An increase of 3 repetitions on the 30 second chair rise test has previously been concluded as a clinically meaningful increase in performance (Johansen et al., 2016).

5.3. Falls and fracture incidence

A fall, using the generally accepted description, was defined as “an event which results in a person coming to rest inadvertently on the ground or floor or other lower level” (World Health Organization, 2018). 3 falls occurred throughout the study period and all falls occurred at a time outside of the scheduled exercise sessions. None of the falls caused serious injury or resulted in a fracture; all fallers recovered from their fall within 24 hours, with minor bruising. 2 of the 3 fallers were male and all fallers were randomized to the placebo group.

Table 5.1: Baseline characteristics of EXVITD participants				
	Whole cohort (n = 24) Mean (SD)	Placebo group (n = 12) Mean (SD)	Vitamin D group (n = 12) Mean (SD)	p value
Demographics				
Sex (% female)	45.83	42.67	50.00	0.682
Age (years)*	70.00 (6.00)	70.50 (5.75)	68.00 (6.50)	0.443
Height (m)	1.66 (0.11)	1.69 (0.11)	1.64 (0.12)	0.006
Weight (kg)	74.68 (18.14)	80.76 (18.63)	72.54 (15.71)	0.005
BMI (kg.m ⁻²)	27.24 (6.31)	28.52 (7.83)	26.99 (4.82)	0.159
SBP (mmHg)	138.06 (21.93)	131.69 (17.66)	132.64 (16.39)	0.740
DBP (mmHg)	79.37 (11.35)	78.50 (13.11)	76.67 (6.37)	0.288
VAS pain sitting*	0.00 (1.00)	0.00 (0.75)	68.00 (6.50)	0.872
VAS pain standing*	0.00 (1.00)	68.00 (6.50)	68.00 (6.50)	0.932
SF-36 Physical function score (%)	75.00 (21.63)	74.17 (19.29)	81.67 (19.46)	0.353
SF-36 General health score (%)	73.27 (14.14)	68.75 (10.25)	80.83 (13.62)	0.022
MMSE*	29.00 (2.00)	29.50 (1.00)	28.50 (2.75)	0.068
Average step count	6708.55 (2527.13)	6518 (2577)	6451 (2463)	0.873
Functional characteristics				
LLEP (W)	128.46 (71.40)	150.75 (70.04)	112.58 (74.22)	0.209
SPPB total score	9.85 (2.09)	9.83 (2.37)	9.83 (2.04)	1.000
TUG (s)	7.93 (2.58)	7.43 (3.17)	8.23 (2.12)	0.475
Peak total power (kW)	0.55 (0.20)	0.56 (0.19)	0.57 (0.22)	0.980
5-time CRT time (s)	7.02 (3.10)	7.50 (4.31)	6.42 (1.37)	0.417
Body composition characteristics				
Total fat mass (kg)	25.72 (10.21)	26.90 (11.90)	24.77 (8.40)	0.216
Total lean mass (kg)	46.45 (9.85)	48.98 (8.67)	44.12 (9.12)	0.001
Femoral neck BMD (g.cm ³)	0.92 (0.14)	0.94 (0.11)	0.93 (0.15)	0.855
Lumbar spine BMD (g.cm ³)	1.27 (0.23)	1.32 (0.23)	1.28 (0.13)	0.654
Biochemical characteristics				
Serum 25(OH)D (nmol/L)	50.21 (19.80)	50.65 (14.85)	51.31 (18.03)	0.817
* values shown for non-normal variables are median (interquartile range) SD: Standard deviation; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; VAS: Visual analogue scale; SF-36: Short form health questionnaire; MMSE: Mini mental state exam; LLEP: Lower limb extensor power; SPPB: Short physical performance battery; TUG: Timed up and go; 5-time CRT: Chair rise test completed using Leonardo Mechanography® ; S: seconds. p refers to the difference between the placebo and vitamin D groups (independent samples t-test or Mann Whitney-U)				

5.4. Functional outcomes

Within-group changes in functional outcomes are displayed in Table 5.2. Absolute power output is presented in Tables 5.1, 5.2 and 5.5. Relative power output was also analysed as there were significant differences in body weight at baseline, although changes in body weight did not significantly differ within- or between-groups.

Standardising for total body weight did not alter the LLEP output results; there was no significant change within the placebo group ($+0.23$ (0.47) W.kg^{-1} , $t(9) = 1.56$, $p = 0.153$) and the change in relative LLEP within the vitamin D group remained significant ($+0.31$ (0.38) W.kg^{-1} , $t(8) = 2.46$, $p = 0.040$). Similarly, there were no between group differences in change in relative LLEP; difference in placebo – vitamin D pooled mean result at 6 months = -0.008 (0.16), $F(1,13) = 0.19$, $p = 0.674$.

Overall, improvements were observable in all functional outcomes measured, with the exception of gait speed. When analysing within-group changes, the vitamin D group significantly increased their LLEP by 19.11% ($p = 0.038$), and the placebo increased their power output by 11.35% (not significant). SPPB total score significantly increased in the vitamin D group and decreased in the placebo group. The 5-time chair rise time was significantly reduced in both groups by 35.60% and 44.69% in the placebo and vitamin D groups, respectively. The TUG test time was faster in both groups at month 6 in comparison to baseline; -8.16% and -18.56% in the placebo and vitamin D groups, respectively, with statistical significance observed in the vitamin D group ($p = 0.034$). Peak total power significantly increased within the whole cohort and the placebo ($+27.71\%$) and vitamin D ($+25.93\%$) groups. The 5-time chair rise test assessed using Leonardo Mechanography® was significantly increased in both the placebo and vitamin D groups, with no between-group differences.

As with LLEP, relative peak power was calculated to account for body weight. Change in peak relative power was significant within the whole cohort (+3.03 (1.85) W.kg⁻¹, $p < 0.0001$), placebo group (+3.17 (2.19) W.kg⁻¹, $p = 0.002$) and vitamin D group (+2.86 (1.45) W.kg⁻¹, $p = 0.002$); there were no between-group differences (difference in placebo – vitamin D pooled mean result at 6 months = 0.93 (0.96), $F(1,5) = 0.94$, $p = 0.378$).

	Whole cohort				Placebo group				Vitamin D group			
	Baseline (n = 24)	Month 6 (n =19)*	Change	p	Baseline (n = 12)	Month 6 (n = 10)	Change	p	Baseline (n = 12)	Month 6 (n = 9)	Change	p
LLEP (W)	137.26 (74.34)	157.00 (87.75)	19.74 (31.28)	0.006	159.30 (70.98)	177.40 (97.17)	18.10 (36.72)	0.153	112.78 (74.09)	134.33 (73.78)	21.56 (26.02)	0.038
SPPB total score	9.95 (2.25)	11.21 (1.32)	1.26 (1.76)	0.002	10.00 (2.41)	9.09 (6.22)	- 0.91 (6.20)	0.637	9.89 (2.03)	11.56 (0.53)	1.67 (1.87)	0.028
Gait speed (s)	3.78 (1.63)	3.57 (0.97)	- 0.21 (1.47)	0.528	3.66 (1.72)	3.61 (1.16)	- 0.05 (1.65)	0.925	3.91 (1.60)	3.52 (0.78)	- 0.39 (1.32)	0.397
5-time chair rise (s)	11.69 (3.01)	6.99 (2.56)	- 4.70 (2.63)	< 0.001	11.01 (1.48)	7.09 (3.43)	- 3.93 (2.48)	0.001	12.44 (3.06)	6.88 (1.23)	- 5.56 (2.67)	<0.001
TUG (s)	7.82 (2.98)	6.78 (2.15)	- 1.04 (1.46)	0.002	7.48 (3.50)	6.87 (2.84)	- 0.61 (0.98)	0.081	8.19 (2.42)	6.67 (1.14)	- 1.52 (1.79)	0.034
Peak total power (kW)	0.60 (0.21)	0.82 (0.31)	0.22 (0.14)	<0.001	0.60 (0.20)	0.83 (0.30)	0.23 (0.15)	0.001	0.60 (0.25)	0.81 (0.34)	0.21 (0.14)	0.008
5-time CRT time (s)	7.21 (3.49)	4.19 (2.49)	- 3.01 (2.16)	<0.001	7.85 (4.68)	4.64 (3.40)	- 3.21 (2.84)	0.006	6.49 (1.32)	3.75 (0.68)	- 2.75 (1.14)	<0.001

Values shown are pooled mean (standard deviation). * N = 16 at month 6 for peak total power and 5-time CRT due to technical issues with the Leonardo Mechanography® Ground Reaction Force Plate
LLEP: Lower limb extensor power; SPPB: Short physical performance battery; TUG: Timed-up and go; 5-time CRT: Chair rise test completed using Leonardo Mechanography®; S: seconds. *p* refers to the change from baseline to month 6 within each group (paired sample t-test)

5.5. Body composition outcomes

Within-group changes in body composition outcomes are displayed in Table 5.3. All outcomes were found to change non-significantly, with the exception of total lean mass, which significantly increased within the placebo group (+ 1.37%). Lean mass was shown to increase within the vitamin D group, but by a smaller and non-significant amount (+ 0.31%, $p = 0.678$). The changes in bone parameters (femoral neck and lumbar spine BMD) were small and non-significant in both groups. A small amount of fat mass was lost in the placebo group (- 1.37%) and gained in the vitamin D group (+ 0.31%), however both of these changes were non-significant. Within-group change in ASM and SMI was greater in the placebo group than within the vitamin D group, although this was also non-significant.

	Whole cohort				Placebo group				Vitamin D group			
	Baseline (n = 24)	Month 6 (n =19)	Change	p	Baseline (n = 12)	Month 6 (n = 10)	Change	p	Baseline (n = 12)	Month 6 (n = 9)	Change	p
Total body weight (kg)	75.81 (17.45)	75.57 (15.53)	- 0.23 (3.44)	0.769	80.90 (18.56)	80.08 (16.15)	- 0.82 (4.62)	0.580	70.14 (13.98)	70.57 (15.15)	0.42 (1.67)	0.470
Femoral neck BMD (g.cm³)	0.91 (0.13)	0.91 (0.13)	0.00 (0.02)	0.407	0.92 (0.10)	0.92 (0.09)	0.00 (0.02)	0.414	0.91 (0.09)	0.91 (0.09)	0.00 (0.02)	0.850
Lumbar spine BMD (g.cm³)	1.30 (0.19)	1.30 (0.20)	0.00 (0.03)	0.963	1.31 (0.24)	1.32 (0.24)	0.01 (0.02)	0.647	1.31 (0.18)	1.31 (0.19)	0.00 (0.04)	0.955
Hip T-score	- 0.59 (0.90)	- 0.59 (0.95)	0.00 (0.18)	0.897	- 0.61 (0.82)	- 0.64 (0.94)	- 0.03 (0.24)	0.703	- 0.57 (1.04)	- 0.54 (1.01)	0.02 (0.07)	0.347
Total fat mass (kg)	25.61 (9.96)	25.65 (9.51)	0.04 (1.12)	0.915	27.04 (12.70)	26.80 (12.38)	- 0.25 (1.13)	0.509	24.03 (6.04)	24.37 (5.26)	0.34 (1.26)	0.448
Total lean mass (kg)	45.90 (9.65)	46.31 (9.96)	0.41 (0.79)	0.023	48.89 (8.58)	49.56 (8.91)	0.67 (0.61)	0.007	42.57 (10.15)	42.70 (10.30)	0.13 (0.91)	0.678
ASM (kg)	19.18 (5.62)	20.23 (4.97)	1.05 (3.68)	0.275	19.79 (1.48)	21.76 (4.06)	1.97 (4.99)	0.244	18.50 (5.57)	18.53 (5.56)	0.02 (0.50)	0.890
SMI	25.83 (6.01)	26.74 (4.20)	0.91 (3.64)	0.215	25.67 (8.14)	27.54 (5.01)	1.87 (4.85)	0.254	25.99 (2.59)	25.84 (3.11)	-0.15 (0.91)	0.626

Values shown are pooled mean (standard deviation). BMD: Bone mineral density; ASM: Appendicular skeletal muscle mass; SMI: Skeletal muscle index. p refers to the change from baseline to month 6 within each group (paired sample t-test)

5.6. Physical activity outcomes

Within-group changes physical activity outcomes are displayed in Table 5.4. No significant changes in any of the physical activity outcomes assessed by ActivPAL accelerometry were observed. Average daily step count assessed over 7 days increased in the whole cohort, as expected (+ 128.07%). The largest increase was attributed to the vitamin D group, which increased their average daily step count by 16.61%. Average time spent sitting/lying increased non-significantly in both groups at month 6, consistent with a small decrease in energy expenditure in both groups. Average time spent standing and average up/down transitions were also decreased in both groups. Average time spent stepping per day decreased marginally in the placebo group but was increased by 24.79% within the vitamin D group at month 6, however this increase was non-significant.

Table 5.4: Within group changes in physical activity outcomes												
	Whole cohort				Placebo group				Vitamin D group			
	Baseline (n = 24)	Month 6 (n =19)	Change	<i>p</i>	Baseline (n = 12)	Month 6 (n = 10)	Change	<i>p</i>	Baseline (n = 12)	Month 6 (n = 9)	Change	<i>p</i>
Average daily step count	5820.34 (2295.44)	6234.23 (2889.79)	413.87 (2121.61)	0.396	6258.40 (2872.73)	6296.10 (3010.35)	37.70 (1656.74)	0.944	5283.33 (1417.13)	6160.86 (2929.80)	877.53 (2573.38)	0.336
Average time spent sitting/lying (hours/day)	16.52 (4.70)	18.95 (3.64)	2.43 (5.07)	0.075	16.08 (5.78)	19.07 (2.91)	2.99 (5.60)	0.127	17.41 (1.84)	19.12 (4.12)	1.71 (3.64)	0.197
Average time spent standing (hours/day)	4.72 (1.67)	4.67 (1.75)	- 0.05 (1.00)	0.823	4.33 (1.53)	4.17 (1.71)	- 0.16 (1.26)	0.707	4.86 (1.77)	4.64 (1.89)	- 0.22 (1.04)	0.546
Average time spent stepping (hours/day)	1.39 (0.49)	1.50 (0.58)	0.11 (0.54)	0.454	1.49 (0.55)	1.40 (0.67)	- 0.09 (0.45)	0.540	1.17 (0.36)	1.46 (0.70)	0.29 (0.67)	0.223
Average up/down transitions per day*	40.23 (12.00)	44.00 (17.00)	3.77	0.117	41.74 (9.50)	46.00 (18.50)	- 0.15	0.878	36.50 (26.50)	42.00 (23.25)	- 1.60	0.109
Average daily EE (METs)*	33.09 (1.88)	33.04 (2.00)	- 0.05	0.528	33.31 (1.70)	33.09 (2.12)	- 0.05	0.959	32.35 (2.50)	33.02 (4.00)	- 0.84	0.401

Values shown are pooled mean (standard deviation). * Median (interquartile range) and Z-scores are reported from the Wilcoxon Signed Ranks Test for within group differences due to non-normal distribution. EE: Energy expenditure; METs: Metabolic equivalents. *p* refers to the change from baseline to month 6 within each group (paired sample t-test)

Table 5.5: Between group comparisons for functional, body composition and physical activity outcomes					
	Adjusted month 6 pooled mean (SE)		Pooled mean change (SE)	ANCOVA results	
	Placebo group (n = 10)	Vitamin D group (n = 9)	Placebo - vitamin D	F(df)	p
Functional outcomes					
LLEP (W)	154.54 (7.16)	159.74 (7.58)	- 5.20 (10.89)	0.12 (1,13)	0.737
SPPB total score	9.40 (1.65)	11.62 (1.54)	- 2.23 (2.29)	1.21 (1,13)	0.291
Gait speed (s)	3.71 (0.30)	3.40 (0.32)	0.31 (0.46)	0.47 (1,13)	0.504
5-time chair rise (s)	7.40 (0.73)	6.52 (0.77)	0.88 (1.09)	0.62 (1,13)	0.444
TUG (s)	7.09 (0.33)	6.43 (0.35)	0.66 (0.48)	1.87 (1,13)	0.194
Peak total power (kW)	1.63 (0.65)	1.13 (0.66)	0.50 (0.93)	0.36 (1,13)	0.557
5-time CRT time (s)	3.83 (0.60)	4.59 (0.67)	- 0.76 (0.95)	0.46 (1,13)	0.510
Body composition outcomes					
Total body weight (kg)	80.08 (16.15)	70.57 (13.98)	- 0.61 (1.24)	0.21 (1,13)	0.654
Total BMC (kg)	2.69 (0.01)	2.66 (0.01)	0.03 (0.02)	2.76 (1,13)	0.121
Hip T-score	- 0.62 (0.06)	- 0.57 (0.07)	- 0.05 (0.10)	0.37 (1,13)	0.552
Total fat mass (kg)	25.45 (0.37)	25.87 (0.40)	- 0.42 (0.57)	0.53 (1,13)	0.481
Total lean mass (kg)	46.47 (0.24)	46.13 (0.26)	0.33 (0.37)	0.92 (1,13)	0.355
ASM (kg)	21.30 (0.96)	19.03 (1.01)	2.27 (1.43)	2.53 (1,13)	0.136
SMI	27.31 (0.73)	26.10 (0.78)	1.20 (1.12)	1.16 (1,13)	0.300
Physical activity outcomes					
Average daily step count	5972.16 (694.80)	6532.27 (742.10)	- 560.11 (1039.04)	0.24 (1,14)	0.633
Average time spent sitting/lying (hours/day)	19.20 (1.11)	19.15 (1.17)	0.06 (1.64)	0.01 (1,13)	0.939
Average time spent standing (hours/day)	4.17 (0.42)	4.60 (0.46)	- 0.43 (0.67)	0.06 (1,13)	0.810
Average time spent stepping (hours/day)	1.27 (0.21)	1.54 (0.23)	- 0.27 (0.34)	1.04 (1,14)	0.325
Average up/down transitions per day	43.34 (3.26)	48.14 (3.47)	- 4.80 (4.84)	0.94 (1,13)	0.351
Average daily EE (METs)	33.21 (0.72)	33.14 (0.81)	0.05 (1.13)	0.04 (1,11)	0.841
Model adjusted for baseline value, sex, baseline 25(OH)D and baseline average daily step count. SE: Standard error; LLEP: Lower limb extensor power; SPPB: Short physical performance battery; TUG: Timed up and go; BMC: Bone mineral content; ASM: Appendicular skeletal muscle mass; SMI: Skeletal muscle index; EE: Energy expenditure; METs: Metabolic equivalents. <i>p</i> refers to the between group mean difference (ANCOVA)					

5.7. Between-group comparisons

Table 5.5 shows the primary comparisons of interest; between group comparisons for functional, body composition and physical activity outcomes. In summary, no significant differences between the placebo and vitamin D groups were observable at month 6 after the completion of 6 months of RET and vitamin D or placebo supplementation daily in any of the outcome measures assessed. For functional outcomes, the vitamin D group exhibited higher LLEP and SPPB total score and a faster gait speed, 5-time chair rise and TUG time than the placebo group. Peak total power was higher and 5-time CRT was faster within the placebo group at month 6.

With regards to body composition outcomes, ASM, SMI, total lean mass and total BMC were all marginally higher within the placebo group at month 6 in comparison to the vitamin D group. Hip T-score was marginally higher within the vitamin D group in comparison to the placebo group at month 6. Very small between-group changes were observable in physical activity outcomes; average daily step count, average time spent standing and stepping and the number of up/down transitions were all marginally higher within the vitamin D group at month 6. Energy expenditure and average time spent sitting/lying were marginally higher within the placebo group.

Table 5.6: Between-group comparisons in biomarker concentrations at different time points throughout EXVITD										
	Placebo group (n = 9) Adjusted pooled mean (SD)				Vitamin D group (n = 9) Adjusted pooled mean (SD)				Between-group comparisons	
	Baseline	Month 1	Month 3	Month 6	Baseline	Month 1	Month 3	Month 6	F (df)	p
25(OH)D (nmol/L)	50.44 (16.25)	60.20 (26.09)	50.95 (15.92)	59.69 (17.14)	55.63 (19.60)	74.83 (17.14)	69.33 (15.67)	79.83 (13.77)	7.21 (1,17)	0.016
PTH (pmol/L)	5.57 (1.99)	6.35 (3.17)	6.06 (2.79)	5.81 (1.98)	5.54 (1.42)	5.13 (1.48)	5.46 (1.43)	5.81 (1.36)	0.21 (1,14)	0.651
Calcium (mmol/L)	2.40 (0.12)	2.41 (0.10)	2.43 (0.05)	2.32 (0.08)	2.39 (0.15)	2.46 (0.11)	2.44 (0.09)	2.33 (0.25)	0.48 (1,13)	0.502
CTX (µg/L)	0.32 (0.18)	0.31 (0.19)	0.31 (0.20)	0.28 (0.17)	0.25 (0.11)	0.30 (0.16)	0.35 (0.22)	0.27 (0.13)	0.11 (1,9)	0.752
Model adjusted for baseline value, sex, baseline 25(OH)D and baseline average daily step count. PTH: Parathyroid hormone; CTX: Carboxterminal collagen crosslinks. p refers to the between group mean difference (ANCOVA)										

Table 5.7: Whole group results and within-group comparisons of inflammatory markers at baseline and 6-months												
	Whole group				Placebo group				Vitamin D group			
	N	Baseline	Month 6	Change	N	Baseline	Month 6	Change	N	Baseline	Month 6	Change
IL-6 (pg/ml)	6	2.39 (2.63)	2.96 (3.82)	0.57 (1.24)	4	2.72 (3.33)	3.55 (4.77)	0.83 (1.46)	2	1.73 (0.04)	1.78 (0.69)	0.05 (0.38)
IL-8 (pg/ml)	8	5.64 (3.69)	4.77 (3.16)	0.05 (0.38)	5	6.45 (4.27)	5.67 (3.50)	-0.78 (1.80)	3	4.29 (2.60)	3.27 (2.24)	-1.02 (0.38)
IL-10 (pg/ml)	14	27.65 (33.48)	21.43 (20.77)	-8.82 (26.68)	6	47.94 (44.19)	26.79 (30.32)	-21.51 (29.66)	8	18.14 (12.09)	18.56 (13.87)	0.42 (19.83)

5.8. Blood marker outcomes

Table 5.6 shows the between-group comparisons in biomarker concentrations at baseline and months 1, 3 and 6. As expected, the vitamin D group significantly increased their serum 25(OH)D concentrations at month 6 from baseline ($F(3,24) = 3.79, p = 0.023$); no significant increases in serum 25(OH)D concentration were observed within the placebo group ($F(3,27) = 1.30, p = 0.294$). Additionally, serum 25(OH)D concentrations were significantly higher at 6 months within the vitamin D group in comparison to the placebo group ($p = 0.033$). PTH, calcium and CTX serum concentrations did not significantly differ between the 2 groups.

Table 5.7 shows the whole cohort, placebo and vitamin D group results and within-group comparisons of IL-6, IL-8 and IL-10 inflammatory markers at baseline and 6-months. No significant changes in any of the inflammatory markers were observed in the whole cohort nor the 2 groups between baseline and month 6.

CHAPTER 6

DISCUSSION OF EXVITD RESULTS

The primary aim of this study was to determine between-group differences in musculoskeletal function and health after 6 months of RET and/or 800 IU vitamin D3 or placebo supplementation daily. Secondary aims included comparisons within- and between groups of body composition, physical activity, blood and bone turnover markers (including vitamin D, calcium, CTX), and falls/fractures.

6.1. Primary outcome measures: Functional outcomes

In summary, gains in power or improvements in functional ability were generally higher within the EXVITD study compared with previous exercise and vitamin D supplementation studies. For example, gains in LLEP, SPPB total score, peak total power and TUG time were all greater than previously observed. There were no significant between-group differences to support the additive effect of vitamin D and RET for any of the outcome measures. However, within-group differences revealed the largest changes in functional outcomes were within the vitamin D group and several outcomes achieved a statistically significant change within this group only. The total study sample size was substantially lower than required, as estimated by the sample size calculation, suggesting that the EXVITD study was simply under-powered to detect any possible between-group differences.

6.1.1. Lower limb extensor power (LLEP)

Lower-limb extensor power output (LLEP) was chosen as the primary outcome measure for the EXVITD study, since decline in muscle power is known to be strongly associated with mobility limitation (Byrne et al., 2016), which in turn is associated with reduced quality of life and independence in older adults (Pahor et al., 2014). Although both the placebo and vitamin D groups improved their LLEP, this change reached significance only within the vitamin D group, in which a large effect

size was observed (+18.10W or +11.35%, $p = 0.153$ Vs +21.56W or +19.11%, $p = 0.038$, $d = 0.83$). An estimated additional +19% increase in LLEP (i.e., up to 38%) in the vitamin D group would have been needed to detect a significant difference between the vitamin D and placebo groups.

At baseline, the relative LLEP of the whole EXVITD cohort (1.81 (0.91) W.kg⁻¹) was precisely equivalent to the 50th percentile of leg power for the 65-74 years age category reported by the Allied Dunbar National Fitness Survey (ADNFS) (Sports Council and Health Education Authority, 1992), a UK national study of physical activity and fitness conducting physical testing in 1318 older adults including LLEP via the Nottingham rig. Analysing the baseline relative LLEP of each of the intervention groups separately, the placebo group average relative power was 2.06 (0.97) W.kg⁻¹, placing them above average in comparison to the ADNFS cohort, whilst the vitamin D group average relative power was 1.53 (0.80) W.kg⁻¹, placing them between the 10th and 50th percentile (1.10 and 1.81 W.kg⁻¹, respectively) (Sports Council and Health Education Authority, 1992). By month 6 of the study, the placebo group increased their relative power to 2.29 (1.30) W.kg⁻¹, an increase of 10.04%, and still above average compared to the ADNFS, although this within-group change was non-significant ($p = 0.153$). The vitamin D group significantly increased their relative power to 1.84 (0.72) W.kg⁻¹ (an increase of 16.85%, $p = 0.040$), meaning that their relative power was now comparable to the 50th percentile reported by the ADNFS (Sports Council and Health Education Authority, 1992). There were no significant between-group differences ($p = 0.674$).

The gains in LLEP within the RET and vitamin D group of the EXVITD study were greater than other RET studies not incorporating vitamin D3 supplementation, yet baseline power was lower within the EXVITD study in two of the three studies

(Vestergaard et al., 2008, Cegielski et al., 2017). A 24 week RET study including older women of mean age 67.5 years resulted in a significant +15.7% increase in peak knee isometric knee extensor power measured (Edholm et al., 2017).

Furthermore, other exercise training studies of differing duration report lower muscle power gains measured using the Nottingham power rig as follows; + 14.00% in 12 men and women (mean age = 63 years) after 4 weeks home-based exercise training (Cegielski et al., 2017), +10.19% in 650 women (mean age = 81 years) after 24 weeks home-based exercise video training (Vestergaard et al., 2008) and +15.84% in 38 men and women (mean age = 76.6 years) after 52 weeks strength training (Capodaglio et al., 2007).

Comparisons with other studies is challenging since muscle power measurement methodology, training regimen and duration and participant population group vary; additionally, few studies incorporating vitamin D supplementation and RET in older adults currently exist, and fewer still assess muscle power over muscle strength. However, 2 of the studies included in the systematic review reported in Chapter 2 measured muscle power. One of these explored the effects of 12 weeks of strength, power or no training on 69 older men and women (mean age = 77 years) and included a “run-in” phase of 8 weeks prior to training, in which all participants were supplemented with 1000 or 2000 IU vitamin D3 daily, dependent on baseline 25(OH)D status. After the run-in phase of 8 weeks of vitamin D3 supplementation, muscle power (measured as sit-to-stand transfer power) was significantly increased in the cohort (+3.58%, $p = 0.017$). Participants were then randomized to 1 of the 3 groups and completed a 12-week training period; power was measured again and was shown to increase in both the strength training (+8.25%) and control (+3.58%) groups, although there were no significant between-group differences ($p = 0.308$)

(Drey et al., 2011). In the second study included in the systematic review, functional muscle power, assessed using the timed stair climb test, increased significantly after 12 months of 1000 IU vitamin D3 supplementation per day and RET compared with controls in a study of 162 older adults (mean age = 67 years). However, since all participants were supplemented with vitamin D3, any changes were attributed to RET (+4.5%, $p < 0.005$) (Gianoudis et al., 2014).

A more recent study not included in the 2017 published systematic review, which reported the effect of vitamin D supplementation and a 24 week exercise intervention in older adults (mean age = 68.8 years) demonstrated that muscle power (assessed using the 5-time sit-to-stand test) significantly increased in all 3 intervention groups (exercise alone, vitamin D supplementation alone and combined exercise and vitamin D). As with EXVITD, there were no significant differences between the groups, although the within-group change was shown to be highest in the combined intervention group, in which 5-time sit-to-stand time improved by 19.41% compared with exercise alone (14.69%). Interestingly, vitamin D supplementation alone resulted in comparable increases in muscle power (13.68%) (Aoki et al., 2018), although this study supplemented with a higher daily dose of vitamin D3 (1000 IU/d) than EXVITD.

6.1.2. Total SPPB score

The EXVITD cohort significantly increased their SPPB total score after the 6-month intervention, however, this increase could be attributed to the vitamin D group which improved their score by an average of 14.45% or +1.67 points, which was statistically significant ($p = 0.028$). The average SPPB score within the placebo group decreased by -10.04% (or -0.91 points, $p = 0.637$), although the between-group difference was not significant. The increase in SPPB for the whole cohort is comparable to the

findings of a systematic review of physical activity interventions for physical function in adults ≥ 65 ; evidence from 5 studies ($n = 266$ men and women) of moderate quality evidence and an average duration of 36 weeks, concluded exercise interventions resulted in a significant increase in total SPPB score of 1.87 points (95% CI 1.17, 2.57, $p < 0.0001$) (Giné-Garriga et al., 2014a). A study of 3 sessions of RET per week for 5 months in 55 older men and women (mean age = 69.1 years) reported a mean increase in total SPPB score of 5.26%, with a large heterogeneity of individual responses to the intervention; changes in SPPB score ranged from a $\leq -20\%$ decrease and $\geq +20\%$ increase in performance (Chmelo et al., 2015). This study serves to highlight that a limitation of some functional tests such as the SPPB and 5-time chair rise is a ceiling effect and therefore they cannot discriminately assess functional change in higher functioning individuals (Sayers et al., 2006); 8 individuals (33.33%) taking part in EXVITD scored the maximum 12 points in the SPPB test at baseline, meaning that only a decrease in performance would demonstrate any change at 6 months.

Again, the study was under-powered to detect any significant changes in SPPB score; it was estimated that an additional 1.0-point increase in total score above the 1.5 point increase expected with RET alone (i.e. 2.5 points) would need to be observed in order to detect a significant between-group difference in the vitamin D group; so it is not possible to state confidently that vitamin D3 supplementation resulted in the increased SPPB score. However, it is possible to conclude that the increase in average SPPB score within the vitamin D group could be classified as a clinically meaningful improvement; effect size calculations concluded that an increase of 0.71 – 1.50 points on the SPPB test was associated with a substantial increase in 1-year self-perceived mobility in a group of 424 older adults aged 70 to 89 years

(Kwon et al., 2009). Additionally, a further study concluded that a 0.99 – 1.34 point increase in total SPPB score was equal to a substantially meaningful change based on secondary data analysis of studies involving older adults (mean age range = 69.8 to 77.6 years) from which an effect size or standard error of measurement was calculated (Perera et al., 2006).

Changes in total SPPB score within the EXVITD study were greater compared with one previous exercise and vitamin D supplementation study (although the duration of the current study was longer), 12 weeks of exercise training resulted in a 1-point increase in SPPB score (+10.20%) in comparison with sedentary controls ($p = 0.009$); there was no significant effect of vitamin D supplementation ($p = 0.065$) (Drey et al., 2011). The change in total SPPB score within a 9-month study randomizing into 4 groups (1. RET and vitamin D, 2. RET and placebo, 3. control and vitamin D and 4. control and placebo) was significantly higher in trained participants (groups 1 and 2) than non-trained (groups 3 and 4, $p = 0.002$) and higher still in the RET and vitamin D group than the RET and placebo group (+14.7% and +10.5%) (Bunout et al., 2006a). Although the change in SPPB score in the RET and vitamin D group in the earlier study was comparable to the change in the vitamin D group of the EXVITD study, the placebo group of the EXVITD study exhibited a non-significant decline in SPPB score, which does not support the results from the RET and placebo group from the earlier study (Bunout et al., 2006a).

6.1.3. SPPB test components: 5-time chair rise test

5-time chair rise test time was significantly reduced in the whole cohort and within both intervention groups; placebo group: -3.93 seconds (55.29%), $p = 0.001$ and the vitamin D group: -5.56 seconds (80.81%), $p < 0.001$. Between-group comparison was

non-significant ($p = 0.444$). The changes in the 5-time chair rise test observed in the EXVITD study were greater than comparable exercise training studies; 53 older women (mean age = 81 years) who completed 3 weekly sessions of home-based video exercise training for 5 months significantly improved their 5-time chair rise time by 36.88% ($p = 0.023$), however this was not significantly different from the improvements observed within the sedentary control group (Vestergaard et al., 2008).

Comparing previous RET and vitamin D supplementation studies, 2 reported the 5-time chair rise test separately to total SPPB score; one of these studies (Uusi-Rasi et al., 2015) reported decreases in 5-time chair rise test in both RET intervention groups. Placebo and RET resulted in a 7.80% decrease ($p = 0.03$) and 100 IU/d vitamin D and RET resulted in a borderline significant decrease in 5-time chair rise time of 6.95% ($p = 0.05$); there was no change in 5-time chair rise time associated with sedentary groups supplemented with placebo or vitamin D after 24 months (Uusi-Rasi et al., 2015). In the other study, a 24-week study combining vitamin D3 supplementation and RET (Aoki et al., 2018), a 19.41% improvement in 5-time sit to stand test time with the combined intervention ($p < 0.001$) was reported; however, this was not significantly different from the significant improvements observed with exercise alone and vitamin D3 supplementation alone.

A possible explanation for the large improvements in the 5-time chair rise test is that a modified chair rise test, a component of the SPPB test, was included in the EXVITD exercise intervention; at each session attended, participants performed a 30 second chair rise test which served to increase muscle strength and also to monitor the intervention progression (to ensure that the intervention could truly be classified as “training”). A small but significant learning effect has been observed with the 30

second chair rise test, regardless of rater and with significantly higher repetitions after the fourth test in comparison with the first (Johansen et al., 2016). This was also a limitation of an earlier study (Drey et al., 2011), who then performed a sub-analysis of the SPPB test including only the balance parameters and gait speed (the maximum resultant score was therefore 8 points). The same analysis was completed with the EXVITD cohort; there were still no between-group differences ($F(1,8) = 2.64, p = 0.143$), however the significant improvement within the vitamin D group remained ($t(8) = 2.40, p = 0.043$). Additionally, the within-group change in the placebo group remained non-significant ($t(9) = 1.08, p = 0.309$), although the average change in total score increased (+0.40 points, $SD = 1.17$) rather than decreased after 6 months.

One further limitation of this test may have been that chair height was not adjusted for the lower limb length of each individual participant. Although most reports of the chair rise test do not specify chair height, those which do most frequently report chair heights of between 40-44cm (Bohannon, 1995). However, it has been suggested that the height of the chair be adjusted dependent on the length of the lower limb of participants since this affects the load applied to the lower limb during the test (Yamada and Demura, 2004).

6.1.4. SPPB test components: Gait speed

Gait speed, a component of the SPPB test, was marginally improved within both intervention groups although neither change was significant; placebo group: -0.05 seconds (1.39%), $p = 0.925$; vitamin D group: -0.39 seconds (11.08%), $p = 0.397$.

Similarly, there were no significant differences in changes in gait speed between the groups.

Although the changes in gait speed were seemingly small, changes within the whole cohort and placebo group could be categorised as a small, clinically meaningful change (within the range of 0.04 – 0.06 m/s. Whole cohort = 0.07 m/s, placebo group = 0.02 m/s) and the change in gait speed within the vitamin D group was considered a substantial, clinically meaningful change (within the range of 0.08 – 0.14 m/s; vitamin D group = 0.13 m/s). This is based on effect sizes calculated to define meaningful change in physical function measures during a secondary data analysis of 692 older adults (mean age range = 69.8 - 77.6 years) who took part in both observational and randomized trials of exercise and stroke rehabilitation (Perera et al., 2006).

One study investigating the mode of exercise intervention and effects in older adults concluded that a combination of aerobic and resistance exercise was more effective at improving gait speed after 12 weeks in older men and women (n = 84, mean age = 69.3 years) than aerobic or resistance training alone (16.8%; 95% CI 7.8, 25.6, $p < 0.001$), with significant effects observable as early as halfway through the intervention (Timmons et al., 2018). Conversely, a systematic review of resistance, coordination and multimodal exercise interventions in a total of 1297 older adults and their effects on habitual gait speed concluded that all 3 modes of exercise similarly increased gait speed by 8.4% (Hortobágyi et al., 2015).

Of the comparable RET and vitamin D supplementation studies, only one analysed a variant of gait speed independent of total SPPB score (Uusi-Rasi et al., 2015). 4 meter normal walking speed was analysed in 409 women (mean age = 74.1 years) who took part in a 2-year study of 800 IU/d vitamin D3 supplementation and exercise; walking speed was maintained in both exercise groups (placebo and exercise and vitamin D3 and exercise) but declined in both sedentary groups (placebo alone and

vitamin D3 alone) (Uusi-Rasi et al., 2015). The effect of exercise on gait speed in older adults is not consistent; a systematic review of combined nutritional and exercise interventions and muscle outcomes in older adults over 65 years found no effect of either exercise or vitamin D supplementation on gait speed (Denison et al., 2015).

6.1.5. Timed up and go test (TUG)

Within-group comparisons revealed both groups improved their TUG time (placebo group 8.88%, $p = 0.081$; vitamin D group 22.79%, $p = 0.034$), although this change was significant only within the vitamin D group. Between-group comparisons were non-significant ($p = 0.194$).

The improvement in TUG observed within the vitamin D group was comparable to an earlier study which implemented 2 sessions of RET per week for 24 weeks in 63 older women (mean age = 67.5 years); after the intervention, there was an approximate 19.05% improvement in TUG time within the resistance training group and an approximate 16.92% improvement within the non-trained control group (Edholm et al., 2017). In a further study, 38 older men and women (mean age = 76.6 years) were randomized to 3 sessions of strength training per week for 1 year or a control group; men (18.6%) and women (20.50%) in the strength training group significantly improved their TUG, with no significant effect of sex observed, however, TUG time within both male (-2.00%) and female (-0.60%) deteriorated (Capodaglio et al., 2007). A systematic review including 12 studies of older adults ($n = 691$ aged ≥ 60 years) concluded that progressive RET significantly improved TUG time in comparison to controls (mean difference = -0.69 seconds, 95% CI -1.11 to -0.27, $p =$

0.0012); studies conducted 2-3 exercise sessions per week for a range of 2 to 104 weeks (Liu and Latham, 2009).

Three RET and vitamin D3 supplementation studies included within the Chapter 2 systematic review assessed TUG (Bunout et al., 2006a, Gianoudis et al., 2014, Uusi-Rasi et al., 2015). In a group of 96 older adults (mean age = 77 years) randomized to 9 months of 1) 400 IU/d vitamin D3 supplementation and RET, 2) RET and placebo, 3) control and vitamin D3 or 4) control and placebo, TUG time was significantly improved in trained participants and supplemented participants improved more than participants receiving the placebo (2.4%, $p = 0.004$) (Bunout et al., 2006a). There was no between group difference in TUG time after 12 months between the intervention (3 sessions per week of RET combined with 1000 IU/d vitamin D3) and control (usual care, general osteoporosis information and 1000 IU/d vitamin D3) groups in an earlier study; there was an approximate change of 0% and 3% within the intervention and control groups, respectively, although this study implemented a secondary cognitive task alongside the TUG (counting backwards from 100 by 3) (Gianoudis et al., 2014). A further study found that TUG time improved within the exercise and vitamin D group after 24 months (1.75%) and deteriorated within the placebo and exercise group (-0.35%), although there was no significant difference between the groups (Uusi-Rasi et al., 2015).

6.1.6. Leonardo Mechanograph® force plate outcomes: Peak total power and 5-time CRT

Peak total power increased significantly within both the placebo (+27.71%, $p = 0.001$) and vitamin D (+25.93%, $p = 0.008$) groups; there were no between-group differences ($p = 0.557$). Similarly, 5-time CRT time improved significantly within the

placebo (69.18%, $p = 0.006$) and vitamin D (73.07%, $p < 0.0001$) groups with no between-group differences ($p = 0.510$).

The change in peak total power observed within the control group of a study of 74 COPD patients after 3 sessions of strength, endurance and squat training for 3 weeks was +0.61 W (95% CI 0.17, 1.06) (Gloeckl et al., 2017); this was higher than the change within both the placebo (0.23 (0.15)) and vitamin D (0.21 (0.14)) groups at month 6 in the EXVITD study. A further study assessed peak relative power after 3 sessions of supervised balance and strength training per week for 12 weeks in 66 older adults (mean age = 73 years) (Lacroix et al., 2016); change in relative peak power within the intervention group (+17.30%, $p < 0.017$) was lower than observed within both the placebo (+28.14%, $p = 0.002$) and vitamin D (+25.63%, $p = 0.002$) groups in the EXVITD study.

Cross-sectional functional performance was assessed via Leonardo Mechanography in 293 community-dwelling older men (mean age = 71.6 years) and women (mean age = 71.4 years) from Germany. Reference data reported average 5-time chair rise test relative peak power in women ($n = 130$) to be 8.9 (SD = 2.1) W.kg⁻¹ and 11.1 (SD = 2.4) in men ($n = 124$) (Dietzel et al., 2015). Women within the placebo group of the EXVITD study ($n = 5$) had lower peak relative power at baseline (6.74 (0.83) W.kg⁻¹) than reported above (Dietzel et al., 2015), however, at month 6 peak relative power was comparable (8.82 (0.32) W.kg⁻¹). Women within the vitamin D group of the EXVITD study ($n = 6$) had similarly lower peak relative power at baseline (6.73 (1.79) W.kg⁻¹), however, relative power was higher than the reference data at month 6 (9.45 (1.77) W.kg) and the within group change for women in the vitamin D group was significant ($p = 0.041$). Men within the placebo group of the EXVITD study ($n = 7$) exhibited lower peak relative power at baseline than previously reported (Dietzel et

al., 2015) (8.53 (2.91) W.kg⁻¹), however, at month 6, power was higher than the reference data (12.44 (4.91 W.kg⁻¹). Similarly, within the vitamin D group of the EXVITD study, men (n = 6) were assessed as having lower peak relative power than the reference data above (Dietzel et al., 2015) (8.72 (1.58) W.kg⁻¹), but this value was increased to 13.44 (2.11) W.kg⁻¹ at month 6.

5-time CRT within the EXVITD study was substantially higher than reported in exercise studies; for example, after 12 weeks of supervised balance and strength training, average 5-time CRT was significantly improved in the intervention group by 30.29% ($p < 0.017$) in comparison with the passive control group, which experienced a deterioration of 5-time CRT time (-1.20%, non-significant) (Lacroix et al., 2016). A further study of 74 COPD patients completing 5 sessions per week of endurance, strength and squat training for 3 weeks reported a non-significant improvement in 5-time CRT time of 1.4 (2.9 – 0.0)% ($p = 0.053$) (Gloeckl et al., 2017).

One other study of vitamin D3 supplementation and exercise training study measured muscle power utilising a force plate; after 12 weeks of strength training, a cohort of 69 older adults (mean age = 77 years) did not significantly alter their muscle power as assessed by sit to stand transfer power; absolute power at baseline was reported as 467 W, with an increase of 42 W after 12 weeks of strength training and also a 12 W improvement evident within the sedentary control group ($p = 0.308$) (Drey et al., 2011). This is much lower than both the baseline (600 W) and change in power (220 W) measured within the EXVITD cohort, which reported a significant increase in power assessed using Leonardo Mechanography within the whole cohort ($p < 0.001$), the placebo ($p = 0.001$) and vitamin D ($p = 0.008$) groups, although no between-group differences were found. The participants within the aforementioned study had a lower SPPB score at baseline (8.8 within the strength training group) than the

EXVITD cohort (9.85), suggesting that these participants may have been frailer than included within the EXVITD study and also the intervention was shorter by 12 weeks.

The improvement in 5-time CRT time as measured by Leonardo Mechanography in the EXVITD study was substantially higher than in previous exercise training studies involving older adults, although, as there were no between-group differences, this cannot be ascribed to vitamin D supplementation alone.

As with the chair rise test completed as part of the SPPB test, the large improvement in 5-time CRT time measured using Leonardo Mechanography may be due to a learning effect; the 30-second sit to stand test was completed at each exercise session by all participants as a way to track progression. Task-specific training has been shown to significantly improve performance; 161 disabled supported housing residents (mean age = 82 years) completing 3 hour-long sessions of task-specific bed and chair rise resistance training exercises per week for 12 weeks (e.g. heel raise, supine to sit and raise from a chair) incorporating additional weight (weighted vest or ankle weights) to increase resistance and challenge balance. Training significantly improved the time taken to perform these tasks (11-20%, $p < 0.0001$) and also performed a significantly greater number of bed or chair rises than the flexibility control group ($p = 0.030$) (Alexander et al., 2001). Additionally, technical issues with the Leonardo mechanography equipment meant that 3 participants were unable to complete their month 6 measurements, further decreasing the representativeness of the already small sample.

Although the Nottingham power rig has been cited as the “gold standard” for muscle power evaluation in older adults (Gray and Paulson, 2014), the use of Leonardo Mechanography in the assessment of muscle function and power has the potential to

be more accurate than other commonly used tests. However, there appear to be no examples of direct comparison or validation studies for this specific brand of equipment. Other brands of force plates have been shown to be correlated with the Nottingham power rig; a sample of 33 older adults (mean age = 67.8 years) completed the 5-time chair rise test (a component of the SPPB) and had their muscle power evaluated as sit-to stand transfer power utilising a force plate (Soehnle, Germany) and power assessed by the Nottingham power rig. Power assessed by the force plate and the Nottingham rig showed a moderate association ($r = 0.6$), however, the 5-time chair rise test was poorly correlated with power measured using the force plate ($r = -0.08$) (Lindemann et al., 2003). Within the EXVITD study, relative power measured using the Nottingham power rig and Leonardo Mechanography showed equal associations at baseline ($r = 0.60$, $p = 0.001$) within the whole cohort, however, the association between the 2 types of equipment at month 6 was higher ($r = 0.836$, $p < 0.001$) and comparable to values reported in an earlier study involving the two-legged jump ($r = 0.86$, $p < 0.001$) (Basse and Short, 1990). The lack of a strong correlation between the two measures and the differences in absolute and relative power measured by the two techniques within the EXVITD study is most probably due to the difference in movements with each test; the Nottingham power rig aims to isolate the lower extremities and activate a specific group of muscles whilst the power assessed by the chair rise test and Leonardo mechanography include upper body muscle activation for balance, stability and co-ordination. Additionally the chair rise test is weight-bearing whilst LLEP measured using the Nottingham power rig is not; the study previously mentioned reported power values measured by the force plate to be twice as large as those measured by the

Nottingham rig (Lindemann et al., 2003), although these differences observed within the EXVITD study were four-fold greater.

In the assessment of muscle power, the Nottingham power rig requires a maximal effort from a small group of muscles, i.e. the extremities, but Leonardo Mechanography combines this intensity of muscle work with the co-ordination of several different muscle groups during both the chair rise test and jump testing; some groups suggest this combination is preferable in the assessment of interventional effects in older adults (Buehring et al., 2015). Additionally, when assessing the reliability of Leonardo Mechanography and common functional tests in 97 older adults (mean age = 80.7 years) with multiple tests completed over 3 months, Leonardo Mechanography retest reliability (ICC = 0.93) was higher than observed with gait speed and the SPPB test (ICC = 0.76 and 0.77, respectively) (Buehring et al., 2015). Although the 5-time sit to stand test is similar to the chair rise test completed using Leonardo Mechanography, the 5-time sit to stand provides only components of muscle power since it is reported in seconds. Additionally, one earlier study concluded that the 5-time sit to stand test was poorly associated with power assessed by a force plate ($r = -0.08$), suggesting that this may be due to the “ceiling” effect of the test, as discussed earlier (Lindemann et al., 2003). It is possible to estimate power from the 5-time chair rise test from participants height, weight and height of the chair used, which has been found to be strongly correlated with muscle power assessed using a leg press machine (BH Fitness, Serie TR, Spain), $r = 0.72$, $p < 0.001$ (Alcazar et al., 2018). That said, although non-significant, this method of power calculation over-estimated muscle power by an average of 7.0 W in 40 community-dwelling older adults (mean age = 77.6 years) (Alcazar et al., 2018), with

the measurement of each variable required for the calculation introducing an element of human error, albeit small (Gómez-Cabello et al., 2012).

6.2. Secondary outcome measures: Body composition

In summary, there were no within-group changes in any body composition outcomes, with the exception of a significant increase in lean mass within the placebo group; as with functional outcomes, there were no between-group differences in any of the body composition outcomes assessed.

6.2.1. Total body weight

Changes in total body weight within the placebo (-0.82 (4.62) kg or -1.02%) and vitamin D (+0.43 (1.67) kg or +0.61%) groups were small and non-significant; additionally, there were no between group differences ($p = 0.654$).

Exercise interventions have previously demonstrated conflicting results regarding total body weight. In a study of 91 community-dwelling older adults (mean age = 83 years) considered mild to moderately frail, total body weight was significantly reduced after a period of 9 months of combined RET, balance and endurance training (-0.8kg, $p = 0.005$) but remained unchanged after 9 months of balance and low-intensity strength training (-0.0 kg, $p = 0.920$); as with the EXVITD study, BMI categorized participants in both groups as overweight at baseline (Binder et al., 2005). In a systematic review and meta-analysis of the effects of exercise (including RET, aerobic or both) in sarcopenic obese older adults (mean age range = 66.8 – 81.4 years) total body weight was marginally and non-significantly reduced as a results of the intervention; - 0.29kg (95% CI -1.44, 0.86, $p = 0.620$) including a total of 258 participants from 5 studies (Hita-Contreras et al., 2018).

4 previous combined RET and vitamin D supplementation studies report total weight at baseline and follow-up; 2 studies found no significant change in body weight within any of the groups included (Bunout et al., 2006a, Uusi-Rasi et al., 2015), comparable with the findings of the EXVITD study. In a study of 18 older women (mean age = 69.2 years) living in a retirement community in Florida, USA randomized to complete 3 sessions per week for 32 weeks of exercise training using weighted vests and 400 IU vitamin D3/day or a sedentary control group also supplemented with 400 IU/d vitamin D3, participants in the training group reduced their weight by 5.69% (-4.20 kg) on average, whilst the control group increased their body weight by 0.71% (+0.20 kg, between-group difference $p = 0.008$) (Jessup et al., 2003). Body weight was non-significantly decreased by -0.2% in the exercise and control groups of a study recruiting 162 older adults (mean age = 67 years) randomized to 12 months of RET and vitamin D3 supplementation (1000 IU/d) or a control group also supplemented with vitamin D3 (Gianoudis et al., 2014).

One limitation for the measurement of total body weight was that the prandial state and the clothing worn during each measurement was not standardised; measurements were taken during the morning, but participants were not instructed to remain in a fasted state.

6.2.2. Femoral neck and lumbar spine BMD

The average change in both femoral neck and lumbar spine BMD within the whole cohort, and the individual placebo and vitamin D groups was very small. The only gain in BMD was within the placebo group at the lumbar spine (+0.01 g.cm³, $p = 0.647$), otherwise BMD was maintained following the 6-month intervention which could be attributed to RET rather than vitamin D supplementation, as suggested by

the lack of between-group differences. The gain in BMD at the lumbar spine within the placebo group was small, but at 3.23%, was higher than the coefficient of variation for the model of DXA machine used within the EXVITD study (reported as 0.56% for BMD by the manufacturer (Toussirot et al., 2007)).

Systematic reviews and meta-analyses of the effect of exercise training on BMD differ in their conclusions; a review of 24 RCT studies including postmenopausal women (age range 50.5 to 69.6 years) found that participants in the 14 studies involving RET significantly increased both their femoral neck (SMD = 0.303, 95% CI 0.127, 0.479, $p = 0.001$) and lumbar spine (SMD = 0.311, 95% CI 0.115, 0.507, $p = 0.002$) BMD. However, additional gains were observed in the studies that utilised “combined RET” (RET combined with balance and jump exercises, for example) as the training method; femoral neck BMD (0.411, 95% CI 0.176, 0.644, $p = 0.001$) and lumbar spine BMD (0.431, 95% CI 0.159, 0.702, $p = 0.002$) (Zhao et al., 2015).

Conversely, an earlier systematic review of the effects of exercise on BMD in older adults (mean age range = 65 to 83 years) included 19 RCTs with 1577 participants; low impact and RET exercise regimens were consistently found to result in no positive and non-significant effects on BMD at the lumbar spine, although small, non-significant gains were observable at the femoral neck (Marques et al., 2012)

There are some limitations to the conclusions of meta-analyses involving BMD measurement; many studies involving bone health and BMD include mainly or solely female participants, for example of the 17 studies included within the meta-analysis by (Marques et al., 2012), 15 included 100% women, 1 did not report the sex of included participants and only 1 included an equal split of male and female participants. Additionally, both of the meta-analyses cited above reported intermediate to high levels of heterogeneity, attributed to differences in the exercise

regimens between different studies (i.e. type, intensity, frequency, duration), but also, regional BMD estimates have been shown to vary between different DXA models and manufacturers (Fan et al., 2010).

The majority of the studies included within the systematic review in Chapter 2 of the thesis reported femoral neck and lumbar spine BMD as outcome measures. Several of these studies found gains in femoral neck BMD (Jessup et al., 2003, Bunout et al., 2006a, Verschueren et al., 2011, Gianoudis et al., 2014), ranging from 0.6% (Gianoudis et al., 2014) to 9.46% (Jessup et al., 2003). Additionally, lumbar spine BMD was reported by 4 of the studies included within the systematic review in chapter 2, with 3 of these studies finding gains in lumbar spine BMD as a result of the combined RET and vitamin D3 supplementation intervention (Jessup et al., 2003, Bunout et al., 2006a, Gianoudis et al., 2014). The gains observed within the placebo group of the EXVITD study were within the range of lumbar spine BMD gains reported within the systematic review in Chapter 2 (1.5% (Gianoudis et al., 2014) to 12.5% (Jessup et al., 2003)).

Only one study reported an overall loss in BMD following the combined intervention; following 12 months of RET and 800 IU/d vitamin D3 supplementation, a group of 409 older women (mean age = 74 years) lost 0.51% BMD at the femoral neck, although this was a significantly smaller loss than experienced by the placebo and no exercise group (1.25%, p 0.04) (Uusi-Rasi et al., 2015). Additionally, the same study reported small percentage increases in lumbar spine BMD within the combined intervention group (0.33%), although this was not significantly different to the placebo and no exercise group.

Although the majority of comparable RET and vitamin D3 supplementation studies reported increased in BMD at the femoral neck and lumbar spine, it is worth mentioning that baseline BMD of the participants included within all of the studies reporting on BMD included within the systematic review reported in chapter 2 were lower than those of the EXVITD cohort; for example, the lowest baseline mean BMD of the femoral hip and lumbar spine were reported as 0.63 g.cm³ and 0.77 g.cm³, respectively by the same study (Jessup et al., 2003), which was also the study that observed the largest increase in both femoral neck and lumbar spine BMD. Baseline mean BMD of the femoral neck within the EXVITD cohort was 0.92 g.cm³. This was higher than reported in a group of 620 healthy Danish adults (age range = 20 to 89 years), in which the BMD of the femoral neck was estimated in women aged 60 to 69 years and 70 to 79 years to be 0.69 (0.11) g.cm² and 0.61 (0.13) g.cm², respectively; in men of the same age ranges, BMD was reported to be 0.79 (0.12) g.cm² and 0.73 (0.09) g.cm², respectively (Warming et al., 2002). At baseline, the mean BMD of the femoral neck in women and men participating in the EXVITD study was 0.88 g.cm³ and 0.96 g.cm³. Although there were no significant increases in BMD at any site observable within the EXVITD study, maintenance of BMD at the femoral neck and lumbar spine may be a clinically relevant finding since “normal” bone loss in healthy older adults has been determined as 0.002 to 0.006 g.cm² per year at all sites (Warming et al., 2002).

Additionally, the duration of all studies that reported BMD as an outcome measure was longer than the EXVITD study, with the exception of (Verschuere et al., 2011). This is important as it has been suggested that the minimum period for RET interventions should be 6 months since this is the amount of time taken for bone remodelling to be detectable on a DXA scan (Zhao et al., 2015). In addition, follow-up

DXA scanning to monitor the response to osteoporosis treatments typically takes place a minimum of 2 years after the initial scan, as any changes before this period are unlikely to be detectable (The National Osteoporosis Society, 2011). Although the precision of the GE Lunar iDXA has been quoted 0.56% for BMD by the manufacturer (Toussirot et al., 2007), it is important to consider that these figures are for short-term precision calculated from measurements taken within hours or days apart; long-term precision of DXA calculated from measurements repeated annually over 7 years at the femoral neck and lumbar spine have been reported to be 2.21% and 1.12%, respectively, with measurements at year 7 significantly different from those taken at baseline for the femoral neck (Patel et al., 2000).

Regarding markers of bone turnover, CTX was not significantly changed within either group following the 6-month intervention, nor were there any between-group significances observed. CTX concentration was non-significantly decreased within the placebo group and non-significantly increased within the vitamin D group (-14.29% vs +7.41%, $p = 0.752$). Baseline CTX concentrations were comparable with those previously reported in similar populations (Marques et al., 2013, Gombos et al., 2016).

CTX concentration was not significantly altered in 47 older adults (mean age = 68.2 years) following 32 weeks of resistance (2 sessions per week) and impact (1 session per week) training; mean CTX concentration remained unaltered in the 24 female participants, with a small non-significant decrease in CTX concentration observed in the 23 male participants (-2.78%, $p = 0.722$) (Marques et al., 2013). A further study assessed the acute responses of CTX concentration in 150 older women (mean age = 60.2 years) considered to have low bone mass (mean T-score = -2.1, range = -1.0 to -4.7) to a single bout of either 30 minutes RET ($n = 50$), 46 minutes of outdoor

walking on a flat surface (n = 50) or a sedentary control (n = 50). A significant decrease in CTX concentration was observed within the RET group (-11.11%, $p < 0.01$), but the CTX concentration observed within both the walking and control groups remained unaltered (Gombos et al., 2016).

Regarding falls as events, 3 falls occurred throughout the study period and all falls occurred at a time outside the scheduled exercise sessions. None of the falls caused serious injury or resulted in a fracture; all fallers recovered from their fall within 24 hours, with minor bruising. 2 of the 3 fallers were male and all fallers were randomized to the placebo group. One limitation of the study was that previous falls history was not collected, and so analyses could not be conducted to assess the effects of the intervention, if any, on this parameter.

6.2.3. Total fat mass

Total fat mass was not significantly altered as a result of the EXVITD study; the placebo group lost 0.25 (1.13) kg on average ($p = 0.509$), which was equivalent to 0.90% of their total fat mass and the vitamin D group gained 0.34 (1.26) kg of fat mass ($p = 0.448$), equivalent to 1.40% of their total fat mass. There were no between-group differences in change in fat mass ($p = 0.481$). The changes in fat masses within both groups were within the coefficient of variation of the GE Lunar iDXA, which has been reported as 0.59% for fat mass (Toussiro et al., 2007).

One study of 9 months RET in frail older adults reported a decrease in percentage fat mass assessed by DXA in both the RET (-1.0 (1.7)%) and flexibility and low-intensity strength training control (-0.4 (1.9)%) groups with no between-group differences ($p = 0.220$) (Binder et al., 2005). A systematic review and meta-analysis of the effects of exercise on body composition on older adults with sarcopenic obesity reported small

and non-significant losses in both total and percentage fat mass; meta-analysis data from 194 participants included in 5 studies found a total fat mass loss of 0.97kg (95% CI -2.94, 1.00, $p = 0.220$) and data from 377 participants from 8 studies concluded a percentage fat mass loss of 0.26% (95% CI -0.74, 0.22, $p = 0.290$) (Hita-Contreras et al., 2018).

Of comparable RET and vitamin D3 supplementation studies, 3 studies reported no significant differences between groups in fat mass; 2 studies analysed body composition using DXA and merely reported that there were no significant changes in any body composition parameters (Bunout et al., 2006a, Uusi-Rasi et al., 2015).

However, 1 study reported results in more detail. After 12 months of either RET and 1000 IU/d vitamin D3 supplementation (intervention group) or vitamin D3 supplementation alone (control group), there were no between-group differences in percentage fat mass loss in either the intervention (-0.8%, range = -2.6 – 1.1%) or control (-1.00%, range = -3.3 – 1.3%) group; participants were 162 older adults (mean age = 67 years) classified as overweight (BMI = 27.4 kg.m⁻²) (Gianoudis et al., 2014).

6.2.4. Total lean mass, ASM and SMI

Total lean mass differed significantly between the groups at baseline; placebo group mean total lean mass was 48.98 (8.67) kg and the vitamin D group average lean mass was 44.12 (9.12) kg, $p = 0.001$. Change in lean mass at 6 months was significant within the placebo group (+0.67 (0.61) kg or +1.35%, $p = 0.007$), but not within the vitamin D group (+0.13 (0.91) kg or +0.30%, $p = 0.678$), although the vitamin D groups change in lean mass was within the GE Lunar iDXA precision error,

which has been reported as 0.45% for lean mass (Toussiro et al., 2007). There were no between-group differences ($p = 0.355$).

Exercise studies assessing total lean mass report similar overall changes to the EXVITD study; after 9 months of RET, lean body mass estimated using DXA was increased by 0.8 (1.4) kg, but was unchanged within the flexibility and low-intensity strength training control (0.0 (1.5) kg, between-group difference, $p = 0.005$) (Binder et al., 2005). In a meta-analysis examining the influence of RET on lean body mass, 49 studies were eligible for inclusion, including 1328 older adults (mean age = 65.5 years) completing an average of 2.8 sessions of resistance exercise per week for an average duration of 20.5 weeks. Lean body mass was found to be significantly increased as a result of resistance exercise (pooled mean estimate = 1.1 kg, 95% CI: 0.9, 1.2 kg, $p < 0.001$) (Peterson et al., 2011). Additionally, increased volumes of training were associated with larger gains ($\beta = 0.05$, $p = 0.01$) and increasing age associated with lower gains ($\beta = -0.03$, $p = 0.01$) in lean body mass, although sex, study design, exercise frequency, intensity and duration were not significantly associated with lean body mass (Peterson et al., 2011). The mean age of the EXVITD cohort was 70.83 years, which was younger than the mean age of participants included within the above meta-analysis, which also included participants ≥ 50 years of age; this may be one explanation accounting for the lower gains in lean mass observable within EXVITD participants in both groups.

Again, 2 similar RET and vitamin D3 supplementation studies utilising DXA to estimate body composition concluded that there were no significant changes in lean mass in any group as a result of the intervention (Bunout et al., 2006a, Uusi-Rasi et al., 2015). Percentage change in lean mass within the placebo and vitamin D groups of the EXVITD study were comparable to a 12 month study comparing RET and 1000

IU/d vitamin D3 supplementation and vitamin D3 alone; the combined intervention of RET and vitamin D supplementation resulted in a 0.8% (95% CI -2.6, 1.1) gain in total lean mass and vitamin D supplementation alone resulted in a 0.4% (95% CI -0.6, 1.5) gain in total lean mass with no between-group differences (Gianoudis et al., 2014). A more recent study estimated lean mass of the right leg using bioelectrical impedance after 24 weeks of RET and 1000 IU/d vitamin D3 supplementation in 148 older adults (≥ 60 years) with vitamin D deficiency at baseline (mean 25(OH)D = 28.33 nmol/L). Lean mass significantly increased in all groups, with increases in the RET alone and vitamin D alone groups comparable to the EXVITD placebo and vitamin D groups ; RET alone (+1.88%), vitamin D alone (+0.5%) and RET and vitamin D (+3.10%), with no between-group differences ($p = 0.208$) (Aoki et al., 2018).

Appendicular skeletal muscle mass (ASM) nor skeletal muscle index (SMI) were not significantly altered as a result of the EXVITD intervention in either group. The placebo group gained 1.97 (4.99) kg (equivalent to +9.05%, $p = 0.244$) and the vitamin D group gained 0.02 (0.50) kg (equivalent to +0.29%, $p = 0.890$) ASM at month 6. There were no between-group differences ($p = 0.136$). At baseline, 2 participants in the placebo group and 5 within the vitamin D group were considered to have low ASM according to the EWGSOP2 criteria (Writing Group for the European Working Group on Sarcopenia in Older People 2 et al., 2018); at month 6, 1 participant within the placebo group and 4 within the vitamin D group had low ASM. SMI at baseline did not significantly differ between the groups ($p = 0.774$). The change in SMI at month 6 was small and non-significant within and between the groups; placebo group +1.87 (4.85), equivalent to a 6.79% increase ($p = 0.254$) and the vitamin D group -0.15 (0.91), equivalent to a 0.55% decrease ($p = 0.626$).

Exercise intervention studies report contrasting results relating to body composition. For example a systematic review and meta-analysis of the effects of exercise interventions on anthropometric measures in older adults (mean age range 66.8 to 81.4 years) with sarcopenic obesity found small, non-significant increases in both ASM and SMI; change in pooled mean ASM in a total of 119 participants was 0.22 (95% CI -0.69, 1.13 kg), $p = 0.630$ and change in pooled mean SMI in 209 participants was 0.35 (95% CI -0.20, 0.89), $p = 0.210$ (Hita-Contreras et al., 2018). Conversely, a systematic review of exercise intervention and body composition outcomes in sarcopenic older adults (mean age 67.1 to 81.1 years) found a significant increase in ALM measured by bioelectrical impedance in 116 participants from 2 studies after an average range of duration of 3 to 6 months; 2.11 (95% CI 0.03, 0.87 kg), $p = 0.04$ (Vlietstra et al., 2018).

Among similar RET and vitamin D supplementation studies, only 1 reported ASM. After an 8-week period of vitamin D3 supplementation, 69 older adults (mean age = 77 years) completed 12 weeks of power or strength training or were randomized to a sedentary control; ASM change in each group was +0.56%, +1.62% and 0%, respectively with no significant between-group changes (Drey et al., 2011). Power training in the aforementioned study resulted in gains in ASM comparable to those measured within the vitamin D group of the EXVITD study; the changes in ASM within the placebo group surpassed those observed in the strength training group, although the duration of the EXVITD intervention was longer. Participants in the study by (Drey et al., 2011) exhibited comparable serum 25(OH)D concentrations to the EXVITD cohort at baseline (50.21 nmol/L). No comparable studies reported SMI, and participant weight in the study by (Drey et al., 2011) was not included and as such, calculation of SMI was not possible.

Although the placebo group showed a significantly higher increase in lean mass and also higher gains in ASM and SMI than the vitamin D group, hypertrophy did not result in increased power or function; LLEP, SPPB total score and TUG time were significantly improved within the vitamin D group but not in the placebo group. Furthermore, gait speed improved more within the vitamin D group, although this change was not significant. One previous study has in fact attributed gains in leg power to be significantly associated with clinically meaningful changes in both the SPPB test and gait speed in a secondary analysis of a group of 138 older adults (mean age = 75.2 years) who completed either strength training with free weights or RET with weighted vests for 16 weeks (Bean et al., 2010).

This finding is in agreement with an earlier study which randomized 45 older adults (mean age = 74.8 years) with self-reported difficulty in ADL to 12 weeks progressive strength or power training or control. Although power increased significantly (range of +19.9 to +41.4%) in the power group in comparison to the strength and control groups, there were no between-group differences in lean mass or ASM; lean mass gains were +1.22%, 2.77% and 1.47% and ASM gains +2.88%, +3.54% and 1.00% within the power training, strength training and control groups, respectively (Marsh et al., 2009). Furthermore, after 12 weeks of RET, 9 elderly women (median age = 80 years) increased their lean muscle tissue, as estimated by MRI, by 2.5%, however, isometric knee flexion muscle strength was increased by 17% (Greig et al., 2011a).

Recent experimental evidence has suggested that hypertrophy may not be the main mechanism for increases in strength, although this work was completed in younger adults; 38 untrained younger adults (mean age = 22 years) were recruited to take part in an 8-week study of 2 sessions per week of exercise. Participants randomized to the HYPER group aimed to increased muscle mass and strength via high volume

RET, which included 4 sets of knee extension and chest press exercises (using a Hammer strength plate by Life Fitness) to volitional failure or the TEST group which aimed to minimise hypertrophy but increase strength via 1-RM testing of the same exercises. Although muscle mass was significantly greater at most sites tested in the HYPER versus the TEST group, changes in muscle strength in the upper and lower body were not statistically different between the groups (Mattocks et al., 2017). In older adults, longitudinal losses in muscle strength have been shown to be greater than losses in muscle mass (Marcell, 2003), and one review has suggested that muscle hypertrophy and increased strength as a result of resistance exercise “may not necessarily be mechanistically linked”, suggesting an observable increase in both muscle mass and strength is an association and cannot be assumed to be a causal relationship (Loenneke et al., 2019).

6.3. Secondary outcome measures: Physical activity monitoring

There were no significant effects of the EXVITD intervention on any of the parameters of habitual physical activity within or between groups. Average daily step count over 7 days was marginally increased within both the placebo and vitamin D groups (+37.70 and +877.53 steps, respectively), as were the average time spent sitting/lying (+2.99 and +1.71 hours) and the number of up/down transitions (+4.26 and +5.50 transitions). Time spent standing and energy expenditure remained relatively constant across both groups, as was average time spent stepping within the control group. Time spent stepping was increased by 0.29 hours within the vitamin D group ($p = 0.223$).

Average daily step count of the EXVITD cohort at baseline (5820.34 (2295.44 steps per day) revealed that the guideline 7000 – 10,000 steps per day recommended for

improved quality of life, maintenance of a healthy weight and immune function (Tudor-Locke et al., 2011, Department of Health and Social Care, 2019) was on average not achieved, although individual variation was high (1811 to 11,867 steps per day). Despite this, the average daily step count of the EXVITD cohort at baseline was higher than reported previously by a UK population-based study which analysed 7-day accelerometer data from 2540 older adults (mean age = 78 years, BMI not reported); average step count was measured to be 4662 (95% CI 4567, 4757) steps per day (Jefferis et al., 2014). The average daily step count of 24 Scottish older adults (mean age = 68 years, mean BMI = 26.2 kg.m⁻²) assessed via 5-day ActivPAL accelerometry was higher than that of the EXVITD cohort; average daily step count was 8493 (2291) steps per day, although average age of included participants was lower than the EXVITD study and 29.00% of the cohort still maintained Monday-Friday employment despite being above the retirement age (Fitzsimons et al., 2013).

Comparing other physical activity outcomes, the aforementioned study involving Scottish older adults reported higher mean time spent sitting/lying (18.06 (1.54) hours per day), time spent stepping (1.75 (0.42) hours per day) and number of up/down transitions (54 (15)), although time spent standing (4.19 (1.34) hours per day) was higher within the EXVITD cohort (Fitzsimons et al., 2013). A rapid review of the use of ActivPAL to measure physical activity in older adults (mean age range = 66.0 to 84.2 years) reported mean ranges for time spent stepping (0.13 to 5.02 hours per day), standing (0.8 to 3.4 hours per day) and sitting/lying (minimum time was 8.2 hours per day), of which the EXVITD cohort lay within; 24 papers were included within the review, but poor quality and a lack of reporting consistency prevented data pooling (Chan et al., 2017).

Exercise training studies report both increases and decreases in average daily step count following completion of the intervention. One study of 12 weeks balance training in 91 older adults (mean age = 75.5 years) with osteoporosis found a small decrease in average daily step count measured over 7 days using an Actigraph accelerometer at 3-month follow-up post-intervention (6209 (2842) steps to 6064 (2430) steps, non-significant) (Dohrn et al., 2017). In 49 older adults (mean age = 70.55 years) with mild cognitive impairment (MMSE average score = 24), average daily step count was estimated from a Kenz Lifecorder accelerometer worn daily throughout the intervention of 110 minutes per week of aerobic and balance exercises for 24 weeks; change in post-intervention step count was significantly higher in the intervention group than within the sedentary control group (+3017 steps Vs -1392 steps, $p < 0.01$) (Park et al., 2019).

As with EXVITD, following 9 months of progressive resistance training, one study reported no significant changes in self-assessed physical activity (Physical Activity Scale for the Elderly) in 91 mild to moderately frail older adults with a mean age of 83 years (Binder et al., 2005). Additionally, energy expenditure (33.02 (4.00) METs) and percentage increase in energy expenditure (+2.03%) within the vitamin D group of the EXVITD study was comparable to values reported by the intervention group of a 12-month strength training study in 38 older adults (mean age = 76.6 years); energy expenditure assessed via the 7-day Paqap, a computerised questionnaire, was estimated to be 32.8 (5.5) Kcal kg⁻¹ day⁻¹, which increased by 2.74% following the intervention period (Capodaglio et al., 2007).

Of comparable RET and vitamin D supplementation studies, only one reported similar physical activity outcomes to EXVITD; average daily step count of 409 older women (mean age = 74.1 years) estimated via a pedometer worn daily throughout

the 24-month intervention was recorded as 5930 (2512) steps, but with a large individual range of 1200 to 16,500 steps (Uusi-Rasi et al., 2015). Step count was comparable to both EXVITD groups, as was the large range of individual values reported.

6.4. Blood biomarker outcomes

Regarding serum 25(OH)D, as expected, concentrations within the vitamin D group were increased significantly from baseline at month 6 ($t(8) = 2.56, p = 0.034$) and between-group differences were also significant ($F(1,17) = 7.21, p = 0.016$). The degree to which vitamin D3 supplementation raised serum 25(OH)D concentrations within the vitamin D group (55.63 to 79.83 nmol/L) was comparable to the exercise study by (Uusi-Rasi et al., 2015), which also supplemented with 800 IU D3 per day; after 6 months, serum 25(OH)D concentrations had risen by approximately 21.00 nmol/L within the exercise and vitamin D group (baseline 25(OH)D approximately 66 nmol/L, 87 nmol/L at month 6, estimated from a graph). By month 6, all participants within the vitamin D group of EXVITD had achieved serum 25(OH)D concentrations considered to be sufficient (> 50 nmol/L), while this was achieved by 70% of participants within the placebo group.

Parathyroid hormone (PTH) concentrations were not significantly altered within either group and between-group differences were also non-significant ($p = 0.651$). The largest increase in serum 25(OH)D concentration within the vitamin D group, between baseline and month 1, was concurrent with a fall in serum PTH concentration and an increase in calcium concentration. This is in agreement with a systematic review and meta-analysis of 1077 adults (mean age range = 28.7 to 63.8 years) which reported that vitamin D supplementation was associated with a small

but significant decrease in PTH concentration (standardized mean difference = - 0.04 (95% CI – 0.06, - 0.02 pmol/L), $p < 0.001$) (Lotito et al., 2017). Under normal conditions, PTH and vitamin D work to maintain calcium homeostasis; PTH stimulates the synthesis of 1,25(OH)₂D in the kidneys, increasing serum calcium concentrations which in turn exerts negative feedback on PTH production. The inverse relationship between serum 25(OH)D and serum PTH in the maintenance of calcium homeostasis is well known, but this relationship reaches a plateau around serum 25(OH)D concentrations of 45 nmol/L after which further increases in serum 25(OH)D exert little additional decreases in PTH concentrations (Steingrimsdottir et al., 2005). Since both groups had serum 25(OH)D concentrations >50 nmol/L at baseline, this may explain why there were no significant changes in PTH or calcium concentrations across the study period.

Regarding inflammatory markers, there were no significant changes from baseline at month 6 in serum IL-6, IL-8 or IL-10 concentrations observed within either group. IL-8 concentration decreased within both the placebo and vitamin D groups, but to a greater degree within the vitamin D group (-31.19%). However, this result was concluded from only 3 participants, so should be interpreted with care.

Vitamin D has previously been considered as an anti-inflammatory agent; for example, a bolus dose of 250,000 IU D3 was found to decrease TNF- α ($p < 0.01$) and IL-6 ($p = 0.09$) concentrations in 15 patients with cystic fibrosis (mean age = 24.9 years, serum 25(OH)D concentration at baseline = 76.38 nmol/L), whilst the 15 patients assigned to the placebo group exhibited no significant change in either of the inflammatory markers after 12 weeks. However, there was no change in IL-8 or IL-10 concentration in either group (Grossmann et al., 2012). Similarly, vitamin D deficiency has been associated

with higher concentrations of inflammatory cytokines including TNF- α , IL-6 and CRP (Laird et al., 2014, Elizondo-Montemayor et al., 2017).

6.5. Target population

The original working title of the EXVITD study in protocol version 1.0 was “The influence of combined vitamin D supplementation and resistance exercise training on musculoskeletal health in frail older men and women”. The rationale for selecting older men and women aged ≥ 70 years living in a supported housing environment was that this population were more likely to be frail and susceptible to sarcopenia/functional deficits and therefore stood to benefit from interventions aimed to improve musculoskeletal health. The cohort of participants eventually recruited via the Clinical Research Network did not reside in sheltered housing and were seemingly more able and active than originally planned.

However, as described above, the placebo group of the EXVITD cohort just achieved an average relative LLEP measurement and the vitamin D group fell below the 50th centile for power compared to the ADNFS reference data (Sports Council and Health Education Authority, 1992). Looking at individual relative LLEP measurements at baseline for each group (presented in Table 6.1), 58.33% of participants within the placebo group and 66.67% of participants within the vitamin D group had a lower than average relative power of the lower limbs at baseline. Although LLEP improved by month 6 within both groups, 4 individuals (40.00%) in the placebo group and 5 individuals (55.56%) in the vitamin D group were still considered to have below average relative power output.

Table 6.1: Cumulative baseline relative LLEP in the placebo and vitamin D groups of the EXVITD study in comparison to UK reference data for older adults (65-75 years) from the Allied Dunbar National Fitness Survey

	Placebo group N=12 Count (%)	Vitamin D group N=12 Count (%)
Average relative LLEP (SD (W.kg ⁻¹))	2.06 (0.97)	1.53 (0.80)
≤ 5 th centile (0.90 W.kg ⁻¹)	1 (8.33)	3 (25.00)
≤10 th centile (1.10 W.kg ⁻¹)	3 (25.00)	4 (33.33)
< 50 th centile (< 1.81 W.kg ⁻¹)	7 (58.33)	8 (66.67)
≤ 50 th centile (1.81 W.kg ⁻¹)	7 (58.33)	8 (66.67)
≤ 90 th centile (2.60 W.kg ⁻¹)	10 (83.33)	11 (91.67)
≤95 th centile (2.84 W.kg ⁻¹)	10 (83.11)	11 (91.67)
> 95 th centile (> 2.84 W.kg ⁻¹)	12 (100.00)	12 (100.00)

Additionally further analysis of the cohort's SPPB total scores at baseline revealed that 9 of the 24 participants (37.50%) randomized could be considered to have lower extremity function limitations, defined as a total SPPB score of ≤9; participants with SPPB total scores between 7 and 9 had a relative risk for mobility-related disability at follow-up of 1.2 - 2.0, compared to participants scoring 10-12 points (Guralnik et al., 2000). 5 participants from the placebo group and 4 participants from the vitamin D group were considered to have functional limitations, however, the 6-month intervention, only 1 participant within the placebo group still had a total SPPB score of ≤9.

Finally, low ASM, as defined by the EWGSOP2 (Writing Group for the European Working Group on Sarcopenia in Older People 2 et al., 2018) was evident from DXA scans in 7 participants (29.17% of the cohort) at baseline. 100% of the participants with low ASM were female; 40.00% and 83.33% of women in the placebo and vitamin D groups, respectively, had low ASM, reflected by the significant difference in lean mass between the groups at baseline ($p = 0.001$). At month 6, no participants

within the placebo group could be classed as having low ASM, although 4 participants within the vitamin D group still had low ASM (44.44% of the group, all 4 participants were women).

At baseline, 2 participants (8.33% of the cohort) were found to be sarcopenic; 1 participant each in the placebo and vitamin D groups, based on the EWGSOP2 2018 definition of sarcopenia being a combination of low muscle strength (> 15 seconds for 5 chair rises) and low muscle quality (ASM <15kg for women and <20kg for men). Neither case could be categorized as severe based on functional performance (gait speed \leq 0.8 m/s) (Writing Group for the European Working Group on Sarcopenia in Older People 2 et al., 2018); The sarcopenic participants were female. At month 6, no participants were sarcopenic.

6.6. EXVITD attrition, exercise and supplement compliance

Both the completion (79.17%) and attrition (20.83%) rates in the EXVITD study were comparable to (Kelley and Kelley, 2013, Picorelli et al., 2014, van der Deijl et al., 2014) or higher (Jancey et al., 2007) than those reported in meta-analyses of exercise interventions in older adults. This is encouraging, as recruiting and enrolling older adults onto an exercise intervention and maintaining their interest in participation is challenging; numerous factors affect this decision, including the participant-instructor relationship (Hawley-Hague et al., 2013) and the sense of community and supportiveness created by a group exercise programme promotes adherence (Farrance et al., 2016).

89.08% of prescribed exercise sessions were attended by participants (Appendix J), which is higher than previously reported by other groups (Kelley and Kelley, 2013, Picorelli et al., 2014, van der Deijl et al., 2014). Additionally, a total of 92.95% of

prescribed supplements were taken by participants (Appendix K), which is comparable with similar studies involving older adults (Verreijen et al., 2014, Trabal et al., 2015). Of the 48 RET sessions prescribed, there were no significant between-group differences in number of sessions attended; placebo group mean sessions attended = 41.90 (4.38) and vitamin D group mean sessions attended = 42.56 (3.21) ($p = 0.717$). Of the 168 placebo or vitamin D supplements prescribed over the course of the 6-month intervention, the placebo group took 155.60 (11.66), whilst the vitamin D group took 154.44 (13.67) on average ($p = 0.845$). Additionally, there were no differences between male and female participants in the average number of exercise sessions attended (42.92 (3.87) vs 41.00 (3.56), respectively. $p = 0.299$) attended or the average number of supplements taken (158.50 (9.99) vs 149.14 (14.38), respectively. $p = 0.112$).

CHAPTER 7

THESIS SUMMARY AND GENERAL DISCUSSION

7. Summary and general discussion

The aim of the work reported in this thesis was to determine whether vitamin D3 supplementation was any more effective in improving musculoskeletal function when combined with exercise training compared with exercise training alone. This research has expanded current knowledge within this area via the publication of the first systematic review and meta-analysis of effects of the combined intervention, a secondary data analysis of a large cohort study with novel findings regarding skeletal muscle health and associations with 25(OH)D and via the EXVITD study itself, which adds valuable data to an under-researched area, additionally reporting novel outcomes such as markers of bone turn over and muscle power mechanography. However, the role of vitamin D in combatting the onset and progression of factors influencing sarcopenia, such as muscle mass, strength and function and whether vitamin D supplementation can boost responsiveness to resistance exercise training, remains unclear.

7.1. Summary of findings

Chapter 2: The effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health and function in older adults: a systematic review and meta-analysis. This was the first review to report on the combined effects of RET and vitamin D3 supplementation. The main finding was that muscle strength of the lower limb showed a small but significant improvement as a result of the combined intervention over RET alone (0.98, 95% CI 0.73, 1.24, $p < 0.001$). Additionally, muscle function (TUG) and BMD of the hip and spine were more improved as a result of the combined intervention than observed with RET alone.

The small number of included studies highlighted the lack of available literature in the area, further justifying the rationale for completing an additional randomized controlled trial of exercise and vitamin D3 supplementation implementing an appropriate design and sample size.

Chapter 3: Is serum 25(OH)D concentration associated with lean mass and strength and is sarcopenic status seasonal-dependent in a subgroup of postmenopausal women from the D-FINES cohort? This secondary data analysis presented novel findings of the transient nature of sarcopenic status, although the strength of conclusions drawn were limited by the very small sample of sarcopenic participants present within the D-FINES cohort. Muscle strength was positively associated with 25(OH)D across all seasons and in all 3 groups, with significance detected during the summer and autumn months for the whole cohort and postmenopausal women <65 years following adjustment for seasonal BMI. Sarcopenia prevalence varied seasonally in both postmenopausal women <65 years and ≥65 years, with prevalence highest in spring and participants with transient sarcopenic status most likely to be sarcopenic in spring in both subgroups

Chapters 4, 5 & 6: The EXVITD study. The main findings of this study were that all muscle function outcome measures were significantly improved within the vitamin D group, with the exception of gait speed, which improved non-significantly. The 5-time chair rise test, peak power and 5-time chair reaction time using Leonardo Mechanography® were significantly improved within the placebo group, all other functional outcomes were non-significantly improved within this group, with the exception of total SPPB score, which declined (-0.91 points, $p = 0.637$). Despite the substantially greater increases in muscle power and functional outcomes observed within the vitamin D group compared with placebo, the conclusions drawn from the

EXVITD study are limited as potential between-group differences were not detected, most likely due to underpowering.

7.2. Implications of the findings

The role of vitamin D3 supplementation in relation to boosting responsiveness to exercise in older adults remains unclear. Although the results of the EXVITD study indicated that aspects of muscle power and function improved to a greater degree within the vitamin D supplemented group, the small sample size meant that the study was underpowered to detect any potential between group differences. All fall incidences throughout the 6-month duration of the intervention (n=3) occurred in participants assigned to the placebo group; while this is an interesting observation, it is impossible to make any assumptions based on the very small number of observations. Although controversy is still evident regarding the role of vitamin D in falls prevention, moderate-quality evidence from a recent Cochrane review suggests that vitamin D may play a role in reducing the rate but not risk of falls, particularly in individuals with low 25(OH)D concentrations (Cameron et al., 2018).

Evidenced by the very small number of studies (7) identified in the novel systematic review presented in chapter 2, very few data exist in this area, and fewer still (1) of the studies identified truly implemented a methodological design most suited to test this relationship. However, a meta-analysis including studies of a suitable design concluded that the combined intervention indeed resulted in a significant additional benefit in muscle strength gains above and beyond that of RET alone (Antoniak and Greig, 2017). As a result of these preliminary findings, further research examining the potential additive effect of vitamin D supplementation on aspects of musculoskeletal health in older adults when combined with exercise is warranted. As stated in a

recent systematic review “elucidation of the real potential of a cheap drug such as vitamin D could be invaluable to public health worldwide” (Autier et al., 2017). The next section includes suggestions regarding future research.

7.3. Future research

Vitamin D supplementation has come under heavy criticism in recent years, principally by one research group from the Bone and Joint Research Group based at the University of Auckland, New Zealand, which has been quite vocal about their lack of support for vitamin D supplementation, particularly in the prevention of falls or fractures (Bolland et al., 2018b). However, their study, entitled “Effects of vitamin D supplementation on musculoskeletal health: systematic review, meta-analyses, and trial-sequential analyses” had a focus on falls, fractures and BMD, with no mention of muscle mass, strength or function outcomes. Other reviews report mixed results for vitamin D supplementation and muscle strength and/or function; one review reported that although clinical evidence of benefit on muscle strength was suggested, a substantial effect on lower extremity muscle strength was unlikely (positive effect but no significance observed from 3 RCTs), despite meta-analyses from 3 studies reporting a significant reduction in postural sway (SMD = -0.20 . 95% CI -0.39 to -0.01 , $p = 0.04$) (Theodoratou et al., 2014). Furthermore, 2 large systematic reviews by one research group examined the effects of vitamin D supplementation on non-skeletal health and reported no significant effects; the earlier study reported improvements but no significant effect in favour of vitamin D supplementation on leg strength, TUG or balance (Autier et al., 2014) and neutral effects on muscle strength from a total of 9 RCTs including 1205 participants in a more recent study (Autier et al., 2017). However, both reviews included participants of all ages, rather than just older adults.

The choice to recruit vitamin D insufficient participants as part of the EXVITD cohort was justified as it was deemed unethical to withhold treatment from deficient participants randomized to the placebo group. That said, the recruitment of insufficient or sufficient participants has been pronounced as “research waste” by one study, so said because any beneficial effect of vitamin D is most likely to be observable in participants with vitamin D deficiency, which in this instance was defined as serum 25(OH)D concentration <25 nmol/L, with insufficiency defined as <50 nmol/L (Bolland et al., 2018a). As the most beneficial effects of vitamin D supplementation alone on muscle strength and functional outcomes have been observed in participants with deficiency (Stockton et al., 2011, Beaudart et al., 2014b), it would be of future interest to investigate whether deficient participants had benefits or additional benefits above insufficient or sufficient participants in a study design comparable with EXVITD. However, as evidenced in the systematic review and meta-analysis in chapter 2 (Antoniak and Greig, 2017), significant improvements in muscle strength were observed with vitamin D3 supplementation combined with exercise in studies including either vitamin D insufficient participants (Bunout et al., 2006a, Uusi-Rasi et al., 2015) or vitamin D sufficient (Agergaard et al., 2015b). Additionally, a more recent study not included within the systematic review found that vitamin D3 supplementation significantly improved postural and anteroposterior steadiness in comparison to exercise training or vitamin D3 supplementation alone in participants who were vitamin D sufficient (72 nmol/L) at baseline (Aoki et al., 2018).

The high supplement compliance reported within the EXVITD study (92.95%) demonstrated that a daily supplementation regimen was feasible within community-dwelling older adults., There is evidence demonstrating that a daily or weekly vitamin D supplement is more effective than single or multiple bolus doses on certain health

outcomes such as respiratory tract infection risk (Martineau et al., 2017).

Recommendations regarding dose of vitamin D supplement is more difficult, since the optimum dose to potentially benefit musculoskeletal outcomes has not yet been determined (Beaudart et al., 2014b). That said, the dose of 800IU/d utilised within the EXVITD study which was below the recommended safety limit of 1000IU/d by the UK Food Standards Agency (Food Standards Agency, 2003) and the 4000IU/d Tolerable Upper Intake Level recommended by the Institute of Medicine (Ross et al., 2011a), was nevertheless sufficient to significantly increase 25(OH)D concentrations from baseline, and was well tolerated with no occurrences of Adverse or Serious Adverse Events.

One important recommendation, and one of the strengths of the EXVITD study, was the use of liquid chromatography-tandem mass spectrometry to measure 25(OH)D concentration. The use of one, highly accurate and validated gold-standard methodology (Volmer et al., 2015) could alleviate difficulties comparing serum 25(OH)D concentration data between studies due to high variability between different assays (Salti et al., 2012). The measurement of serum 25(OH)D concentration is the best determinant of vitamin D status (including intake from the diet and cutaneous production) (Holick, 2009) due to the stable half-life (2 – 3 weeks) of the metabolite (Thacher and Clarke, 2011). Although 1,25(OH)D₃ is the biologically active metabolite of vitamin D, its unstable nature, very low circulating concentration and the influence of PTH make it unsuitable when quantifying vitamin D status (Holick, 2009).

However, 1,25(OH)D₃, and not 25(OH)D has previously been associated with psoriasis severity and cardiovascular disease risk factors in 122 middle-aged adults with psoriasis (mean age = 49.6 years) (Playford et al., 2019), indicating that the measurement of 1,25(OH)D₃ may have some clinical relevance.

Muscle power was selected as the primary outcome measure since muscle power has been shown to be a superior predictor of functional status and decline than muscle strength (Foldvari et al., 2000, Suzuki et al., 2001, Bean et al., 2010, Byrne et al., 2016). Additionally, muscle power is more generally relatable to activities of daily living in older adults, such as rising from a chair and climbing stairs (Foldvari et al., 2000), although relatively few of the studies included within the meta-analysis in chapter 2 (Antoniak and Greig, 2017) included measures of muscle power (Drey et al., 2011, Gianoudis et al., 2014).

Regarding the design of the exercise component of future RCT studies of combined exercise and vitamin D supplementation, the initial protocol stated that 3 sessions of exercise per week were to be completed for 6 months; the frequency of the exercise sessions was one of the major reasons for refusal to participate. Not only was participant recruitment more successful once 2 exercise sessions per week were implemented, this frequency was sufficient to result in clinically meaningful changes in 2 important functional outcomes; the SPPB score and gait speed, with improvements in muscle power outcomes, gait speed, TUG and 5-time chair rise test observed in both groups. 2 exercise sessions per week would also fulfil the physical activity guidelines for older adults in the recently produced UK Chief Medical Officer's report (Department of Health and Social Care, 2019), whilst the observed compliance to the exercise sessions was high (89.08), suggesting that 2 sessions of exercise per week was feasible for the selected population.

A minimum duration of 6 months would be recommended for future interventions, particularly if BMD is to be included as an outcome measure, since this has been suggested as the minimum period for remodelling to be evident on DXA scans (Zhao et al., 2015). Additionally, it has been suggested that new behaviours taking around 6

months to be adopted in the long-term (Hawley-Hague et al., 2013), since older adults are most likely to abandon new exercise commitments within the initial 6 months, largely dependent on whether their expectations are met within this period (Stiggebout et al., 2006, Jancey et al., 2007).

Significant improvements in muscle strength and function have been observed after less frequent exercise sessions; a group of 46 older adults (aged 65 to 79 years) completed 24 weeks of RET, with no difference in any strength or function outcome measure between participants exercising one, two or three times per week for the study duration (Taaffe et al., 1999b). Mode as opposed to frequency appears to be of more importance to muscle strength in the design of exercise studies involving older adults (Taaffe et al., 1999a, Henwood and Taaffe, 2006, DiFrancisco-Donoghue et al., 2007), with resistance training (more explosive concentric phase with increasing percentage of 1RM) producing superior improvements in function in older adults than strength training (slower and equal concentric and eccentric phases and static percentage of 1RM) (Henwood and Taaffe, 2006). A dose-response relationship has been shown between exercise intensity and muscle strength, with high intensity training confers more benefit than moderate or low intensity training (Beneka et al., 2005, Steib et al., 2010).

The justification for not using quantifiable measures of exertion, exercise progression or muscle loading was sound since the use of objective measures such as heart rate monitors would add additional participant burden, however, implementing rate of perceived exertion scales may have been a good compromise. Additionally, the rationale that the use of more familiar exercise equipment would be more readily adapted and maintained in daily practice appeared to come to fruition at the close of the study, since many of the participants continued to attend exercise sessions with

the specialist exercise instructor. That said, exertion, exercise progression or muscle loading data would have been very informative, particularly as exercise intensity has been found to be of importance to outcome measures including strength (Beneka et al., 2005, Steib et al., 2010).

One suggestion which would be informative for the design of future studies would be preliminary observations regarding the vitamin D status of the target population, a recommendation made previously by (Bolland et al., 2018a). Looking retrospectively at the EXVITD study, this approach would have been informative regarding functional ability also; the participants recruited were not necessarily those we were intending to target initially since sheltered-housing accommodation residents were much frailer, with more comorbidities than expected. For example, of the 23 screening failures, 47.83% were due to low serum 25(OH)D concentration, whilst 56.52% were due to comorbidities identified via the health questionnaire. Difficulties in recruiting from this population would have been better anticipated had some form of preliminary observations been completed.

7.4. Personal reflections

From a personal stand point, the dynamics of the study team supported the protocol presented in Chapter 4. The support and flexibility of the staff at the Clinical Research Facility (research nurses, radiologists and receptionists) was one factor which helped ensure that study data were collected accurately and on time.

Recruitment was immeasurably quicker and more efficient subsequent to the support of the local Clinical Research Network; if their support had been gained when recruitment commenced, the target sample size (n=127) would have been more achievable.

Logistic issues were one of the main, time consuming challenges encountered once the intervention had begun. On one hand, overseeing the coordination of transport for participants was a method of monitoring exercise session attendance in addition to the attendance register; however, the quality of the taxi service received was intermittent, and on a small number of occasions resulted in missed sessions when taxis did not arrive.

The personal interaction with participants and encouraging socialising both after and outside of the sessions was helpful from not only a compliance and monitoring perspective but getting to know the participants was something I personally enjoyed. Several participants remarked to me after study completion that they had considered dropping out in the early weeks of the intervention, but continued due to the social aspect and rapport built with myself, the specialist exercise instructor and other participants. This is in agreement with previous studies which found that drop-outs were most likely to occur in the initial stages of an intervention (Stiggelbout et al., 2005, Hawley-Hague et al., 2013) and group cohesion and social interaction and support has been shown to promote adherence (Hawley-Hague et al., 2013, Farrance et al., 2016), particularly in older adults (Stiggelbout et al., 2005).

7.5. Conclusions

Observational and interventional data propose a relationship between vitamin D and musculoskeletal health. The body of research presented here collectively provides tentative support for the use of vitamin D supplementation in combination with resistance training to improve aspects of sarcopenia. Future research is justified as vitamin D could prove to be a cost-effective method of maintaining musculoskeletal health and function in older adults, should its potential be confidently deduced.

Not only is it uncertain whether vitamin D boosts the responsiveness of muscle tissue to exercise, but the knowledge that is evidently still missing is one of basic science; what is the potential mechanism by which vitamin D interacts with exercise to boost the muscle anabolic response?

It may be that vitamin D protects muscle and bone from the increase in inflammatory cytokines observed during “inflammageing” and these cytokines “block” the effects of resistance training. Vitamin D deficiency has been shown to predispose older adults to a pro-inflammatory environment (Laird et al., 2014, Elizondo-Montemayor et al., 2017), which in turn enters the body into a catabolic state of muscle mass loss, potentially linked to sarcopenia (Dalle et al., 2017). Moreover, vitamin D replete older adults have a better response to resistance training in terms of muscle strength than older adults considered to be vitamin D deficient (Fuller et al., 2011); it may be that vitamin D “primes” muscle ready for protein synthesis.

8. Bibliography

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APPENDIX A

Appendix A: Narrative analysis summary of findings for Group 1 secondary outcome measures									
Category	Outcome measure	Assessment point	Study	Intervention group % change from baseline			Control group % change from baseline		
				M	SD	N	M	SD	N
Body composition	CSA of quadriceps muscles (cm ²)	16 weeks	Agergaard et al., 2015	+4.94	5.28	7	+8.46*	6.80	10

CSA: Cross-sectional area

APPENDIX B

Appendix B: Narrative analysis summary of findings for Group 2 primary outcome measures									
Category	Outcome	Assessment point	Study	Intervention group change from baseline (%)			Control group change from baseline (%)		
				Mean	SD	N	Mean	SD	N
Muscle strength	Isometric knee extensor strength (Nm)	6 months	Verschueren et al., 2011	+3.01	2.67	28	+0.11	3.18	28
Muscle power	Sit-to-stand transfer power (W)	12 weeks	Drey et al., 2011	+8.99*	5.51	23	+2.61	2.49	22
	Functional stair climbing muscle power (W)	12 months	Gianoudis et al., 2014	+10.40*	13.00	81	+6.20	12.70	81
Muscle function	30 second sit-to-stand (number of stands)	12 months	Gianoudis et al., 2014	+18.30*	23.60	81	+2.70	17.2	81
	5-time chair stand time (s)	24 months	Uusi-Rasi et al., 2015	-6.95	2.50	102	-3.49	3.30	102
	Normal walking speed (m/s)	24 months	Uusi-Rasi et al., 2015	-1.80	0.20	102	-3.30	0.21	102
	Endurance: 12-minute walk (m)	9 months	Bunout et al., 2006	+8.80	17.60	22	+20.90	27.70	24
Balance	Romberg ratio (%)	9 months	Bunout et al., 2006	+2.80	33.80	22	-0.60	35.80	24
	Four square step test (s)	12 months	Gianoudis et al., 2014	-12.00*	14.10	81	-5.20	14.90	81
	Body sway (cm)	32 weeks	Jessup et al., 2003	-26.39*	0.52	9	+2.90	0.49	9
	Backwards walking (% able to complete)	24 months	Uusi-Rasi et al., 2015	+25.47*	13.59	102	+9.48	15.58	102

APPENDIX C

Appendix C: Narrative analysis summary of findings for Group 2 secondary outcomes									
Category	Outcome measure	Assessment point	Study	Intervention group change from baseline (%)			Control group change from baseline (%)		
				M	SD	N	M	SD	N
Body composition	Appendicular lean mass (Kg)	12 weeks	Drey et al., 2011	+1.65	0.71	23	+0.00	0.87	22
	Muscle mass of upper limb (cm ³)	6 months	Verschueren et al., 2011	-0.16	0.57	28	-0.25	0.38	28
	BMD of femoral neck (g/cm ²)	6 months	Verschueren et al., 2011	+0.71	0.42	28	+0.99	0.51	28

APPENDIX D

Appendix D: Unadjusted associations between serum 25(OH)D concentration and lean mass and muscle strength									
	All postmenopausal women			Postmenopausal women aged <65 years			Postmenopausal women aged ≥65 years		
	N	r _s (95% CI)	p	N	r _s (95% CI)	p	N	r _s (95% CI)	p
25(OH)D x relative appendicular skeletal muscle index Autumn	145	-.054 (-0.22, 0.12)	0.534	111	-.221 (-0.42, -0.04)	0.018	31	-.171 (-0.48, 0.21)	0.333
25(OH)D x relative appendicular skeletal muscle index Spring	148	.037 (-0.13, 0.20)	0.655	103	-.218 (-0.39, -0.05)	0.026	28	-.094 (-0.43, 0.27)	0.615
25(OH)D x HGS Summer	167	.285 (0.14, 0.41)	<0.001	123	.300 (0.14, 0.42)	0.001	44	.318 (0.07, 0.51)	0.035
25(OH)D x HGS Autumn	157	.194 (0.03, 0.35)	0.015	118	.233 (0.03, 0.36)	0.011	39	.176 (-0.19, 0.50)	0.284
25(OH)D x HGS Winter	145	.180 (0.01, 0.34)	0.030	108	.216 (0.02, 0.33)	0.025	44	.190 (-0.11, 0.46)	0.260
25(OH)D x HGS Spring	144	.201 (0.04, 0.35)	0.016	110	.237 (0.05, 0.35)	0.013	34	.037 (-0.33, 0.39)	0.835

ASM: Appendicular skeletal muscle mass; Relative appendicular skeletal muscle index is ASM/h² HGS: Handgrip strength

APPENDIX E



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THE INFLUENCE OF STRENGTH TRAINING AND VITAMIN D3 SUPPLEMENTATION ON MUSCLE AND BONE FUNCTION IN OLDER ADULTS

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

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

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1

What is the purpose of the study?

Muscle and bone loss are inevitable consequences of ageing, even if our health is good. We are looking at ways of reducing muscle and bone loss in order to help improve the ability to perform tasks and activities important for independent living. The purpose of this research study is to find out whether a period of strength training combined with a vitamin D3 supplement is any more effective at improving muscle and bone function than strength training alone. In order to do this, we would like to measure the size of your muscles, along with their strength and power, do some tests to see how well you can perform tasks and activities important for daily living plus answer some questions about how you feel about your life just now. We would also like to take blood samples. We would like to see if any of these measurements change after strength training and whether taking vitamin D3 can help to boost these changes.

Why have I been chosen?

You have been chosen because you are 65 years of age or over and are living independently.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You will still be free to withdraw at any time in the future 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 that you receive.

What will happen to me if I take part?

If you are interested in taking part, we would like you to complete a brief screening health questionnaire. We will use your responses to the questionnaire to help determine whether you are eligible to take part in the study. If you are eligible to participate, we will ask you to sign a consent form and then we will take a small sample of blood (just over a tablespoon full). We will also ask you to answer a few short questions about thinking and memory. The purpose of these procedures is to help us determine whether it is appropriate for you to continue with your participation. We will then ask you to wear a small monitor which measures physical activity for 7 days. After this we will then ask you to visit either your communal room or a facility close by (we will provide taxi transport if necessary) to take part in an exercise training study. This will involve getting together in a group with a specialist exercise instructor to do some strength and balance training exercises. This will take place 3 times a week for 6 months. You will also be asked to take either a vitamin D tablet or a placebo tablet once a day for 6 months. We will provide you with tablets 4 weeks at a time. Neither you nor we will know which type of tablet you are taking until the end of the study. Before and after the 6 months of exercise training combined with the tablets, we will ask you to come to the Wellcome Trust Clinical Research Facility at the Queen Elizabeth Hospital, Birmingham, in order to make some physical function measurements. We will provide taxi transport. At the end of the study you will be given a DVD to keep which includes the exercises you have been doing during the study. If something should happen whereby you do not fulfil the eligibility criteria any longer, then we would not continue with the collection of data but you may still participate in the exercise at the discretion of the study team's medical expert.

‘What tests and procedures will be carried out as part of this study’

Measurement of bone and body composition (DXA scan): This gives us information about your bone strength and body composition (that is, the amount of muscle, bone and fat in your body). The scan takes about 30 minutes and involves X-rays. However, the radiation exposure you will receive by taking part in this study is equivalent to 4 days of naturally occurring background radiation which is an extra 1% of the annual naturally occurring background radiation. This additional risk is comparable to travelling 50 miles by car.

Test of muscle power: Your leg muscle power will be measured using specialised equipment and will involve simple pushing movements which you will be asked to hold for a few seconds. These tests will take about 10 minutes so you may feel a little tired afterwards but the tests should not cause any pain or discomfort. We will also measure the power you exert when you get out of a chair using a special force platform. This will take only a minute.

Test of functional ability (short physical performance battery): This is a short series of tests of balance, walking speed (over a few feet) and chair rise. It will take about 10 minutes.

Test of functional ability (timed up-and-go): This test will measure your ability to get up out of a chair and walk a few feet then turn around and go back to your chair. It will take 5 minutes.

Questionnaires: You will be asked to complete two questionnaires; one asks about your quality of life and the other about pain/ discomfort in muscles and bones. They will take approximately 20 minutes to complete.

Measurement of physical activity: We will measure this just before you do the other tests. We will attach a small physical activity monitor to your thigh with sticky pads and ask you to wear it for 7 days. We would ask you to remove some of your clothing for a few minutes so that we can attach the monitor. The monitors are very small (1.5x2.5 inches) and lightweight (less than 1oz). You may bathe/ take a shower during this time (although if you wish to bathe we would ask you to remove the monitor while you are bathing). We will collect the monitor from you or provide you with a stamp-addressed envelope to return the monitor to us after 7 days.

Food diary: We will give you a special diary so that you can record the things you eat and drink over the course of 2 days (in other words 2 days at the beginning of the study and 2 days at the end).

A blood test: We will take small samples of blood from a vein (a total of four blood samples of 20ml which is just a bit more than a tablespoon). We will take a sample at the beginning (which we mentioned earlier), two during the study and one at the end. The samples will be used to check your vitamin D and calcium levels as well as other important chemicals in your blood.

Measurement of blood pressure: This test will allow us to measure your blood pressure whilst seated and also whilst standing.

What are the possible disadvantages and risks of taking part?

We have taken every step in the design of this study to minimise any possible disadvantages and risks.

What are the possible benefits of taking part?

Regular exercise has many benefits not only in terms of improving muscle and bone function but also improving the ability to perform normal everyday tasks as well as helping to improve quality of life. We cannot guarantee that you will receive all of these benefits but we would be surprised if you didn't receive any. Taking vitamin D3 supplements may confer a small additional benefit. Even if you do not benefit directly from taking part in the study, the information we receive may help us to find ways of slowing down muscle and bone loss, not just in relatively healthy older people but in people with certain diseases characterized by muscle wasting.

Part 2

What if relevant new information becomes available?

If any clinically significant information comes to light as a consequence of taking part in this study, we will inform your GP (with your permission).

What will happen if I don't want to carry on with the study?

You can withdraw from the study at any time without having to give a reason. However, we may still choose (with your permission) to use any data obtained as a result of your participation. Any identifiable data will be anonymised.

What if there is a problem?

Complaints: If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this by contacting Dr Sean Jennings, Research Support Group, University of Birmingham (0121 415 8011 or s.jennings@bham.ac.uk).

Harm: In the event that something does go wrong and you are harmed during the research study, the University has in force a Public Liability Policy and/or Clinical Trials policy which provides cover for claims for "negligent harm" and the activities here are included within that coverage.

Will my taking part in this study be kept confidential?

Yes. All information which is collected about you during the course of the research will be kept strictly confidential. However, we would like to inform your GP of your involvement in this study but we will require your permission to do this. All other information about you which leaves the University of Birmingham will have your name, address and date of birth removed so that you cannot be recognised from it.

What will happen to the results of the current research study?

The results of this study will be published in medical journals, reports and textbooks. You will not be identifiable in any publication or report.

Who is organising and funding the research?

The research is being organised and sponsored by the University of Birmingham. The study is funded by the National Osteoporosis Society.

Who has reviewed the study?

This study has been given a favourable ethical opinion for conduct in the NHS by the Black Country Research Ethics Committee.

Contact details

You may contact me (one of the Principal Researchers) directly by telephoning 0121 414 8743 or email c.a.greig@bham.ac.uk for further information at any time. Alternatively, you may contact Dr Alison Rushton (School of Sport, Exercise and Rehabilitation Sciences) who is acting as an independent advisor. Her contact telephone number is 0121 415 8597 (email a.b.rushton@bham.ac.uk)

Many thanks for taking the time to read this information.

Dr Carolyn Greig PhD

School of Sport Exercise and Rehabilitation Sciences

MRC-ARUK Centre for Musculoskeletal Ageing Research

The University of Birmingham

APPENDIX F

Studies of Human Performance in Older People: Health Questionnaire

Name:

Address:

Date of Birth:

Telephone no.:

If the answer is YES to any of the following questions, please give some details including dates where possible.

Have you any history of heart trouble?

(such as heart attack, angina, valve disease, palpitations, pains in chest, dizzy spells)

Have you any history of problems with blood vessels?

(such as thrombosis, embolus, claudication, aneurysm, dizzy spells, stroke, blood clots)

Have you any history of chest problems?

(bronchitis, asthma or wheezy chest)

Have you ever smoked?

(if YES please state whether you are a current or ex-smoker and how much)

Do you suffer from diabetes?

(if YES please state if insulin dependent)

Have you any history of major illness now or in the last 20 years?

(such as rheumatoid arthritis, blood disorders, cancer)

Have you any history of emotional or psychiatric problems?

Do you suffer from osteoarthritis?

(if YES please state joints affected and indicate mild, moderate or severe and any medication regularly taken)

Have you broken or fractured any bones? If so, when?

Do you have any problems with your bones?

(osteoporosis, loss of height)

Have you any history of back problems? If so, when did they start and do they still affect you in any way?

Have you had any surgery on your joints? If so, when?

Do you suffer from high blood pressure?

Have you had any acute illness in the last six months?

(such as influenza, recurrent sore-throat, bronchitis)

Please state any medication, prescribed or over the counter, regularly taken for any condition

Name of medication

How often medication is taken

Have you been in hospital in the last 5 years? If so, why and for how long?

Do you have any physical disabilities?

(such as visual or hearing problems)

Is there any other illness or condition that affects your general health or interferes with your mobility?

Approximately how tall are you?

Approximately how much do you weigh?

Your Doctor's Name:

Your Doctor's Address:

Thank you for completing this questionnaire

APPENDIX G



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CONSENT FORM

THE INFLUENCE OF STRENGTH TRAINING AND VITAMIN D3 SUPPLEMENTATION ON MUSCLE AND BONE FUNCTION IN OLDER ADULTS

Please initial box

1. I confirm that I have read and understand the information sheet dated 13 July 2016 (version 6.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.
3. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from the University Hospitals Birmingham NHS Foundation Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

4. I understand that personal data collected during the study will be kept anonymised and stored securely at the University of Birmingham.

5. I agree to my GP being informed of my participation in the study. I agree to take part in the above study.

6. I agree to the storage of samples taken during the course of this study so that they may be considered for use in future research studies (pending a favourable ethical opinion).

Name of Participant Date

Signature

Name of Investigator Date

Signature

APPENDIX H

Case Report Form

EXVITD: Influence of combined vitamin D3 supplementation and resistance exercise training on musculoskeletal health in frail older men and women

Participant ID:

Chief Investigator:

Dr Carolyn Greig

School of Sport, Exercise and Rehabilitation Sciences
University of Birmingham
Edgbaston
Birmingham
B15 2TT
UK

Telephone: [0121 414 8743](tel:01214148743)

Email: c.a.greig@bham.ac.uk

Sponsor reference number: RG_14-185

CRF study number: 1464

RRK number: 5293

GENERAL GUIDELINES FOR THE CASE REPORT FORM COMPLETION

All clinical data collected during the study has to be documented in this case report form.

Please use a black ball-point pen for filling in the case report form.

Incorrect entries should be deleted with a single line. The original entry must be kept legible. All changes and/or corrections made to the case report form must be initialled and dated by the investigator or by another responsible person of the respective study centre.

Please answer all questions and write clearly.

Identification of subject throughout the study is done by subject number only.

Please enter only one digit in one box. Data should be entered at the right hand margin. If not enough digits are available to fill all fields, please prefix the number by recording "0".

Please enter complete dates wherever possible. If day or month of a date should be unknown, please enter "NK" in the respective fields. e.g.: NK/05/2004

Times should be entered using the 24-hour clock. Please enter times from 00:00 to 23:59. Do not use 24:00.

If a question is not applicable, please record "NA" for "not applicable".

If an examination was not done, please record "ND" for "not done".

If a result is zero, please enter "0".

If a page has not been used, please enter the subject number and cross out the page.

Comments should be as short as possible. Please do not enter comments outside the predefined areas (Final Comment Page).

The case report form has to be signed by the investigator. If a premature termination of the trial occurs in a subject, the case report form should be completed up to this day and the termination form must be filled in.

DEMOGRAPHIC DATA

Date of Assessment

Informed Consent Yes No

Date of Consent

--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Date of Birth

--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Age

--	--	--

 yrs

Sex Male Female

Health Questionnaire completed? Yes No

MMSE completed? Yes No

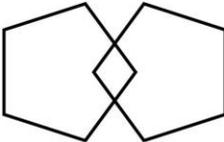
Venous blood sample collected? Yes No

GP notified? Yes No

Accelerometer attached? Yes No

MINI MENTAL STATE EXAMINATION (MMSE)

Name:
DOB:
Hospital Number:

One point for each answer	DATE:		
ORIENTATION Year Season Month Date Time Country Town District Hospital Ward/Floor/ 5/ 5/ 5
REGISTRATION Examiner names three objects (e.g. apple, table, penny) and asks the patient to repeat (1 point for each correct. THEN the patient learns the 3 names repeating until correct)./ 3/ 3/ 3
ATTENTION AND CALCULATION Subtract 7 from 100, then repeat from result. Continue five times: 100, 93, 86, 79, 65. (Alternative: spell "WORLD" backwards: DLROW)./ 5/ 5/ 5
RECALL Ask for the names of the three objects learned earlier./ 3/ 3/ 3
LANGUAGE Name two objects (e.g. pen, watch). Repeat "No ifs, ands, or buts". Give a three-stage command. Score 1 for each stage. (e.g. "Place index finger of right hand on your nose and then on your left ear"). Ask the patient to read and obey a written command on a piece of paper. The written instruction is: "Close your eyes". Ask the patient to write a sentence. Score 1 if it is sensible and has a subject and a verb./ 2/ 2/ 2
COPYING: Ask the patient to copy a pair of intersecting pentagons/ 1/ 1/ 1
			
TOTAL:/ 30/ 30/ 30

MMSE scoring
 24-30: no cognitive impairment
 18-23: mild cognitive impairment
 0-17: severe cognitive impairment



INCLUSION CRITERIA

Aged 65 years and over

YES

NO

Living independently

Ambulatory

EXCLUSION CRITERIA

History of myocardial infarction within the previous 2 years

YES

NO

Cardiac illness: moderate/ severe aortic stenosis, acute pericarditis, acute myocarditis, aneurysm, severe angina, clinically significant valvular disease, uncontrolled dysrhythmia, claudication, within the previous 10 years

Thrombophlebitis or pulmonary embolus within the previous 2 years

History of cerebrovascular disease (CVA or TIA) within the previous 2 years

Acute febrile illness within the previous 3 months

Severe airflow obstruction

Uncontrolled metabolic disease (e.g. thyroid disease)

Significant emotional distress, psychotic illness or depression within the previous 2 years

Major systemic disease active within the previous 2 years (e.g. cancer, rheumatoid arthritis)

Significant emotional distress, psychotic illness or depression within the previous 2 years

Lower limb fracture sustained within the previous 2 years/ upper limb fracture within the previous 6 months

Non arthroscopic lower limb joint surgery within the previous 2 years

Any reason for a loss of mobility for greater than 1 week in the previous 2 months or greater than 2 weeks in the previous 6 months

Resting systolic blood pressure > 200mmHg or resting diastolic blood pressure > 100mmHg

Poorly controlled atrial fibrillation; poor (chronic) pain control

YES

NO

Moderate/severe cognitive impairment (mini mental state examination (MMSE) score <23)

Vitamin D deficient (serum 25(OH)D3 <30nmol/l); current antiresorptive or anabolic treatment for osteoporosis

Treatment with bisphosphonates for osteoporosis in the past two years

Current use of glucocorticoids; known primary hyperparathyroidism; hypercalcaemia (albumin-adjusted serum calcium >2.60 mmol/l)

Renal impairment (Stage 4 or 5)

☞ If any of the shaded boxes are ticked, the participant is not eligible for the study

Previously taken vitamin D3 supplement? Yes No

If 'yes' please state dose _____ IU.day⁻¹

ELIGIBILITY

Is the subject eligible to participate in the study Yes No

If no, please comment:

VITAL SIGNS

Date of Assessment

Height cm

Weight kg BMI kg/m²

Pulse per minute *☞ Measured after 5 minutes rest, sitting, on the dominant arm*

Blood Pressure Sitting

Measurement 1: Systolic mmHg Diastolic mmHg

Measurement 2: Systolic mmHg Diastolic mmHg

Measurement 3: Systolic mmHg Diastolic mmHg

Blood Pressure Standing

Measurement 1: Systolic mmHg Diastolic mmHg

Measurement 2: Systolic mmHg Diastolic mmHg

Measurement 3: Systolic mmHg Diastolic mmHg

☞ Blood pressure to be measured in 5 minutes intervals on the dominant arm

Concomitant Medication present? Yes No

☞ If yes, please complete Concomitant Medication page

Has the participant had a loss of mobility in the last 2 months? Yes No

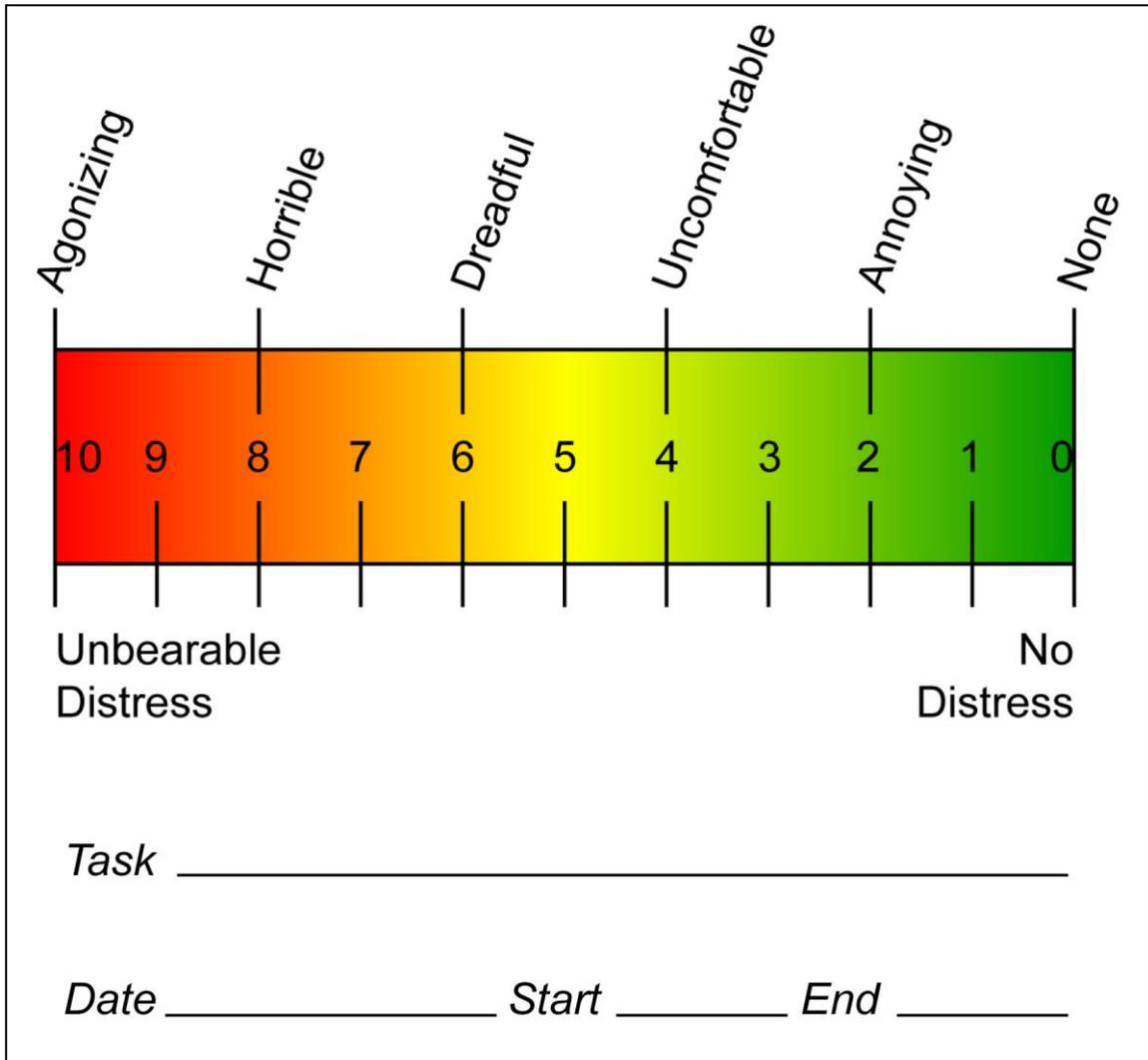
☞ If yes, please tick Exclusion Criterion 13 as "No" and exclude the subject

Accelerometer returned? Yes No

3-day food diary handed out? Yes No

DXA scan performed? Yes No

VAS Scale for musculoskeletal pain and comfort



	Sitting	Standing
Score		

Date of Assessment

Has the Concomitant Medication changed since the previous visit? Yes No

If yes, please complete Concomitant Medication page

3-day Food Diary returned? Yes No

Accelerometer returned? Yes No

Venous Blood sample taken? Yes No

Musculoskeletal Pain/Comfort Questionnaire completed? Yes No

ACCELEROMETER RESULTS

Start of physical monitoring

Date Time

End of physical monitoring

Date Time

No. of 24h Time Periods	Average No. of steps (24 h period)	Average Time lying and sitting (h/day)	Average Time standing (h/day)	Average Time stepping (h/day)	No. of u/d transitions	EE (MET/h)

MUSCLE EXAMINATION

Lower Limb Extensor Power Output

Dominant limb (L/R)	
Seat length (cm)	
Position of non-dominant foot	

	Power Output (Watts.kg ⁻¹)
Measurement 1	
Measurement 2	
Measurement 3	
Measurement 4	
Measurement 5	
Best measurement	

SHORT PHYSICAL PERFORMANCE BATTERY TEST

Balance Tests: A and B. Side-by-Side Stand and Semi-Tandem Stand

	Held for 10 secs (1 point)	Held for <10 secs (0 points, please state time)	Not attempted/failed. 0 points (please select reason from key)
Side-by-Side Stand			
Semi-Tandem Stand			

C: Tandem Stand

	Held for 10 secs (2 points)	Held for 3 – 9.99 secs (1 point)	Held for <3 secs (0 points)	Not attempted/failed. 0 points (please select reason from key)
Tandem Stand				

Key

- 1. 1 Tried but unable
- 2. 2 Participant could not hold position unassisted
- 3. 3 Not attempted – you felt it was unsafe
- 4. 4 Not attempted – participant felt unsafe
- 5. 5 Participant unable to understand instructions
- 6. 6 Participant refused
- 7. 7 Other (please specify)

D: Total Balance Test Score _____ (sum points)

Comments: _____

Gait Speed Test

	Time for 3m walk (secs)	Walking aid? (None, cane, other)	Not attempted/failed (Please select reason from key)
Attempt 1			
Attempt 2			

Key

1. Tried but unable
2. Participant could not hold position unassisted
3. Not attempted – you felt it was unsafe
4. Not attempted – participant felt unsafe
5. Participant unable to understand instructions
6. Participant refused
7. Other (please specify)

Scoring

Participant unable to complete 5 chair stands or completes stands in >60 sec: 0 points

If chair stand time is 16.70 sec or more: 1 point

If chair stand time is 13.70 to 16.69 sec or more: 2 points

Repeated Chair Stand

If chair stand time is 11.20 to 13.69 sec: 3 points

	Time taken to complete 5 stands (secs)	With or without using arms?	Not attempted/failed (Please select reason from key)
Attempt			

Key

1. Tried but unable
2. Participant could not hold the position unassisted
3. Not attempted – you felt it was unsafe
4. Not attempted – participant felt unsafe
5. Participant unable to understand instructions
6. Participant refused
7. Other (please specify)

Scoring for Complete Short Physical Performance Battery**Test Scores**

Total Balance Test score _____ points

Gait Speed Test score _____ points

Chair Stand Test score _____ points

Total Score _____ points

Timed 3-metre up and go

	3m up and go [sec]
Repetition 1	
Repetition 2	
Repetition 3	
Best attempt	

POWER REQUIRED TO RISE FROM A CHAIR

Attempt number	CRT5 (seconds)	Power _(Max) (W.kg ⁻¹)
1		
2		
3		
4		
5		

The SF-36v2™ Health Survey

SF1: In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SF2: Compared to one year ago, how would you rate your health in general now?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SF3: The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes limited a lot	Yes limited a little	No not limited at all
Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lifting or carrying groceries	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Climbing several flights of stairs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Climbing one flight of stairs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Bending, kneeling, or stooping	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Walking more than a mile	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Walking several hundred yards	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Walking one hundred yards	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bathing or dressing yourself	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

The SF-36v2™ Health Survey Continued

SF4: During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
Cut down on the amount of time you spent on work or other activities	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Accomplished less than you would like	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Were limited in the kind of work or other activities	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Had difficulty performing the work or other activities (e.g, it took extra effort)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SF5: During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
Cut down on the amount of time you spent on work or other activities	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Accomplished less than you would like	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Did work or other activities less carefully than usual	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SF6: During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

Not at all	Slightly	Moderately	Quite a bit	Extremely
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SF7: How much bodily pain have you had during the past 4 weeks?

None	Very mild	Mild	Moderate	Severe	Very severe
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SF8: During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

The SF-36v2™ Health Survey Continued

SF9: These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks...

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
did you feel full of life?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
have you been very nervous?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
have you felt so down in the dumps nothing could cheer you up?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
have you felt calm and peaceful?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
did you have a lot of energy?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
have you felt downhearted and depressed?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
did you feel worn out?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
have you been happy?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
did you feel tired?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SF10: During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SF11: How TRUE or FALSE is each of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
I seem to get sick a little easier than other people	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I am as healthy as anybody I know	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I expect my health to get worse	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
My health is excellent	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Date of Assessment

Has the Concomitant Medication changed since the previous visit? Yes No

☞ If yes, please complete Concomitant Medication page

Venous blood sample collected? Yes No

Date of

Assessment

--	--	--	--	--	--	--	--	--	--

Has the Concomitant Medication changed since the previous visit? Yes No

☞ If yes, please complete Concomitant Medication page

Venous blood sample collected? Yes No

VITAL SIGNS

Date of Assessment

--	--	--	--	--	--	--	--	--	--

Pulse

--	--	--	--

 per minute *☞ Measured after 5 minutes rest, sitting, on the dominant arm*

Blood Pressure Sitting

Measurement 1: Systolic

--	--	--	--

 mmHg Diastolic

--	--	--	--

 mmHg

Measurement 2: Systolic

--	--	--	--

 mmHg Diastolic

--	--	--	--

 mmHg

Measurement 3: Systolic

--	--	--	--

 mmHg Diastolic

--	--	--	--

 mmHg

Blood Pressure Standing

Measurement 1: Systolic mmHg Diastolic mmHg

Measurement 2: Systolic mmHg Diastolic mmHg

Measurement 3: Systolic mmHg Diastolic mmHg

 *Blood pressure to be measured in 5 minutes intervals on the dominant arm*

Concomitant Medication present? Yes No

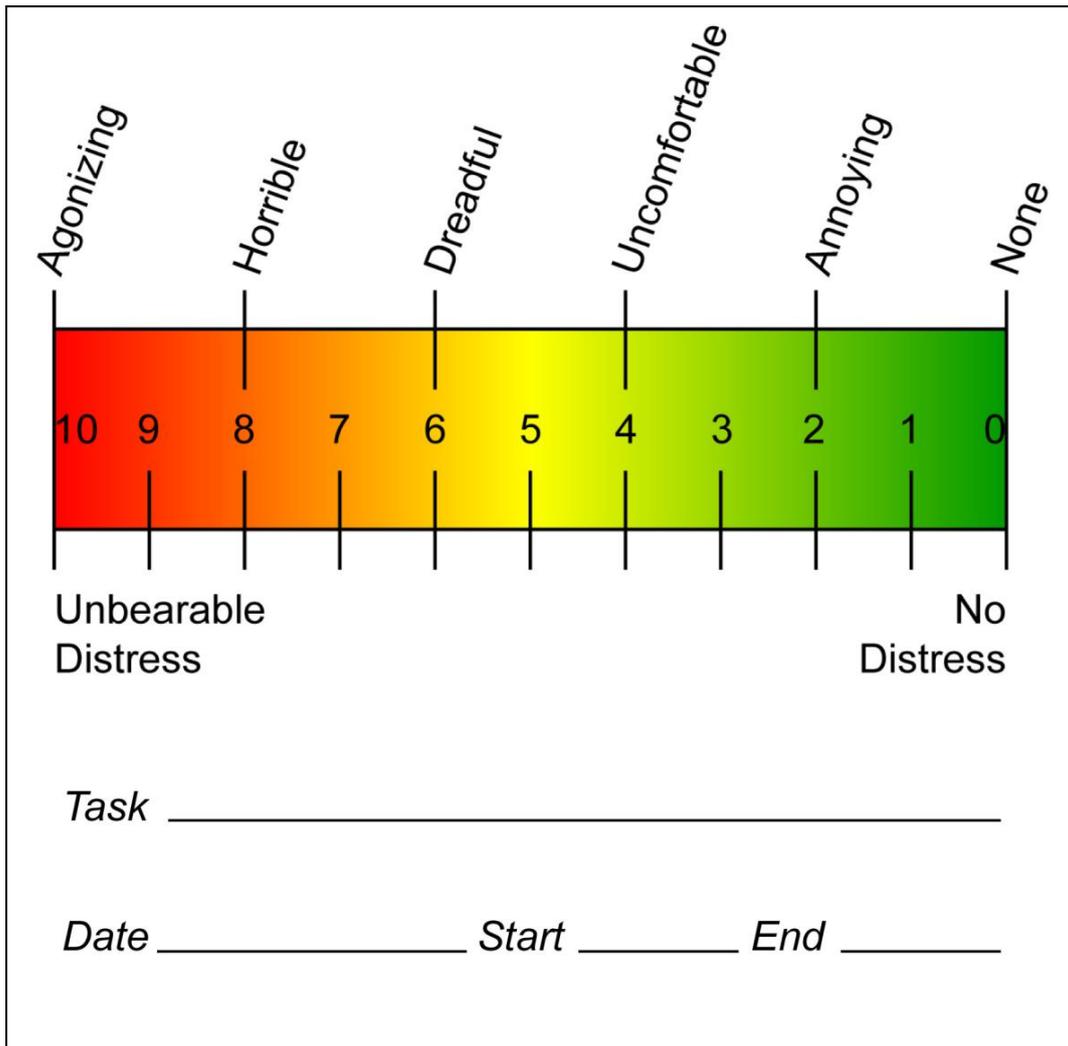
 *If yes, please complete Concomitant Medication page*

Physical Activity Monitor attached? Yes No

3-day food diary handed out? Yes No

DXA scan performed? Yes No

VAS Scale for musculoskeletal pain and comfort



	Sitting	Standing
Score		

Date of Assessment

Has the Concomitant Medication changed since the previous visit? Yes No

 If yes, please complete Concomitant Medication page

3-day Food Diary returned? Yes No

Accelerometer returned? Yes No

Venous Blood sample taken? Yes No

Musculoskeletal Pain/Comfort Yes No

Questionnaires completed?

DXA scan completed Yes No

PHYSICAL ACITIVITY MONITOR RESULTS

Start of physical monitoring

Date

--	--	--	--	--	--	--	--	--	--

 Time

--	--	--	--

End of physical monitoring

Date

--	--	--	--	--	--	--	--	--	--

 Time

--	--	--	--

No. of 24h Time Periods	Average No. of steps (24 h period)	Average Time lying and sitting (h/day)	Average Time standing (h/day)	Average Time stepping (h/day)	No. of u/d transitions	EE (MET/h)

MUSCLE EXAMINATION

Lower Limb Extensor Power Output

Dominant limb (L/R)	
Seat length (cm)	
Position of non-dominant foot	

	Power Output (Watts.kg ⁻¹)
Measurement 1	
Measurement 2	
Measurement 3	
Measurement 4	
Measurement 5	
Best measurement	

SHORT PHYSICAL PERFORMANCE BATTERY TEST

Balance Tests: A and B. Side-by-Side Stand and Semi-Tandem Stand

	Held for 10 secs (1 point)	Held for <10 secs (0 points, please state time)	Not attempted/failed. 0 points (please select reason from key)
Side-by-Side Stand			
Semi-Tandem Stand			

C: Tandem Stand

	Held for 10 secs (2 points)	Held for 3 – 9.99 secs (1 point)	Held for <3 secs (0 points)	Not attempted/failed. 0 points (please select reason from key)
Tandem Stand				

Key

1. Tried but unable

2. Participant could not hold the position unassisted
3. Not attempted – you felt it was unsafe
4. Not attempted – participant felt unsafe
5. Participant unable to understand instructions
6. Participant refused
7. Other (please specify)

D: Total Balance Test Score _____ (sum points)

Comments: _____

Gait Speed Test

	Time for 3m walk (secs)	Walking aid? (None, cane, other)	Not attempted/failed (Please select reason from key)
Attempt 1			
Attempt 2			

Key**Scoring**

- | | | | |
|---|--|---------------------------------|-----------------------------------|
| 1 | Tried but unable | If time is more than 6.52 secs: | <input type="checkbox"/> 1 point |
| 2 | Participant could not hold position unassisted | If time is 4.66 to 6.52 secs: | <input type="checkbox"/> 2 points |
| 3 | Not attempted – you felt it was unsafe | If time is 3.62 to 4.65 secs: | <input type="checkbox"/> 3 points |
| 4 | Not attempted – participant felt unsafe | If time is less than 3.62 secs: | <input type="checkbox"/> 4 points |
| 5 | Participant unable to understand instructions | | |
| 6 | Participant refused | | |
| 7 | Other (please specify) | | |

Repeated Chair Stand

	Time taken to complete 5 stands (secs)	With or without using arms?	Not attempted/failed (Please select reason from key)
Attempt			

Key**Scoring**

- | | | |
|---|---|---|
| 1. Tried but unable | Participant unable to complete 5 chair stands or completes stands in >60 sec: <input type="checkbox"/> 0 points | |
| 2. Participant could not hold the position unassisted | | |
| 3. Not attempted – you felt it was unsafe | | |
| 4. Not attempted – participant felt unsafe | | |
| 5. Participant unable to understand instructions | | |
| 6. Participant refused | | If chair stand time is 16.70 sec or more: <input type="checkbox"/> 1 points |
| 7. Other (please specify) | | |

Scoring for Complete Short Physical Performance Battery

If chair stand time is 13.70 to 16.69 sec or more: 2 points

Test Scores

Total Balance Test score _____ points

Gait Speed Test score _____ points

Chair Stand Test score _____ points

Total Score _____ points

Timed 3-metre up and go

	3m up and go [sec]
Repetition 1	
Repetition 2	
Repetition 3	
Best attempt	

POWER REQUIRED TO RISE FROM A CHAIR

Attempt number	CRT5 (seconds)	Power _(Max) (W/kg)
1		
2		
3		
4		
5		

SAE REPORTING

Serious Adverse Event Form (v2)
(Research drugs, devices and interventions)
University Hospital Birmingham NHS Trust

This form must be completed in the event of a Serious Adverse Event / Incident.
This can be defined as an untoward medical occurrence in a patient during clinical research involving a pharmaceutical product or clinical intervention that: is fatal; is life threatening; results in persistent or significant disability/ incapacity ; requires inpatient hospitalisation or prolongs a current hospitalisation; is a congenital anomaly in offspring; or an event that may jeopardise the patient or may require intervention to prevent one of the outcomes listed above.

(R&D SAE id: _____)

Study Title or Trust RRK Number: _____

Section A - Details of Subject Affected by Serious Adverse Event

Has the Principal Investigator been informed of this event prior to completion of this form?
Yes No

Subject Initials: _____ **Risk Form Number:** _____ **Subject Number:** _____

Section B - Details of the Serious Adverse Event

Date of Onset: _____ **Time:** _____

Hospital: _____ **Exact Location:** _____

Definition of Serious Adverse Event: (tick the appropriate category for the event)

- | | | |
|--|--|--|
| Death <input type="checkbox"/> | Resulted in Disability <input type="checkbox"/> | Congenital abnormality <input type="checkbox"/> |
| Life threatening <input type="checkbox"/> | Required Hospitalisation <input type="checkbox"/> | none of above <input type="checkbox"/> |

Describe Event: (A summary of signs and symptoms (including severity), vital signs, diagnosis, treatment of event, concurrent treatment, other relevant medical history, details of study drug/ device. Please include the time point in the study at which the event occurred.)

Number of additional pages added, if any _____

(R&D SAE id: _____)

Risk Form Number: _____

Section C - Relationship To Study Involvement

1. Was the incident related to the patient's involvement in the study?
Likely Possible Unlikely
2. Is the event related to a break in the study protocol? Possible Unlikely
3. If you answered **Possible** to number 2, please give details below

4. Was the event unexpected (i.e. not consistent with available information about the drug, device or intervention)? Expected Unexpected

5. Action Taken Regarding Participation In Study:
Temporarily Discontinued

Date: _____

Decision taken by: _____

Permanently Discontinued

Date: _____

Decision taken by: _____

Patient Continued In Study (Please Tick Box)

Section D - Outcome Of Serious Adverse Event

- Recovered
- Event Continuing
- Patient Died

If necessary please give additional details below:

Section E - Reporter's Details

(Please Print)

Name: _____ Title: _____ Post: _____

Department: _____ Contact Number: _____

Please attach the completed form to a Trust Incident Report Form and complete sections A (Details Of The Person Affected By The Incident) and D (Details Of The Person Completing The Form). Forward both forms to: The R&D Office, Queen Elizabeth Hospital Birmingham, Edgbaston, Birmingham
Forward one copy of this form to the Principal Investigator and retain one copy in the Study File.

Concomitant Medications Log

A list of concomitant medications. This log should be used for CRF studies as required and where an equivalent document is not provided by the study Sponsor or Chief Investigator.

Study Title: _____ CRF Study ID: _____

Participant _____
Study ID: _____

Medication	Dose	Unit	Indication / Reason for Use	Frequency	Route	Start Date* (dd/mm/yy)	Stop Date* (dd/mm/yy)	Ongoing

*Please also include the time if this is required by the study protocol

STUDY TERMINATION

Did any Serious Adverse Events occur during the study? Yes No

 *If yes, please complete SAE Report*

Did the subject terminate the study as planned Yes

No  *Please state reason below*

Date of last study procedure

--	--	--	--	--	--	--	--	--	--

If the study was not terminated as planned, please state the reason for study termination:

Subject withdrawal of consent

Investigator's discretion

Intercurrent illness

Changes in the subject's condition

Noncompliance with study procedures

Termination of the clinical trial

Other

Please describe _____

INVESTIGATOR STATEMENT

I hereby certify that all information entered by myself or my team is, to the best of my knowledge, correct.

Signature

APPENDIX I: EXERCISE PROGRESSION LOG

ID	Group	30-second sit to stand				Band colour used				Ankle weight used				Number of squats			
		Baseline	Month 1	Month 3	Month 6	Baseline	Month 1	Month 3	Month 6	Baseline	Month 1	Month 3	Month 6	Baseline	Month 1	Month 3	Month 6
001	1	9	14	-	-	Red	Blue	-	-	0	0.75	-	-	10	10	-	-
002	1	9	24	27	30	Red	Blue	Black	Black	0	0.75	1	1	10	10	40	60
004	1	15	27	25	29	Green	Green	Blue	Blue	0.5	0.5	0.75	0.75	16	20	180	320
005	2	12	22	22	24	Green	Green	Blue	Black	0.5	0.5	0.75	1	16	20	180	300
006	1	12	18	21	27	Red	Red	Blue	Blue	0	0.75	0.5	0.5	10	10	40	60
007	2	11	17	22	29	Red	Blue	Blue	Blue	0	0.75	0.5	0.5	10	10	40	60
008	2	16	22	21	21	Green	Green	Blue	Blue	0.5	0.5	0.75	0.75	32	60	160	300
009	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
010	1	4	12	12	13	Red	Green	Green	Blue	0.5	0.5	0.5	0.5	30	60	160	300
011	2	17	18	18	19	Blue	Black	Black	Black	0.75	1	1	1	220	290	300	300
012	1	16	30	31	37	Green	Black	Blue	Blue	0.5	1	1	1	100	150	350	500
013	2	16	26	27	34	Green	Green	Blue	Blue	0.5	0.75	0.75	1	100	150	350	500
014	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
015	1	13	20	25	25	Green	Green	Blue	Blue	0.5	0.75	1	1	100	150	350	500

APPENDIX I: CONTINUED

ID	Group	30-second sit to stand				Band colour used				Ankle weight used				Number of squats			
		Baseline	Month 1	Month 3	Month 6	Baseline	Month 1	Month 3	Month 6	Baseline	Month 1	Month 3	Month 6	Baseline	Month 1	Month 3	Month 6
016	1	26	28	31	34	Black	Black	Black	Black	1	1	1	1	100	150	350	500
017	1	12	29	32	35	Green	Blue	Black	Black	0.5	1	1	1	100	150	350	500
018	2	12	22	31	35	Green	Green	Blue	Blue	0.5	0.5	1	1	100	150	350	500
019	2	7	14	15	15	-	Green	Blue	Black	-	0.75	1	1	70	120	200	500
020	2	25	30	33	38	Blue	Black	Black	Black	0.5	1	1	1	200	350	500	600
021	1	24	28	30	33	Blue	Black	Black	Black	1	1	1	1	150	300	500	1000
024	1	3	15	12	12	-	Green	Blue	Black	-	0.75	1	1	70	120	200	400
026	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
029	2	17	22	24	30	Green	Blue	Blue	Black	0.75	1	1	1	150	200	250	500
030	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Groups: 1 = placebo, 2 = vitamin D

APPENDIX J: EXERCISE COMPLIANCE LOG

ID	Group	Month 1 compliance (%)	Month 2 compliance (%)	Month 3 compliance (%)	Month 4 compliance (%)	Month 5 compliance (%)	Month 6 compliance (%)
001	1	100.00	100.00	N/A	N/A	N/A	N/A
002	1	87.50	87.50	100.00	100.00	100.00	100.00
004	1	100.00	100.00	75.00	25.00	87.50	75.00
005	2	87.50	100.00	75.00	87.50	75.00	75.00
006	1	62.50	75.00	37.50	100.00	100.00	100.00
007	2	100.00	87.50	100.00	87.50	100.00	100.00
008	2	87.50	75.00	75.00	62.50	100.00	100.00
010	1	100.00	87.50	87.50	75.00	75.00	62.50
011	2	100.00	87.50	100.00	87.50	100.00	100.00
012	1	100.00	75.00	100.00	100.00	87.50	100.00
013	2	75.00	100.00	100.00	100.00	100.00	100.00
015	1	100.00	100.00	87.50	87.50	62.50	62.50
016	1	100.00	100.00	100.00	87.50	100.00	100.00
017	1	87.50	100.00	87.50	87.50	87.50	100.00
018	2	75.00	87.50	87.50	87.50	87.50	100.00
019	2	100.00	75.00	100.00	75.00	100.00	100.00
020	2	100.00	100.00	62.50	100.00	62.50	100.00
021	1	87.50	75.00	50.00	87.50	87.50	37.50
024	1	100.00	100.00	100.00	100.00	87.50	75.00
029	2	87.50	75.00	100.00	100.00	100.00	-
Groups: 1 = placebo, 2 = vitamin D							

APPENDIX K: SUPPLEMENT COMPLIANCE LOG

ID	Group	Month 1 compliance (%)	Month 2 compliance (%)	Month 3 compliance (%)	Month 4 compliance (%)	Month 5 compliance (%)	Month 6 compliance (%)
001	1	89.29	82.12	N/A	N/A	N/A	N/A
002	1	100.00	60.71	75.00	92.86	100.00	100.00
004	1	96.43	100.00	50.00	100.00	75.00	42.86
005	2	96.43	92.86	71.43	92.86	92.86	42.86
006	1	100.00	100.00	100.00	100.00	92.86	100.00
007	2	100.00	100.00	100.00	89.29	100.00	100.00
008	2	78.57	89.29	82.12	78.57	75.00	67.88
010	1	71.43	92.86	92.86	100.00	100.00	100.00
011	2	92.86	100.00	100.00	100.00	100.00	100.00
012	1	100.00	100.00	92.86	92.86	85.71	100.00
013	2	100.00	96.43	92.86	92.86	89.29	85.71
015	1	92.86	100.00	92.86	89.29	85.71	100.00
016	1	96.43	96.43	92.86	92.86	92.86	85.71
017	1	82.12	85.71	92.86	75.00	89.29	100.00
018	2	85.71	96.43	96.43	92.86	92.86	100.00
019	2	100.00	100.00	100.00	100.00	100.00	100.00
020	2	89.29	100.00	100.00	100.00	100.00	100.00
021	1	100.00	100.00	100.00	100.00	100.00	100.00
024	1	100.00	100.00	100.00	100.00	100.00	100.00
029	2	100.00	100.00	100.00	100.00	100.00	-
Groups: 1 = placebo, 2 = vitamin D							