

**THE EFFECT OF DIET-INDUCED WEIGHT LOSS ON  
BONE AND MUSCLE STRENGTH IN OVERWEIGHT  
AND OBESE INDIVIDUALS**

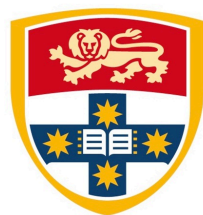
*A thesis submitted by*

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*A thesis submitted in fulfillment of for the degree of*

**Master of Philosophy (Medicine)**

**School of Medicine**



THE UNIVERSITY OF  
**SYDNEY**

**March 2016**

## Declaration

This thesis is submitted to the University of Sydney in fulfillment of the requirement for the Degree of Master of Philosophy (Medicine).

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

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Date: 30/03/16

## **Abstract**

The increasing prevalence of overweight and obesity is an alarming global issue. Not only do elevated body weights influence individual health and mortality, it also has an impact on a larger scale by placing financial burden on society. While diet-induced weight loss is the cornerstone treatment for overweight or obesity, some but not all studies have suggested that it has a harmful effect on bone and muscle strength. Bone and muscle strength are known in unison as the musculoskeletal system and have a parallel relationship, whereby factors affecting one system also tend to affect the other. Negative effects on bone and muscle strength independently, or together, results in an increased risk of disease states such as osteoporosis, dynaopenia and sarcopenia, and thus adversely affect overall health. The research presented in this thesis focuses on elucidating potential harmful implications that diet-induced weight loss may have on bone (Chapter 2) and muscle strength (Chapter 3), explored through two systematic reviews and meta-analyses using a random effects model.

In Chapter 2, included data were from 41 publications of overweight or obese but otherwise healthy adults who followed a dietary weight loss intervention, and which examined total hip, lumbar spine or total body bone mineral density (BMD) via dual energy x-ray absorptiometry, or serum or urinary concentrations of markers of bone turnover, at the start and end of the intervention. There were significant decreases in total hip BMD with dietary interventions of 6, 12 or 24 (but not 3) months' duration. No significant changes in BMD occurred in the lumbar spine or total body following dietary weight loss interventions ranging in duration from 3 to 24 months, except for a significant decrease in total body BMD after dietary interventions lasting 6 months. No significant changes occurred in the serum concentrations of the marker of bone

turnover, N-terminal propeptide of type I procollagen. Interventions of 2 or 3 months in duration (but not of 6, 12 or 24 months' durations) induced significant increases in serum concentrations of the bone turnover markers osteocalcin, C-terminal telopeptide of type I collagen or N-terminal telopeptide of type I collagen, indicating an early effect of diet-induced weight loss to promote bone breakdown. While results from individual studies varies, this meta-analysis shows a clear effect of diet-induced weight loss to significantly reduce total hip BMD in overweight and obese individuals, consistent with the observed increases in circulating levels of bone turnover markers. Since the hip is the gold-standard site for diagnosing osteoporosis and assessing fracture risk, it is now important to determine the long-term effects that diet-induced weight loss may have on fracture risk in overweight and obese adults.

In Chapter 3, the aim was to identify how diet-induced weight loss in adults with overweight or obesity impacts on muscle strength. 27 publications, including 33 interventions, most of which were 8-24 weeks in duration, were included. Meta-analysis of knee extensor strength as measured by isokinetic dynamometry found a significant decrease following diet-induced weight loss, by 7.5% from baseline values. Meta-analysis of handgrip strength showed a non-significant decrease with dietary restriction for weight loss. Due to variability in methodology and muscles tested, no other data could be meta-analyzed, and qualitative assessment of the remaining interventions revealed mixed results. Despite varying methodologies, diets and small sample sizes, these findings suggest a potential adverse effect of diet-induced weight loss on muscle strength. While these findings should not act as a deterrent against weight loss in people with overweight or obesity, due to the known

health benefits of losing excess weight, they call for strategies to combat strength loss – such as weight training and other exercises – during diet-induced weight loss.

From the two studies presented in this thesis, it can be seen that diet-induced weight loss can negatively impact on bone and muscle strength in overweight and obese populations. Bone and muscle are organs that are fundamental to the maintenance of health. They have a dual relationship whereby factors that affect one organ will also affect the other. This parallel regulation of bone and muscle was reflected in the findings from this thesis, where dietary weight loss interventions resulted in decreases in BMD, as well as reductions in muscle strength. Therefore weight loss can induce adverse effects, and this could conceivably increase the risk of problems such as osteoporosis and dynaopenia, but future research is needed to determine relative benefits, as well as the possible risks, of treating overweight and obesity with diet-induced weight loss. Some of the mechanisms that may contribute to this dual detrimental effect of diet-induced weight loss on bone and muscle strength have been proposed in Chapter 4, however future research is necessary to determine the factors contributing to this potential adverse effect, as well as interventions to curb the possible negative consequences.

## **Acknowledgements**

The studies reported in this thesis were conducted as part of the Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders, of the University of Sydney.

Firstly, I would like to extend an enormous thank you to my principle supervisor Associate professor Amanda Salis. Without your continual belief in me, your support, wisdom, encouragement and understanding, I would not have been able to achieve all that I have during my Masters. Thank you for the time you have spent both on this thesis, and on me throughout my Masters. I am so privileged and honored to have been given the opportunity to work alongside and be mentored by such an influential woman. Thank you for pushing me into creating something I can be proud of.

To Radhika Seimon, thank you for being my shoulder to lean on, for all your amazing help and support as well as friendship. Thank you for being someone I look up to and strive to achieve all the wonderful things you have accomplished. Thank you also for taking the time to edit my thesis with me. A warm thank you to Crystal Lee, thank you for your guidance through the statistical world, for your advice and assistance in finalizing and editing this thesis. You made conducting meta-analyses much more pleasant. A special thank you to Michelle Hsu, you provided with me with much needed relief and aid with the papers I have submitted, and for your friendship.

Thank you DREAM Team, Alice Gibson, Arpita Das, Felipe Luz – thank you for working with me as part of our team. Thank you for your advice, feedback and support with conference presentations and for inspiring me. To the PREVIEW team; Roslyn Muirhead, Jessica Honeywood, Shannon Overs, Michelle Whittle, thank you

for allowing me to work alongside you to obtain data, for your patience with me and understanding. I feel honored to have been apart of such a large clinical trial and to have work with such hardworking individuals.

To all other staff and students at the Boden institute, thank you for providing me with support, sharing your office space and help throughout my candidature. To Sue Shapses, Tuan Nguyen, thank you for help with the meta-analysis of the effect of weight loss on bone.

Thank you to all my friends, new and old for your encouragement, belief in me and love. To Luke Petridis, thank you for believing in me and for helping keep me on track. Thank you to my two beautiful best friends Allison Grech and Rochelle Wallace, I am so grateful to have you in my life. Thank you for being on this journey with me, for reading over drafts, for listening to my complaints and for being there for me when no one else was.

Lastly thank you to my family, my father, mother and siblings, Simon and Sabrina. Thank you for your unconditional love, for your support, motivation and for everything that you have done to get me to where I am today.

Thank you.

## **Publication and manuscript arising from this thesis**

The data presented in this thesis have formed the basis for the publication and manuscript listed below:

**Zibellini J**, Seimon RV, Lee CMY, Gibson AA, Hsu MSH, Shapses SA, Nguyen TV, Sainsbury A; 2015 ‘Does diet-induced weight loss lead to bone loss in overweight or obese adults? A systematic review and meta-analysis of clinical trials’ *JBMR*, vol. 30, no. 12, pp. 2168-2178

**Zibellini J**, Seimon RV, Lee CMY, Gibson AA, Hsu MSH, Sainsbury A; 2016 ‘Effect of diet-induced weight loss on muscle strength in overweight or obese adults – a systematic review and meta-analysis of clinical trials’ – revised manuscript re-submitted to *Obesity Reviews* on 26<sup>th</sup> February 2016

## **Statement of Authentication**

### **Chapter 2**

Does diet-induced weight loss lead to bone loss in overweight or obese adults? A systematic review and meta-analysis of clinical trials

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**Jessica Zibellini**, Radhika V Seimon, Crystal MY Lee, Alice A Gibson, Michelle SH Hsu, Sue A Shapses, Tuan V Nguyen, Amanda Sainsbury

### **Journal**

Journal of Bone and Mineral Research, 2015, 30 (12), 2168-2178

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JZ conceived the idea and the study design, developed the search strategy, screened all abstracts and titles, extracted all data from each of the papers, analysed the data using STATA, generated all figures, wrote the manuscript and completed all the re-drafting.

RSV reviewed all accepted studies and was involved in the editing of the manuscript.

CMYL was involved in the statistical analysis of data and editing of the manuscript.

AAG was involved in the structure and editing of the manuscript.

MSHH was involved in screening all articles and extracting data and editing of the manuscript.

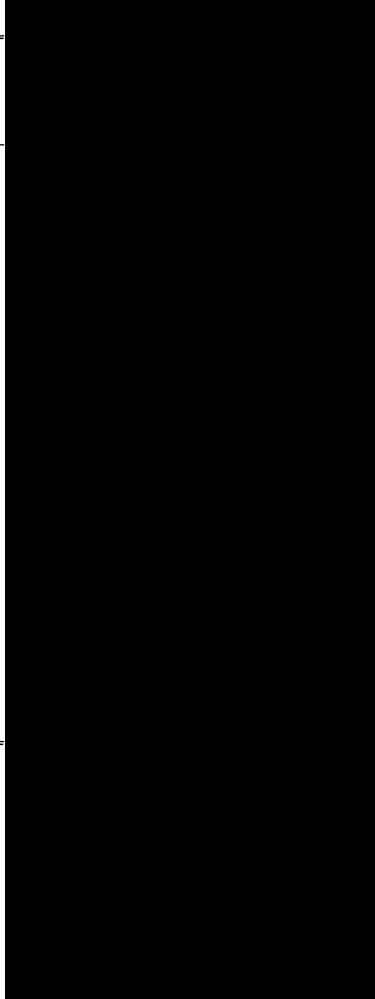
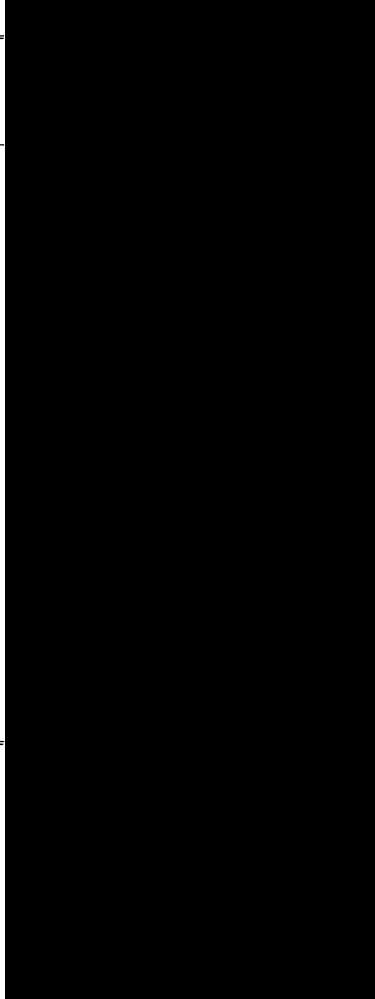
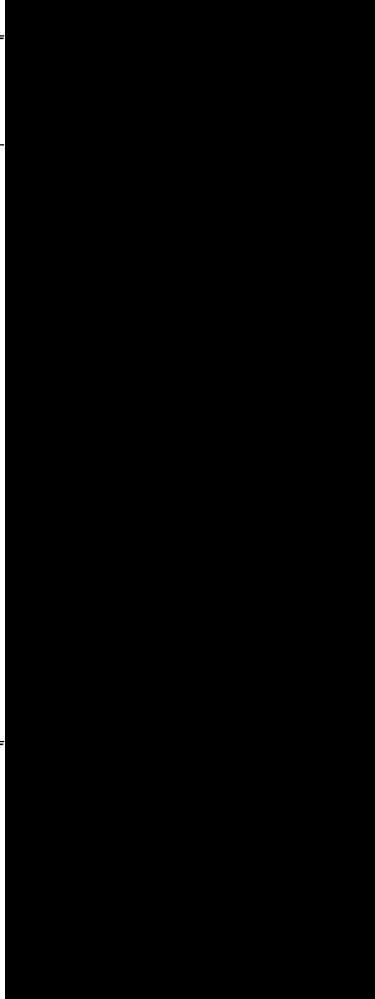
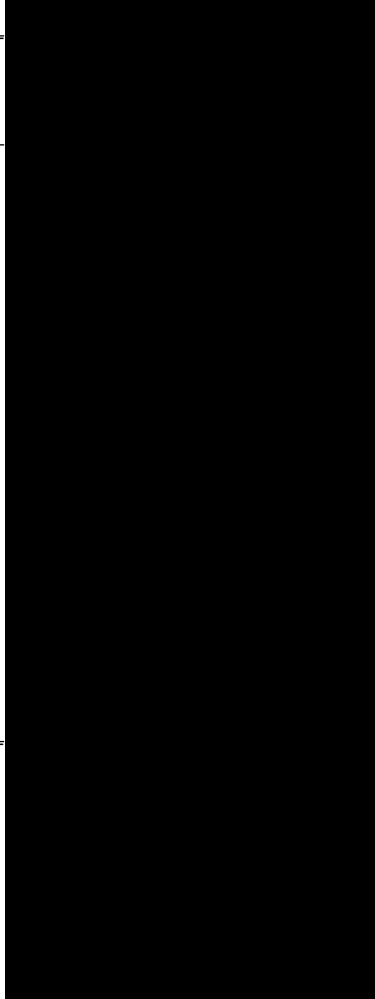
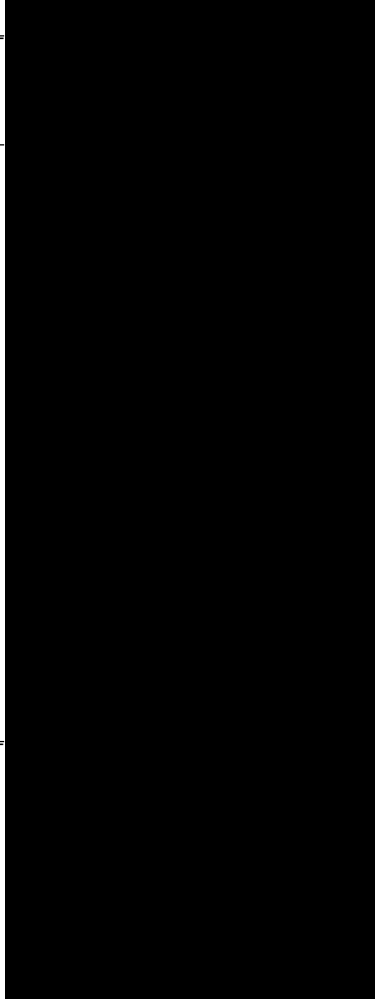
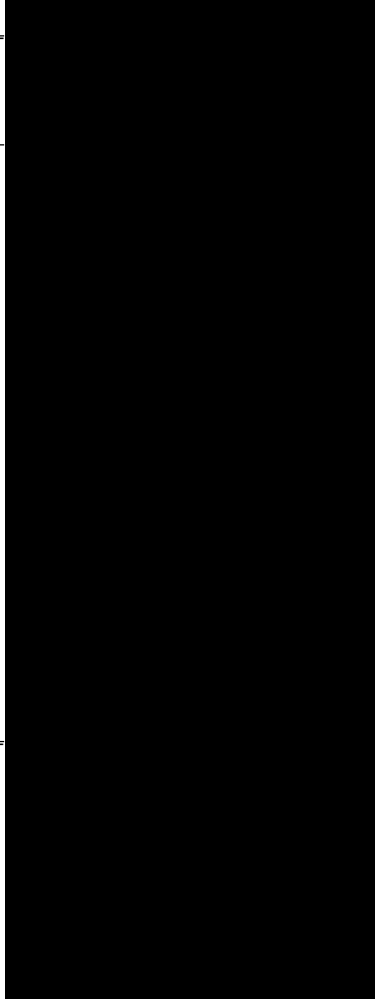
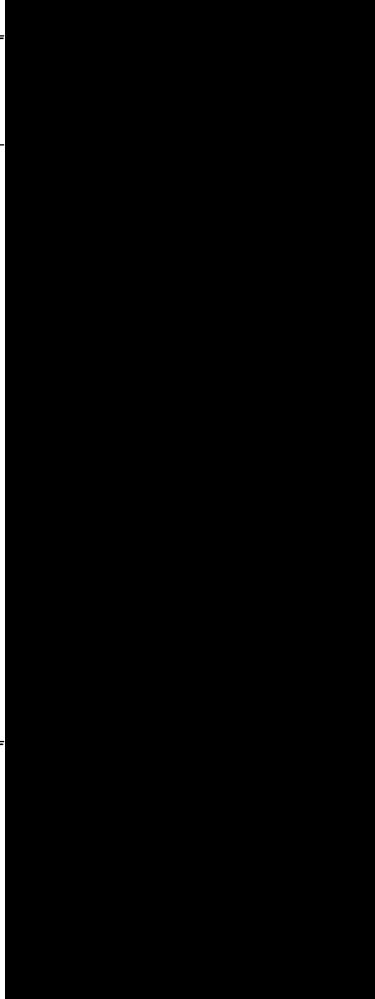
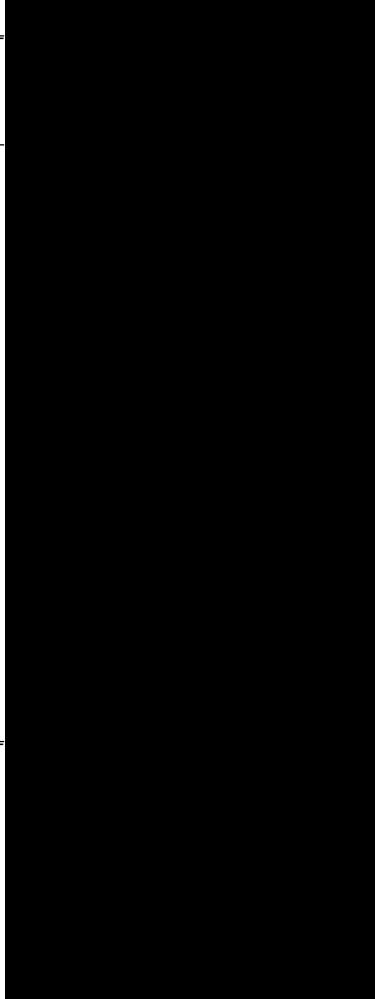
SAS provided various data from selected papers for the data analysis, provided expert input for the manuscript.

TVN provided expert input for the manuscript.

AS supervised the research, assisted with the inclusion/exclusion criteria development, critically reviewed all drafts of the manuscript.

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### **Chapter 3**

Effect of diet-induced weight loss on muscle strength in overweight or obese adults – a systematic review and meta-analysis of clinical trials

#### **Authors**

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#### **Journal**

Revised manuscript re-submitted to Obesity Reviews on 26<sup>th</sup> February 2016

#### **Author contribution**

JZ conceived the idea and the study design, developed the search strategy, screened all abstracts and titles, extracted all data from each of the papers, analysed the data using STATA, generated all figures, wrote the manuscript and completed all the re-drafting.

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## **Chapter 1: General Introduction**

This chapter provides background on the health issue that is obesity, and how weight loss may impact aspects related to body composition, in particular, bone and muscle strength. Obesity has become an increasing dilemma in our society, not only having significant health impacts on an individual scale, but also placing a large financial burden at a population level (1, 2). Diet-induced weight loss is the first line of treatment to reduce the prevalence of obesity (3, 4), with it having positive influences in reducing the risk of mortality and co-morbidities (5). However, during energy deficit, the body undergoes physiological changes that have been eluded to have negative rather than positive effects on bone and muscle (6). The aim of this thesis was to investigate whether diet-induced weight loss results in deleterious effects on bone and muscle in overweight and obese populations.

### **1.1 Definition of obesity**

Obesity is a state of excess adiposity or body fat. The cause of obesity is not singular but rather multifactorial, with influences such as physical inactivity, disordered eating, mental illness and genetics playing a role in this accumulation of excess body fat (5). Overweight and obesity are commonly classified by the body mass index (BMI), which takes into account the weight and height of an individual. BMI is calculated by dividing weight in kilograms (kg) by the height in meters (m) squared ( $\text{kg}/\text{m}^2$ ). A BMI of less than  $18.5 \text{ kg}/\text{m}^2$  is considered by the World Health Organization to be underweight,  $18.5$  to  $24.9 \text{ kg}/\text{m}^2$  is considered to be in the healthy and normal weight range,  $25.0$  to  $29.9 \text{ kg}/\text{m}^2$  is considered to be overweight, and a BMI greater or equal to  $30.0 \text{ kg}/\text{m}^2$  is considered clinically obese (7).

## **1.2 Prevalence and significance of obesity**

The prevalence of obesity worldwide is increasing yearly. A recent global analysis indicated that between 1980 and 2013, there was an increase in the number of overweight and obese individuals, from 857 million to 2.1 billion (8). The worldwide prevalence of overweight or obese males increased from 28.8% to 36.9% between 1980 and 2013, whilst that of females rose from 29.8% to 38.2%. In Australia, results from the Australian Bureau of Statistics' National Health Survey found that in 2011-12, 62.8% of adults (aged 18 years and older) were classified as overweight or obese. Of these overweight or obese adults, 35.3% were categorized as being overweight. Furthermore the proportion of obese individuals grew from 18.7% to 27.5% between 1995 and 2012 (9). Twenty-five percent of children in Australia in 2011-12 were overweight or obese (9). And so, overweight and obesity was the 2<sup>nd</sup> highest contributor to the burden of disease in Australia after dietary risks (9).

Obesity has been identified as a risk factor for a number of co-morbidities including type-2 diabetes, cardiovascular diseases, musculoskeletal diseases, gallbladder diseases as well as cancers varying from kidney, colorectal and pancreatic to prostate, breast and ovarian, as highlighted in a recent meta-analysis (10). Moreover, obesity is associated with around 3.4 million deaths globally each year (8). These risk factors of obesity translate to health, economic and psychological consequences and in 2007-08, the total direct cost of overweight and obesity in Australia was \$18.3 billion (2).

## **1.3 Weight loss strategies**

Due to the multifaceted nature of obesity, various strategies to induce weight reduction have been developed. For example, dietary and/or physical activity

interventions, pharmacotherapy, and in cases of morbid obesity (for those with a BMI of 40 kg/m<sup>2</sup> or greater) or severe obesity (35 kg/m<sup>2</sup> or greater) with at least one obesity related comorbidity (11), bariatric surgery is often suggested.

Dietary interventions are the cornerstone method of weight loss in overweight and obese individuals. Dietary interventions range from those severely restricting energy intake (very low energy diets; VLED) that induce large reductions in body weight, to moderately energy-restricted (MER) diets that often range in macronutrient composition and produce modest weight losses (12, 13). A VLED was defined as a diet providing less than 3.4 MJ per day (12, 14), and a MER was defined as a diet providing greater or equal to 5 MJ per day (13). In 2011-12, 13% of (or 2.3 million) Australians aged 15 years or older reported being on a diet for weight reduction or other health purposes (15). Furthermore, 17% (or 1.6 million) of the 9.2 million overweight or obese Australians were on a diet to induce weight loss in 2011-12 (15).

Several pharmacological treatments have been developed and made available over time, however most do not have long-term efficacy and are often associated with adverse side effects and have thus been taken off the consumer market or not been approved by regulatory bodies. Currently pharmacotherapy options for overweight and obese individuals in Australia are limited, with only three approved drug treatments available for use to reduce body weight; orlistat, phentermine and – since December 2015 – liraglutide. Orlistat is an anti-obesity drug that acts by reducing dietary fat absorption by approximately 30% through gastric and pancreatic lipase inhibition, resulting in an approximate weight reduction of 2.9% after a one-year trial (16). However, side effects include fatty stools and diarrhoea which cause discomfort

in patients (16). The second drug available for purchase with a prescription in Australia is phentermine, which acts to suppress appetite, yet like orlistat also has short term side effects, in this case dry mouth, insomnia and cardiovascular complications, for example (17). Lastly, liraglutide, a newly developed weight loss drug marketed as Saxenda, acts as an analogue of glucagon like peptide 1, which causes a reduction in hunger levels and thus indirectly decreases energy intake, leading to the desired outcome of weight loss (18). This dose of liraglutide however is not to be confused with another dose formulation of liraglutide, which is used for the management of type-2 diabetes and is marketed as Victoza. The long-term effects of liraglutide as used for weight loss are limited, with only one-year clinical trials having thus far been conducted (19, 20). However, short term side effects of liraglutide for weight loss include an increase in heart rate and dehydration (18). Importantly, it should be noted that with all pharmacological interventions for obesity, concomitant lifestyle modification is recommended.

In those individuals with a BMI  $\geq 40$  kg/m<sup>2</sup>, or those with a BMI  $\geq 35$  kg/m<sup>2</sup> and with at least one obesity-related comorbidity, bariatric surgery is the most effective treatment option. Bariatric surgery induces reductions in body weight that are maintained in the long term for the majority of patients, with weight losses of around 15 – 40% being achieved and maintained for a minimum of 10 years post surgery, depending on the particular surgical procedure (21). The three most common surgical interventions are Roux-en Y gastric bypass, sleeve gastrectomy and laparoscopic adjustable gastric banding. These interventions, although differing in the mechanism via which energy intake is reduced, have been proven to be successful and as a

consequence, a greater number of obese individuals are seeking surgical intervention in conjunction with lifestyle modifications to reduce body weight (22, 23).

The above-mentioned methods of weight reduction vary in both procedure and efficiency. However, with any weight loss intervention, dietary modification is at its core, with various positive outcomes of diet-induced weight loss having been well established (24). These include reductions in cardiovascular disease risk (25), hypertension (26), lipid profile (27) and inflammation (28). Until recently, any hazardous side effects of diet-induced weight loss have been largely ignored, with the aforementioned benefits of weight loss outweighing the risks. The potential adverse effects of energy-restriction are related to changes in hormones, such as those regulated by the hypothalamic-pituitary axes. Studies have shown that in both lean animals and in overweight or obese humans an inhibition of the gonadotropic (29, 30) and somatotrophic axes occurs (31, 32). Inhibition of these systems results in downstream consequences. For example, reductions in circulating concentrations of sex steroids and insulin like growth factor 1 (IGF-1), may decrease bone mineral density (6, 33, 34) and muscle strength (6, 35). Therefore, there is a need for any potential negative repercussions of diet-induced weight loss to be explored in order to then identify measures to counteract or minimize these changes. The aims of this thesis were to examine the effect of weight loss through dietary intervention on bone and muscle strength in overweight and obese individuals.

#### **1.4 Bone**

The skeletal system plays a pivotal role in the body providing structural, functional and biochemical support to other organs and cells of the body. Furthermore, from a

clinical perspective, bone, more specifically bone mineral density (BMD), is extremely important due to its association with osteoporosis, incidence of falls and fractures that in turn result in an increased risk of mortality (36-38). Therefore, it is crucial that bone integrity is maintained. In the following section, bone composition and methods of assessing bone integrity are explored.

### **1.4.1 Bone Composition**

Bone is a specialized connective tissue composed of both organic matter (type I collagen, growth factors, blood proteins, osteonectin, osteocalcin) as well as inorganic matter (mineral hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) (39, 40). Histologically, there are two principle forms of mature bone; cortical and trabecular bone. Cortical bone makes up around 80% of the skeleton, covering the outer surface of bones (41). Cortical bone has a high density due to the organization of bone tissue in concentric sheets or lamellae with a central canal containing neurovascular bundles (41, 42). As a result of its highly and tightly organised structure, cortical bone provides resistance to uniaxial stresses (41, 43). Contrastingly, trabecular bone is found in areas exposed to high stress, such as the vertebrae and hip, and is essential for the strength and integrity of bone because of its ability to absorb large mechanical loads (41). The mechanical property of trabecular bone is a consequence of the lamellae having a less compact structure than cortical bone, forming an interconnecting meshwork of trabeculae that form spaces filled with bone marrow, ultimately producing a large surface area of bone (35, 41, 42).

Bone undergoes constant remodeling, which is a tightly coupled balance of bone formation and resorption, through the degradation of bone via osteoclastic cells, and

the laying down of bone by osteoblastic cells (41). Bone remodeling helps to uphold the integrity of bone by repairing ischemic or micro-fractures. It is also important for maintaining calcium homeostasis, and for adjusting and strengthening bone architecture in response to various mechanical loads and strains inflicted on it (44, 45).

### **1.5 Methods of assessing bone integrity**

Bone integrity can be defined as the size of bone, shape, micro-architecture, density and quality of bone (46). Two main techniques are used in the assessment of bone integrity. Clinically, dual energy x-ray absorptiometry (DXA) is the gold-standard technique for the determination of BMD. In contrast, bone turnover – the osteoblastic-osteoclastic homeostatic cycle, can be examined through the concentration of bone turnover markers measured in either serum or urine. There are distinct markers for bone formation or bone resorption and as such, they aid in the determination of the rate at which bone is remodeled, as well as whether formation or resorption is favoured (42). The methods used for the determination of BMD as well as changes in bone turnover, will be described in detail in the next sections.

#### **1.5.1 Assessing bone integrity with DXA**

DXA is a method used for the quantification of BMD, which can provide an overall picture of the integrity of bone. DXA is based on a beam of radiation that is targeted at a radiation detector below a table upon which a patient lies. The beam of radiation then moves relative to the table so that the body is scanned. The weakened radiation ray is then correlated to BMD (47, 48).

DXA measurements of BMD are important in clinical practice, having both a diagnostic and prognostic role (49) and is the primary technology used for the determination of osteoporosis and calculation of risk of falls and fractures (50, 51). However, it should be noted that artifacts might occur, particularly in individuals who are overweight or obese and in those who are undergoing weight changes. Indeed, the accuracy and precision of DXA is known to decrease with increasing BMI (52). This is a consequence of various factors including difficulties in accurately positioning obese patients onto the scanning bed, as well as excess adipose tissue causing measurement errors. One study illustrated the influence of excess fat by layering plastic bags filled with fat around phantom spine models and healthy, non-obese humans (52). When fat was positioned around the phantom spine model, an increase in BMD was detected by DXA, in contrast to the decrease in spine BMD that was registered by DXA when fat was layered around the torso of 13 healthy human volunteers ( $\leq 30 \text{ kg/m}^2$ ) (52). Moreover, DXA results are compromised when participants undergo changes in body weight, as this affects the precision of DXA (53-55). This is related to a technical limitation of DXA, in that its dual energy x-ray sources can only measure two tissue types at any one time (e.g. bone and soft tissue). Thus, being a two-compartment model for the determination of body composition, DXA calculations assume certain ratios of fat to lean tissues in order to calculate the density of bone tissue as well as fat mass and fat free mass (42). However, these assumed ratios may be violated in obesity and with weight changes (e.g. after weight loss) (56). Lastly, BMD as determined by DXA is only able to detect changes in bone turnover 6-12 months after the initiation of an intervention (42). In light of these limitations in the use of DXA to assess bone, particularly during weight loss

interventions in overweight or obese individuals, other forms of bone assessment, such as measurement of bone turnover markers, should be employed.

### **1.5.2 Bone turnover markers**

The importance of bone turnover markers in assessing changes occurring in bone has become increasingly important due to the aforementioned technical limitations of DXA, particularly in overweight or obese people undergoing changes in body mass. In contrast to DXA, bone turnover markers are able to reveal changes in bone physiology 2-3 months after commencement of various treatments, such as diet induced weight loss or medications (57). Moreover, bone turnover markers may predict fracture risk (58) and are able to elucidate whether increases or decreases occur in markers of bone formation and markers of bone resorption. Markers of bone formation include N-terminal propeptide of type I collagen (P1NP) and osteocalcin. On the other hand, markers of bone resorption are C- terminal telopeptide of type I collagen (CTX) and N-terminal telopeptide of type I collagen (NTX). These bone turnover markers will be discussed in this thesis, and were chosen due to international recommendations and associations with detecting changes in bone homeostasis (42).

As mentioned above, bone formation involves the deposition of new bone matrix by osteoblast cells. P1NP is a bone formation marker that is a cleavage product of type I procollagen found in bone matrix (42). Type I procollagen is enzymatically broken down by N-procollagenase, which is secreted by osteoblasts,, and P1NP can be found circulating in blood as a by-product during bone formation (42). The International

Osteoporosis Federation and the International Federation of Clinical Chemistry and Laboratory Medicine recently recommended that serum concentrations of P1NP be measured as a marker of bone formation for clinical practice and research (58-61). Another marker of bone formation is osteocalcin, a 49 amino acid peptide produced directly from osteoblasts during bone formation, which thus has a high specificity for indicating osteoblastic activity (62). Recent evidence from a study of older men highlighted that osteocalcin has a high predictive ability in the determination of fracture risk and incidence of hip fracture compared to other markers such as P1NP (63). Hence, both these markers of bone formation; P1NP and osteocalcin, should be measured to determine bone turnover (63), and were included in this thesis.

Osteoclasts are lytic cells that degrade the bone matrix through secretion of enzymes and proteases. During bone resorption, osteoclasts hydrolyze type I collagen (58). CTX and NTX are two breakdown products of type I collagen from the bone matrix, which can be detected in both serum and urine. These markers of bone resorption are known to be specific and sensitive in detecting bone resorption (62), however their concentrations are known to vary with circadian rhythms (64). The International Osteoporosis Federation and the International Federation of Clinical Chemistry and Laboratory Medicine recommend that serum CTX be measured as a marker of bone resorption (58), because over a time period CTX levels remain more stable in circulation in comparison to other markers of bone resorption (65, 66). In addition to CTX, serum or urinary concentrations of NTX has been measured in a large number of studies and has been shown to be an effective marker in assessing the efficacy of osteoporosis treatment (67). Therefore, it is important that both markers of bone

resorption, CTX and NTX, are analysed in assessing bone, and were thus both included in this thesis.

## **1.6 Muscle**

Muscle and bone are together commonly referred to as the musculoskeletal system due to their inherent interrelated properties and their shared mesenchymal tissue origin as well as parallel changes that occur in both over time with exercise, disuse or age (68). Therefore, if an analysis of bone integrity is undertaken, muscle too should also be examined. Muscle function plays a fundamental role in determining the level of exercise performance, the ease of activities of daily living, and frailty (69). However, diet-induced weight loss may, through changes in hormonal levels and physiological adaptations, cause negative changes in muscle mass and function, including strength (6). Muscle function can also be influenced by the degree of stretch of the muscle, contraction velocity, the specific fibre types within the muscle as well as the level of muscle fiber recruitment (70). The following section describes muscle strength and the forms of assessment used to determine muscle strength.

### **1.6.1 Muscle strength**

Muscle strength can be predicted by fat free mass (71, 72), often referred to as lean body mass, and fat free mass is associated with muscle mass (73). Muscle strength is defined as the ability of a muscle to generate maximal force (74, 75). Muscle strength has been correlated to several health outcomes and is a highly regarded clinical

indicator of an individual's functional capacity, as well as providing an insight into the mass and quality of muscle (76). Age impacts muscle strength, with reductions of 8 – 10% occurring every decade after the age of 40 years (74), and this is related to the disease state known as dynaopenia (71). Another noteworthy muscular disease state related to advancing age is sacropenia, an age related decline in muscle mass (71, 77). Dynaopenia and sacropenia both relate to poor prognostic outcomes (78, 79) and thus, methods of maintaining and strengthening muscle is an important avenue for the maintenance of optimum health.

### **1.6.2 Muscle strength assessments**

Muscle strength is typically assessed through muscular contraction, with the form of contraction varying depending on the technique. There are several methods in use for measuring muscle strength, depending on which groups of muscles are to be examined. The four commonly applied methods of muscle strength testing that will be discussed in this thesis are one repetition maximum (1RM) strength testing, isokinetic dynamometry, isometric dynamometry and handgrip strength testing (a form of isometric dynamometry).

The multiple methods of assessing muscle strength vary in their ability to detect changes in strength, even when the same muscles are investigated. This was shown in a study that investigated three different modes of muscle strength assessment (1RM, isokinetic dynamometry and isometric dynamometry) in the forearm / elbow extensors in men undergoing a 12-week resistance-training program (80). This study found that the degree of increase in muscle strength varied according to the method used to assess it. Strength was found to increase by the greatest extent when the 1RM

procedure was undertaken, followed by isokinetic dynamometry and lastly, with the least force difference from baseline detected, isometric dynamometry (80). This finding highlights the need to cross-examine different muscle strength assessments and to develop a uniform mode of assessment in order to compare intervention validly and reliably.

Handgrip strength has been found to be a marker of nutritional status (81, 82), as well as being a predictor of health outcomes (82, 83). For example, reviews highlight that low handgrip strength is associated with a greater number of post-surgical complications, including loss of physical functionality and longer hospitalization (82, 84). Such correlations between handgrip strength and health give clinical importance to this particular strength test. Moreover, due to the flexibility and ease of obtaining handgrip strength measurements, handgrip strength findings were included in this thesis.

### **1.7 Aims of the study**

Given the importance of bone and muscle strength to long-term health, the aims of this thesis were to examine the effect of diet-induced weight loss on bone and muscle strength in overweight and obese populations. More specifically, the first study (Chapter 2) aimed to quantify the changes in BMD and bone turnover markers in order to establish the effect of diet-induced weight loss on bone. This was achieved through the collation of literature in the form of a systematic review and quantified through meta-analysis. The purpose of the second study in this thesis (Chapter 3) was to reveal the impact of diet-induced weight loss on muscle strength, as well as to clarify and identify suitable methods of assessing muscle strength. To achieve this,

literature was searched systematically and data extracted and meta-analyzed so that the effects of diet-induced weight loss on muscle strength could be highlighted and quantified. Meta-analysis studies are placed at the top of the hierarchy of evidence, and, systematic reviews and randomized control studies are placed underneath. Meta-analyses provide quantitative data from all studies investigating the same effect, by collating all data sets resulting in a single outcome value (85).

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## **Chapter 2: Does diet-induced weight loss lead to bone loss in overweight or obese adults? A systematic review and meta-analysis of clinical trials**

*Published in 2015 Journal of Bone and Mineral Research, vol. 30, no. 12, pp. 2168-2178*

### **2.1 Abstract**

Diet-induced weight loss has been suggested to be harmful to bone health. We conducted a systematic review and meta-analysis (using a random-effects model) to quantify the effect of diet-induced weight loss on bone. We included 41 publications involving overweight or obese but otherwise healthy adults who followed a dietary weight loss intervention. The primary outcomes examined were changes from baseline in total hip, lumbar spine and total body BMD, as assessed by dual energy X-ray absorptiometry. Secondary outcomes were markers of bone turnover. Diet-induced weight loss was associated with significant decreases of 0.010-0.015 g/cm<sup>2</sup> in total hip BMD for interventions of 6, 12 or 24 (but not 3) months' duration ([95% confidence intervals] [-0.014 to -0.005], [-0.021 to -0.008], and [-0.024 to -0.000] g/cm<sup>2</sup> at 6, 12 and 24 months, respectively). There was however no statistically significant effect of diet-induced weight loss on lumbar spine or whole body BMD for interventions of 3 to 24 months' duration, except for a significant decrease in total body BMD (-0.011 [-0.018 to -0.003] g/cm<sup>2</sup>) after 6 months. While no statistically significant changes occurred in serum concentrations of N-terminal propeptide of type I procollagen (P1NP), interventions of 2 or 3 months in duration (but not of 6, 12 or 24 months' durations) induced significant increases in serum concentrations of osteocalcin (0.26 [0.13-0.39] nmol/L), C-terminal telopeptide of type I collagen (CTX, 4.72 [2.12-7.30] nmol/L) or N-terminal telopeptide of type I collagen (NTX, 3.70 [0.90-6.50] nmol/L bone collagen equivalents [BCE]), indicating an early effect of diet-induced weight loss to promote bone breakdown. These data show

that in overweight and obese individuals, a single diet-induced weight loss intervention induces a small decrease in total hip BMD, but not lumbar spine BMD. This decrease is small in comparison to known metabolic benefits of losing excess weight.

## **2.2 Introduction**

Osteoporosis and its consequence of fragility fracture represent a significant burden on public health. Up to 38% of women and 8% of men aged 50 years and above are affected by osteoporosis (1). From the age of 50, the residual lifetime risk of fracture is 47% for women and 22% for men (2). In women, the lifetime risk of hip fracture is equivalent to or higher than the risk of invasive breast cancer (3). More importantly, individuals with a hip fracture are at risk of further fracture and mortality (4). Bone mineral density (BMD) is the most important predictor of fracture risk and mortality following a fracture. Each standard deviation decrease in BMD is associated with an approximately 3-fold increase in fracture risk (5). Therefore, the diagnosis of osteoporosis is based on measurement of BMD.

In recent years, evidence has emerged that osteoporosis and obesity are linked. Obesity is a major public health risk and is now a worldwide epidemic, with a recent global analysis highlighting a 27% increase in overweight and obesity for adults between 1980 and 2013 (6). Despite its association with various metabolic dysfunctions, obesity has been thought to provide some protection against osteoporotic fractures, with high body mass indices (BMIs) said to be correlated with increased BMD (7). However, this view that obesity has bone-strengthening effects is now being questioned, with studies showing that the positive linear relationship between BMI and BMD is weaker at high BMIs (8), and other studies suggesting that severe obesity ( $\text{BMI} \geq 35 \text{ kg/m}^2$ ) may be a risk factor for certain types of fractures (9-11). For instance, a study involving over 60,000 women from 10 countries revealed an association between  $\text{BMI} \geq 30 \text{ kg/m}^2$  and increased risk of ankle and upper leg fractures (with reduced risk of wrist fractures) (10), and a similar correlation

was also found in men, albeit only after correction for the increased BMD generally associated with obesity (11) and not without controversy (12).

Whether or not obesity *per se* has an effect on fracture risk, emerging evidence suggests that obesity treatment, namely bariatric surgery – which induces weight losses of up to 75% of excess body weight which are maintained for up to 10-14 years post surgery (13) – results in bone loss (14, 15). However diet-induced weight loss, not bariatric surgery, with or without concomitant physical activity, is seen as the first treatment option for overweight and obesity (16). Given that weight loss via lifestyle modification is also known to induce hormonal changes which would be expected to reduce both lean body mass (17) and bone mass (18, 19), an increasing number of studies have investigated changes in bone mass in response to diet-induced weight loss in overweight and obese individuals.

Of the studies that have investigated changes in bone mass in response to diet-induced weight loss in overweight or obese adults, the results have been highly variable, with increases (20-24), decreases (25-40) and no change (22, 24, 26, 30, 33, 41-49) in bone mass being reported. Thus, attempting to draw sound conclusions from these mixed results is extremely difficult. However, definitive knowledge about any effects of diet-induced weight loss on bone is becoming increasingly important, given the large numbers of people in our increasingly overweight and obese world population who are dieting for weight reduction. For instance, in 2011-2012, 17% (or 1.6 million) of the 9.2 million people in Australia who were overweight or obese reported being on a weight reducing diet (50). Although there have been a few previous reviews examining the effect of diet-induced weight loss on bone (51-53), a formal quantitative assessment of its effect on bone health has not been performed. In the presence of conflicting findings from studies with limited sample sizes, a meta-analysis can be helpful in resolving the effect size. We thus conducted a systematic review and meta-analysis to quantitatively determine the effects of diet-induced weight

loss interventions of any duration on bone mass as well as on circulating or urinary concentrations of biomarkers ('markers') of bone formation or bone resorption (turnover) in overweight or obese men and women who were otherwise healthy. We included bone turnover markers in order to provide more sensitive assessment of the effects of diet-induced weight loss on bone in overweight and obesity, because measurement of bone turnover markers can reveal treatment effects after only 2-3 months compared to a minimum of 6 months required before significant changes in BMD can be detected (54, 55).

## **2.3 Materials and Methods**

### **2.3.1 Inclusion and exclusion criteria for selecting studies for this review**

Study designs included in this analysis were randomized controlled trials, pilot studies and cohort studies. Only original research studies were included; review articles, as well as abstracts and conference papers, were excluded. Included studies involved participants aged 18 years or above who were overweight or obese (BMI greater than or equal to 25 kg/m<sup>2</sup>) but otherwise healthy. Therefore, studies that specifically recruited participants with diseases such as type 2 diabetes, osteoporosis or cardiovascular disease were excluded. Included studies were required to implement a dietary weight loss intervention involving the restriction of energy intake relative to participants' measured or estimated energy requirements and resulting in a reduction in average body mass of the group overall. No limit was placed on duration of the diet-induced weight loss period. Studies were excluded if participants had undergone bariatric surgery or were taking medications designed to induce weight loss. Studies were also excluded if the intervention involved calcium supplementation or supervised exercise, or if exercise was the primary means of eliciting weight loss, due to potential confounding effects of calcium supplementation (35, 56) or physical activity on bone mass (57). Because physical activity is frequently recommended for weight management, to reduce publication bias we included interventions in which exercise was recommended as part of a healthier lifestyle, provided that the exercise was not supervised or was not the primary focus of

the intervention. Any eligible non-surgical, non-medication, non-supplementation or non-exercise arms of any of the above such studies were included in this review.

Studies were included where one or more of the following outcomes were assessed: BMD of the total hip, lumbar spine (L1-L4 or L2-L4) or total body, as determined by dual energy X-ray absorptiometry (DXA), or serum concentrations of N-terminal propeptide of type I procollagen (P1NP) or osteocalcin (both bone formation markers), serum concentrations of C-terminal telopeptide of type I collagen (CTX), or serum or urinary concentrations of N-terminal telopeptide of type I collagen (NTX) (bone resorption markers). We chose to investigate BMD of the hip and spine because these parameters are clinically relevant and are included in fracture risk algorithms, notably the World Health Organization's Fracture Risk Assessment tool (FRAX®) and the Garvan Fracture Risk Calculator (58). Indeed, the hip is the gold standard site both for the diagnosis of osteoporosis and the assessment of fracture risk (59, 60). The spine, like the hip, is clinically significant due to its correlation with fracture risk (60-62). Both of these sites, due to their high trabecular bone content and thus high bone surface area, are highly susceptible to factors that influence bone metabolism (63). Although total body BMD is not used clinically for the diagnosis of osteoporosis or the estimation of fracture risk (63), many studies have reported total body BMD, making it important to consider this parameter so as not to introduce bias in study selection. We chose to search for the above-mentioned bone turnover markers because while there is currently no standardised set of bone markers for use in clinical practice and research, there have been several recent international recommendations to measure serum P1NP and CTX as the standardised reference markers of bone formation and resorption, respectively (64-66). Although it has not been recommended to determine serum osteocalcin and serum or urinary NTX concentrations as markers of bone formation and resorption, respectively, they are also still commonly measured in conjunction with one or more of the recently recommended markers. Osteocalcin is of particular interest given a recent study demonstrating a significant association between serum osteocalcin –

but not P1NP or CTX – concentrations with incident fracture risk in older men, even after adjusting for other risk factors (67). To be included in this review, studies had to provide a within-subject comparison between baseline (i.e. before commencement of the dietary weight loss regime) and a time point immediately upon completion of the dietary regime.

### **2.3.2 Search strategy**

MEDLINE, PreMEDLINE, Embase, Cinahl, and Sport Discuss were searched from the inception date of each database to March 2014. Both medical subject headings (MeSH) and free text search terms were employed. Limitations were set so that only studies published in English and involving human participants were found. Reference lists of relevant articles as well as review articles were searched to help ensure that all relevant studies were found. The Supplementary Methods shows an example of the specific key words (or MeSH terms) that were used for the search of MEDLINE for population, intervention and outcomes.

### **2.3.3 Data collection, extraction and analysis**

Two independent authors (JZ and MSHS) screened the titles and abstracts of studies identified in the above search strategy. The full texts of potentially relevant studies were retrieved, and the inclusion and exclusion criteria were applied. If discrepancies arose as to which studies to include, consensus was reached by consultation with a third author (RVS). The following data was extracted from each study, as summarized in **Supplementary Table 1**: the number of participants in the study (sample size), the sex, menopausal status, age and baseline BMI of participants (calculated from height and weight where BMI was not reported), duration and details of the dietary weight loss intervention, time points at which outcomes were collected and used in this review, baseline weight, weight change from baseline at the end of the intervention, and results from one or more of the following parameters: BMD of the total hip, lumbar spine (L1-L4 or L2-L4) or of the total body, or circulating or urinary P1NP, osteocalcin, CTX and NTX concentrations.

Some studies included more than one intervention that matched our inclusion criteria (20, 22, 26, 28, 29, 32, 37, 42, 43, 68-70). In these instances, data from the different dietary weight loss interventions were not pooled but were instead treated as independent interventions. For studies that reported both sexes independently (20-22, 24, 29, 42, 44, 71-75), data from both sexes were pooled before inclusion in our analysis. This is because the majority of studies did not report males or females separately, or did not include both sexes within their population.

Heights and weights were converted to m and kg, respectively, when data were not already reported or provided in these formats. The durations of dietary weight loss interventions were expressed in months, with 1 month corresponding to 4 weeks for intervention durations that were reported in weeks. Dietary interventions that were not 2, 3, 6, 12 or 24 months in duration were rounded to the nearest of these durations and included in that group for analysis, as shown in **Supplementary Table 1**. Some interventions measured additional time points between baseline and the end of the dietary intervention. These shorter time points were also included as separate interventions, because they reflect the effect of shorter dietary interventions. Energy prescriptions for dietary weight loss interventions were tabulated in kJ, with studies reporting energy prescriptions in calories being converted to kJ by multiplying by 4.18. A very low energy diet (VLED) was defined as a diet providing less than 3.4 MJ per day (76, 77), and a low energy diet (LED) was defined as a diet providing greater or equal to 3.4 but less than 5 MJ per day (76). A moderately energy restricted diet (MER) was defined as a diet providing greater or equal to 5 MJ per day (78). BMD in all studies was reported as  $\text{g/cm}^2$ . Data for lumbar spine BMD measured between L1-L4 were pooled with data measured between L2-L4. In all studies, serum P1NP concentrations were reported in  $\mu\text{g/L}$ . Serum concentrations of osteocalcin were reported by studies in either  $\text{nmol/L}$  or  $\mu\text{g/L}$ . For this analysis we converted all data for osteocalcin to  $\text{nmol/L}$  using the molecular weight of osteocalcin as 5800 (79). The included studies reported serum concentrations of CTX in  $\text{nmol/L}$  or  $\mu\text{g/L}$ , depending on which manufacturer's assay was used. As these methods are not comparable to

each other, no conversion equation is available. Thus, data for serum CTX concentrations were separated according to the units reported by the original studies (nmol/L or  $\mu\text{g/L}$ ). NTX concentrations were also analysed in two distinct data sets due to measurements having been made either in serum or urine, both of which also have their own units of measurement and non-comparable concentrations; nmol/L bone collagen equivalents (BCE) for serum, and nmol/L BCE / nmol/L creatine for urine. Corresponding authors were contacted if any required data was not available from the publication or was published in a format different from that required for this meta-analysis (20, 22-25, 28, 35-37, 39, 42-47, 69-71, 73, 74). For authors from whom we did not receive a response (24, 49, 75), data for their publications were analysed qualitatively rather than quantitatively.

#### 2.3.4 Data synthesis and analysis

Our primary outcomes were the change in BMD at the total hip, lumbar spine and total body, and secondary outcomes were the change in serum P1NP, serum osteocalcin, serum CTX and serum or urinary NTX concentrations from baseline (i.e. before commencement of the dietary weight loss intervention) to a time point immediately after the dietary intervention. Weight loss between baseline and the end of the dietary intervention was also analysed.

The synthesis of data was performed with a random-effects meta-analysis model. Briefly, we calculated effect size and its variance for each study. The effect size,  $d_i$ , was the weighted mean difference between measures taken before and after the dietary weight loss intervention, with the weight being inverse to the variance for each study. It is assumed that each  $d_i$  is normally distributed with a "true" but unknown mean of  $t_i$  and a within-study variance of  $\sigma_i^2$ . The collection of  $d_i$  across studies is further assumed to follow a normal distribution with an unknown mean of  $\delta_0$  and a between-study variance of  $s^2$ . The random-effects model recognizes the possibility of

heterogeneity of between-study variation (i.e., that  $\tau^2$  could be different from 0) but with a fixed value.

The heterogeneity of effects across studies was assessed by computing the coefficient of inconsistency ( $I^2$ ), which is the proportion of total variation among studies that is due to between-study heterogeneity. An  $I^2$  exceeding 50% is regarded as evidence of substantial heterogeneity, indicating that secondary analyses are required to determine whether differences in primary outcomes may be due to differences in various parameters among participants. We decided a priori that if substantial heterogeneity was observed between studies, we would conduct sub-group analyses for the type of dietary weight loss intervention under study (VLED or LED versus MER) and menopausal status (pre- versus post-menopausal). In order to avoid duplication of data in these sub-group analyses, changes from baseline to the end of the dietary intervention were included in the analysis, but changes from baseline to any additional time points measured before the end of the intervention were not. Because of the limited number of studies available, duration of dietary intervention was not taken into account for such sub-group analyses, with all studies being pooled into their corresponding type of dietary intervention or menopausal status regardless of diet duration. To further explore any heterogeneity identified, we also conducted meta-regression analysis of change in the parameter of interest versus baseline body weight and baseline BMD. It was not possible to assess whether other differences amongst participants (i.e. sex and age) contributed to differences in primary outcomes, because separate data from men or women or from people of clearly defined age groups were not available for most studies.

Publication bias was investigated visually with a funnel plot and confirmed with an Egger's test. Due to the nature of this meta-analysis, where data were compared before and after an intervention, the usual quality filters that apply to randomized controlled trials or observational studies could not

be applied. All statistical analyses were conducted using STATA software version 13.0 (StataCorp, Texas, USA).

## 2.4 Results

### 2.4.1 Characteristics of clinical trials

As seen in **Supplementary Figure 1**, 3145 publications were retrieved from the 5 databases searched, equating to 2765 unique publications. Following screening of titles and abstracts, the full texts of 71 publications were then retrieved and analysed against the inclusion and exclusion criteria, resulting in the exclusion of 30 publications for the reasons shown in **Supplementary Figure 1**. There were no further publications identified from screening references lists of these 71 full-text publications. As a result, 41 publications were included in this report, with 38 of them being included in the meta-analysis and the other 3 (24, 49, 75) being analysed qualitatively; 31 reporting on BMD outcomes (29 being included in the meta-analysis) and 23 reporting on bone turnover markers (21 of which were included in the meta-analysis) (**Supplementary Figure 1**).

The publications included in this report are listed alphabetically in **Supplementary Table 1**. Sample sizes varied from  $n = 9$  to 164, averaging at 46 participants per study. The studies included either males and females or females only, with females being divided in some cases into pre- and post-menopausal status (**Supplementary Table 1**). The lowest reported mean age of any study was 33.7 years, and the greatest mean age was 70 years. BMI was of a minimum mean average of 25.6  $\text{kg/m}^2$  (overweight) and a maximum mean average of 39.3  $\text{kg/m}^2$  (obese). Mean body weight for participants in the included studies ranged from 67.2 to 111.6 kg.

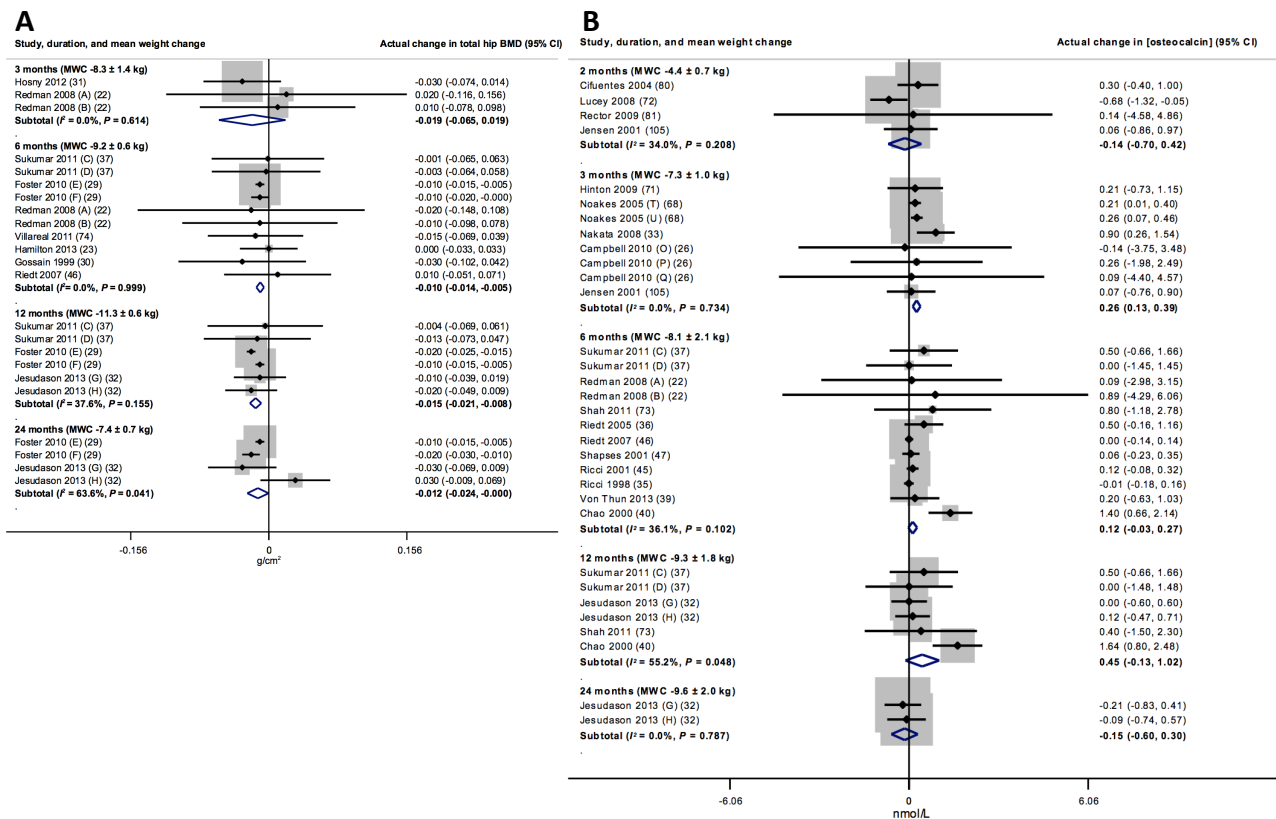
A number of publications reported on multiple dietary interventions, such as normal protein and high protein diets, so the total number of interventions for this meta-analysis reported in the 38 publications was 53. Dietary weight loss interventions differed amongst studies with respect to both

the duration and details of intervention (**Supplementary Table 1**). A number of publications measured outcomes for each intervention at more than one time point (e.g. after 3 and 6 months on a 6-month intervention), and the total number of observations for these 38 publications and 53 interventions was 64.

### 2.4.2 Total hip BMD

*Summary.* This meta-analysis shows significant reductions by 0.010-0.015 g/cm<sup>2</sup> in total hip BMD after dietary weight loss interventions of 6-24 months in duration. There was significant heterogeneity of effects among interventions, partly due to differences in energy restriction (there was a significant reduction in total hip BMD with MER but not with severe energy restriction via VLED or LED). Heterogeneity could not be attributed to differences among participants in menopausal status or baseline body weight or baseline total hip BMD.

Total hip BMD data were available from 13 interventions with 23 observations and 889 participants. The average weight losses ranged from 7 to 11 kg (**Figure 1A**), depending on the duration. Mean  $\pm$  SEM of total hip BMD at baseline were  $1.01 \pm 0.02$  g/cm<sup>2</sup>. Total hip BMD decreased at all time points investigated (3, 6, 12 and 24 months), significantly so at 6 months (-0.010 [-0.014, -0.005] g/cm<sup>2</sup>,  $P = 0.001$ ), 12 months (-0.015 [-0.021, -0.008] g/cm<sup>2</sup>,  $P = 0.001$ ), and 24 months (-0.012 [-0.024, 0.000] g/cm<sup>2</sup>,  $P = 0.047$ ), as shown in **Figure 1A**. Significant heterogeneity was found amongst interventions lasting 12 months and 24 months, but not amongst those of 3- or 6-month duration, as seen from the  $I^2$  statistics in **Figure 1A**.



**Figure 1. Forest plot of change in (A) total hip BMD (bone mineral density) and (B) serum osteocalcin from baseline until the end of dietary weight loss interventions of varying durations.** Mean weight changes (MWC) during the dietary interventions are recorded next to the corresponding duration, ± standard deviations. The letter in parentheses to the right of each study is used to distinguish different dietary interventions from the same publication, with details of all corresponding dietary interventions listed in **Supplementary Table 1**. Plotted values (and the numbers at right) represent the absolute changes in (A) total hip BMD (in g/cm<sup>2</sup>) and (B) serum osteocalcin concentrations (in nmol/L), with 95% confidence intervals (CI) illustrated by the error bars (or the numbers in parentheses at right). Mean ± SEM of total hip BMD and serum osteocalcin concentrations at baseline were 1.01 ± 0.02 g/cm<sup>2</sup> and 1.6 ± 0.5 nmol/L, respectively. *I*<sup>2</sup> indicates the percentage of heterogeneity for the dietary interventions of each duration.

Due to this significant heterogeneity in the data at 12 and 24 months, sub-group analyses were conducted to explore for potential contributing factors. All interventions were divided according to

the degree of energy restriction (VLED or LED versus MER as outlined in the Methods section), regardless of the duration of the dietary intervention. Weight loss was greater with VLED or LED (-11.1 [-14.4, -7.8] kg,  $P = 0.001$  versus baseline), than with MER (-9.6 [-10.8, -8.6] kg,  $P = 0.001$  versus baseline). Although total hip BMD decreased with both types of dietary intervention, the decrease was significant only with MER (-0.013 [-0.018, -0.008] g/cm<sup>2</sup>,  $P = 0.001$ ). Menopausal status had no significant effect on the effect of weight loss on total hip BMD (data not shown). To further explore possible reasons for the heterogeneity in outcomes, we conducted a meta-regression analysis of change in total hip BMD versus baseline body weight and baseline BMD using the 13 studies that investigated total hip BMD after 6 or 12 months. However, this analysis did not show any linear relationship between change in total hip BMD and baseline body weight ( $P = 0.757$ ) or baseline BMD ( $P = 0.467$ ), suggesting that differences in these baseline parameters could not account for variability in the results. This finding also suggests that the reduction in total hip BMD was unlikely due to the statistical phenomenon of regression toward the mean, whereby variables that are higher or lower than the mean upon first measurement will tend to be closer to the mean the next time they are measured, even without intervention. The Egger's test and funnel plot revealed no significant publication bias for the change in weight ( $P = 0.967$ ) or total hip BMD ( $P = 0.492$ ).

### 2.4.3 Lumbar spine BMD

*Summary.* This meta-analysis shows no significant overall effect of diet-induced weight loss on lumbar spine BMD. However, there was significant heterogeneity of effects among interventions. This heterogeneity was related to differences in energy restriction and menopausal status, with a significant decrease in lumbar spine BMD in interventions involving VLED or LED but not MER, and in interventions involving pre- but not post-menopausal women.

BMD at the lumbar spine showed variable responses to diet-induced weight loss, with increases, decreases and no change in this parameter being found from the baseline mean  $\pm$  SEM values of

$1.16 \pm 0.03$  g/cm<sup>2</sup> in a total of 20 interventions and 29 observations in 1,097 participants, with no overall significant differences being identified by this meta-analysis at any time point (**Supplementary Figure 2**). Significant heterogeneity between studies was present at 3 and 12 but not at 6 or 24 months. VLED or LED caused a significant decrease in BMD of the lumbar spine across all time points ( $-0.031$  [ $-0.062, 0.000$ ] g/cm<sup>2</sup>,  $P = 0.05$ ), with no significant change in this parameter in response to MER ( $0.002$  [ $-0.003, 0.007$ ] g/cm<sup>2</sup>,  $P = 0.497$ ). This result was opposite to that found for the total hip as described above, where BMD was significantly decreased only in response to MER and not in response to VLED or LED. When menopausal status was investigated, BMD of the lumbar spine decreased significantly only in pre-menopausal women ( $-0.023$  [ $-0.005, 0.000$ ] g/cm<sup>2</sup>,  $P = 0.05$ ) and not in post-menopausal women ( $0.002$  [ $-0.011, 0.016$ ] g/cm<sup>2</sup>,  $P = 0.726$ ). Therefore, although no significant effect of diet-induced weight loss on lumbar spine BMD was found when the data were pooled according to the duration of dietary interventions, significant effects were found when the studies were analysed according to the diet type and menopausal status of the participants. No evidence of publication bias was found.

#### 2.4.4 Total body BMD

*Summary.* Apart from a significant decrease of  $0.011$  g/cm<sup>2</sup> in total body BMD after dietary weight loss interventions of 6 months in duration, this meta-analysis showed no overall effect of diet-induced weight loss on total body BMD. There was significant heterogeneity of effects among interventions, related to differences in energy restriction (there was a significant increase in total body BMD with VLED or LED but not MER), and differences in menopausal status (there was a significant decrease in total body BMD in post- but not pre-menopausal women).

**Supplementary Figure 3** depicts the forest plot for the change in BMD of the total body in response to diet-induced weight loss in 32 interventions and 37 observations including a total of 883 participants (mean  $\pm$  SEM at baseline of  $1.17 \pm 0.02$  g/cm<sup>2</sup>). For dietary interventions of durations

of 3 and 12 months, no significant changes from baseline in total body BMD were found ( $P = 0.119$ , and  $P = 0.619$ , respectively). For dietary interventions that had a duration of 6 months, however, a significant decrease from baseline in total body BMD was observed ( $-0.011$  [ $-0.018$ ,  $-0.003$ ]  $\text{g}/\text{cm}^2$ ,  $P = 0.004$ ). Significant heterogeneity was observed for interventions of 3 and 12 months' duration. VLED or LED resulted in a significant overall increase in total body BMD ( $P = 0.001$ ) compared to a non-significant increase in this parameter in response to MER ( $P = 0.59$ ). While both pre- and post-menopausal women lost a similar amount of body weight, and while BMD of the total body decreased in both pre- and post-menopausal women, the effect of diet on total body BMD was significant only in the post-menopausal women ( $P = 0.006$ ). This finding is different to that identified above, where no change in total hip BMD was found in either pre- or post-menopausal women, and a significant decrease in lumbar spine BMD was only detected in the pre-menopausal cohort. No publication bias was detected.

#### **2.4.5 N-terminal propeptide of type I procollagen (P1NP)**

There were 8 interventions and 10 observations that investigated the effect of diet-induced weight loss on serum concentrations of the bone formation marker, P1NP, in a total of 176 participants with a mean  $\pm$  SEM at baseline of  $40.8 \pm 3.3$   $\mu\text{g}/\text{L}$ . Serum P1NP concentrations showed no significant change in response to diet at any time point (**Supplementary Figure 4**). No heterogeneity was observed for serum P1NP.

#### **2.4.6 Serum osteocalcin**

*Summary.* This meta-analysis showed that 3-month diet-induced weight loss interventions were associated with a significant increase in serum osteocalcin concentrations. However, the effect was not observed when measured after interventions of 6, 12 or 24 months in duration. There was significant heterogeneity among studies, due at least in part to differences in energy restriction but

not menopausal status, with a significant increase in osteocalcin being observed after MER but not after VLED or LED.

There were 25 interventions and 32 observations that investigated the effect of diet-induced weight loss on serum concentrations of the bone formation marker, osteocalcin, in a total of 827 participants with a mean  $\pm$  SEM at baseline  $1.6 \pm 0.5$  nmol/L. Overall, serum osteocalcin concentrations tended to increase with diet-induced weight loss (**Figure 1B**), with a significant increase occurring in response to dietary interventions of 3 months' duration ( $0.26$  [0.13, 0.39] nmol/L,  $P = 0.001$ ). Heterogeneity was present only at 12 months. Weight reduction was greater with VLED or LED ( $-13.7$  [-23.3, -4.2] kg,  $P = 0.005$  versus baseline) than with MER ( $-6.8$  [-23.3, -6.1] kg,  $P = 0.001$  versus baseline). However, a significant increase in osteocalcin was only found in response to the MER ( $0.10$  [0.01, 0.19] nmol/L,  $P = 0.025$  versus baseline) and not in response to the VLED or LED ( $0.23$  [-0.68, 1.13] nmol/L,  $P = 0.624$  versus baseline). This finding is consistent with that for BMD of the total hip, where a significant decrease occurred in response to MER but not VLED or LED, yet opposite to that for the lumbar spine, which favored MER over VLED for effects on BMD. No significant changes from baseline to the end of the dietary intervention were present with the limited number of interventions having a specifically pre- or post- menopausal status (pre-menopausal,  $0.17$  [-0.14, 0.48] nmol/L,  $P = 0.290$ ; post-menopausal,  $0.06$  [-0.06, -0.18] nmol/L,  $P = 0.320$ ). There was no publication bias.

#### 2.4.7 Serum C-terminal telopeptide of type I collagen (CTX)

Serum CTX was investigated and reported in nmol/L units in 5 interventions and 5 observations with a total of 103 participants, all with a 3-month duration and a mean  $\pm$  SEM at baseline of  $12.5 \pm 1.5$  nmol/L (**Supplementary Figure 5**). A significant increase in this parameter was observed ( $4.72$  [2.12, 7.31] nmol/L,  $P = 0.001$  versus baseline). There was no significant heterogeneity detected, so no secondary analyses were performed for this parameter. Serum CTX was investigated and

reported in  $\mu\text{g/L}$  units in 8 interventions and 10 observations involving 486 participants overall and a mean  $\pm$  SEM at baseline of  $0.51 \pm 0.09 \mu\text{g/L}$  (**Supplementary Figure 6**). There was a non-significant trend for an increase in this parameter for interventions of 2 months' duration, and a significant increase for interventions of 3 months' duration ( $0.21 [0.13, 0.29] \mu\text{g/L}$ ,  $P = 0.001$  versus baseline), albeit that finding stems from the results of a single intervention (71). However, dietary interventions that were 6, 12 or 24 months in duration produced no overall significant change in serum CTX concentrations compared to baseline and reported in  $\mu\text{g/L}$  (**Supplementary Figure 6**). Significant heterogeneity for serum CTX in  $\mu\text{g/L}$  occurred at 6 and 12 months, but subgroup analysis was not performed for this parameter due to the limited number of studies available. Indeed, only 1 study that investigated serum CTX in  $\mu\text{g/L}$  used an LED, and only 1 study investigated a distinctly pre-menopausal population.

#### **2.4.8 Serum and urinary N- terminal telopeptide of type I collagen (NTX)**

There were 14 interventions and 17 observations that investigated serum or urinary NTX concentrations in a total of 306 participants. For both serum (**Supplementary Figure 7**) and urinary (**Supplementary Figure 8**) concentrations of the bone resorption marker NTX, there was an overall trend for an increase from baseline mean  $\pm$  SEM values of  $15.5 \pm 1.2 \text{ nmol/L BCE}$  and  $37.4 \pm 4.1 \text{ nmol/L BCE} / \text{nmol/L creatine}$ , respectively, in response to diet-induced weight loss. A significant increase only occurred for serum NTX in response to a 2-month diet-induced weight loss intervention, albeit this time point only included one study (80) ( $3.70 [0.90, 6.50] \text{ nmol/L BCE}$ ,  $P = 0.010$  versus baseline). No heterogeneity was observed for serum or urinary NTX.

#### **2.4.9 Qualitative analysis of studies that could not be included in the meta-analysis**

For the 3 publications (49, 75, 81) that were not able to be included quantitatively in this meta-analysis, the findings are broadly consistent with results from the meta-analysis. Svendsen (1993) (49) placed 50 overweight post-menopausal women on a diet involving MER for 3 months, thereby

inducing significant weight loss ( $-9.5 \pm 0.4$  kg,  $P = 0001$  versus baseline). Lumbar spine and total body BMD were investigated, with non-significant decreases being observed in both parameters when measured at 3 months. The second study, by Thorpe (2008) (24), investigated the effect of a 12-month high protein or high carbohydrate weight-reducing diet on total hip, lumbar spine and total body BMD in 130 male and female participants. In the cohort on the high protein weight-reducing diet, BMD was significantly increased at all sites investigated, whereas the cohort on the high carbohydrate weight-reducing diet showed a non-significant trend towards decreased BMD at all three sites (total hip, lumbar spine and total body). Hyldstrup (1993) (75) investigated serum osteocalcin concentrations in 44 obese men and women undergoing a VLED for 2 months, with 24 participants continuing to 8 months on the diet. A significant increase in serum osteocalcin concentrations occurred at 2 months, and this increase over baseline values was still present when measured at 8 months, with no significant difference between values recorded at 2 and 8 months.

## **2.5 Discussion**

Whether or not diet-induced weight loss exerts an adverse effect on bone health has been a controversial issue. In this study, by using a meta-analysis approach, we have demonstrated that dietary weight loss interventions were associated with a small but statistically significant reduction in total hip BMD, and the effect could be seen from the sixth month of intervention onwards. We did not, however, find any significant effect of diet-induced weight loss on BMD of the clinically relevant site of lumbar spine. Importantly, we found that the decrease in total hip BMD occurred subsequent to statistically significant increases in serum concentrations of osteocalcin, CTX and NTX, suggesting that weight loss diets increase bone turnover, albeit perhaps only transiently, with consequences for total hip but not spine BMD.

While the effect was small, several lines of evidence support a real effect of diet-induced weight loss to reduce total hip BMD. First, of the less than 25% of studies in this review that included a

control group (22, 25, 36, 40, 45-47, 73, 74, 80, 82), there were no significant changes in BMD (22, 25, 46, 47) or bone turnover markers (22, 36, 45-47, 73, 80) in the control group over the time frames investigated in this review, with the exception of 4 studies (36, 40, 45, 74) investigating post-menopausal women or people older than 65 years of age. Secondly, the Dubbo Osteoporosis Epidemiology Study showed that women with a baseline body weight over 70 kg did not show any reduction in BMD over an average interval of 2.7 years, in contrast to the significant bone losses seen in women weighing up to 70 kg (83). Therefore, as the mean baseline body weight of participants in all but one (34) of the studies included in our meta-analysis was over 70 kg, it is likely that loss of total hip BMD in this population was a real effect of diet-induced weight loss. Thirdly, the significant increases in circulating concentrations of bone turnover markers suggest a bone catabolic state. It is important to consider bone turnover markers in this field of research, because the accuracy and precision of DXA is known to decrease with increasing BMI (84) and when participants undergo changes in body weight (85-88), and because BMD measured at time points earlier than 6 months are not considered to be clinically significant, since a complete cycle of bone remodeling takes 4 to 6 months (53). Thus, diet-induced weight loss induces small but statistically significant losses in total hip BMD that would likely not otherwise be seen in overweight or obese individuals.

It is not clear whether the significant reduction in total hip BMD represents a maladaptive change, or whether it simply represents normalization of BMD relative to reduced body mass after diet-induced weight loss, akin to the finding that a certain loss of percent fat free mass is to be expected in response to reduced BMI (89). The statistically significant decreases in BMD observed in this meta-analysis (-0.010 to -0.015 g/cm<sup>2</sup>) represent an approximately 1 to 1.5% change from baseline values, which is similar to the average yearly BMD loss for elderly women (83, 90). In an untreated population, each standard deviation decrease in femoral neck BMD (which is 0.12 g/cm<sup>2</sup>) was associated with an approximately 3.5-fold increase in the risk of hip fracture (91). Thus, it can be

estimated that a 0.010 to 0.015 g/cm<sup>2</sup> decrease in BMD is equivalent to an approximately 10 to 15% increase in fracture risk. Thus, it would appear unlikely that a single dietary weight loss intervention of 6-24 months' duration would have any prevailing negative impact on bone, and should be safe for most overweight or obese people. However, given that long-term obesity management typically involves multiple periods of diet-induced weight loss, repeated at intervals over many years, potential cumulative adverse effects on bone should be considered, especially in those at risk of accelerated bone loss such as older or inactive women (83). In keeping with this possibility, a prospective cohort study in 6,785 lean and overweight or obese women aged 65 years and over found that losing 5% or more of initial body weight over an average 5.7-year period, whether intentionally or unintentionally and irrespective of current weight, significantly increased the rate of hip BMD loss and doubled the risk of hip fracture (90). Moreover, a population-based study of 20,745 men and women in Tromsø, Norway, showed that women who recalled dieting and losing 11 kg or more, or who recalled 11 or more dieting episodes, had an adjusted hazard ratio for non-vertebral osteoporotic fractures of 1.48 or 1.73 in a mean follow-up period of approximately 12 years, compared to women with no recollection of dieting (92). Thus, while a single diet-induced weight loss intervention may not increase fracture risk by a clinically relevant degree, caution may be warranted for repeated episodes of diet-induced weight loss.

Unlike the total hip, no significant effect of diet-induced weight loss was observed in the lumbar spine. This may be due in part to the greater measurement error that occurs in the lumbar spine in comparison to the hip (53). DXA scans of the spine, in particular the lumbar region (L1- L5), often pick up calcification from other sources besides healthy vertebrae, and this increases apparent BMD readings (93, 94). Such calcification can originate from atherosclerotic lesions within the aorta, or from osteophytes (protrusions of bone tissue that form in response to joint damage from conditions such as in arthritis), masking underlying changes in bone mass due to age or disease or other factors

(93, 95). Therefore the validity of lumbar spine BMD results can be questionable, highlighting the greater reliance on total hip BMD measures for the estimation of fracture risk (58, 60, 64, 82, 96).

The majority of studies identified in this systematic review and meta-analysis investigated total body BMD, which was significantly reduced by 6-month (but not by 3- or 12-month) weight loss interventions. However, measures of total body BMD are not clinically relevant because they do not identify specific areas that are affected by loss of bone mass. In osteoporosis, bones with a high proportion of trabecular as opposed to cortical bone tissue, such as the hip and spine, have an increased likelihood of degradation (63). When sub-regions of whole body DXA scans, such as the pelvis, are used to measure BMD rather than the total hip or proximal femur, under diagnosis of osteoporosis occurs (97, 98). Therefore, local DXA scans of specific bone regions should be performed rather than, or in conjunction with, total body scans when investigating BMD.

We initially hypothesized that any diet-induced reductions in BMD and increases in bone turnover would be more clearly apparent after interventions involving VLED or LED than those involving MER, due to the greater severity of energy restriction used. This is because energy restriction induces hormonal changes that might be expected to decrease bone mass (18, 53), and the effects may be more pronounced with severe than with moderate energy restriction (99). An additional rationale for this hypothesis is that severe energy restriction induces greater weight loss than MER (100), which would lead to greater mechanical unloading of bone, with subsequently greater bone loss. Mechanistic support for this comes from the finding that weight loss-induced reductions in BMD and bone quality in older adults are correlated with increases in circulating concentrations of sclerostin, an osteoblastic inhibitor released from osteocytes in response to mechanical unloading (101). Furthermore, we hypothesized that post-menopausal women would exhibit more detrimental changes in bone mass and turnover in response to diet-induced weight loss than pre-menopausal women, because they are already pre-disposed to detrimental changes in bone. However, our sub-

group analyses did not provide any clear evidence to support or refute these hypotheses. The conflicting results identified emphasize the need for further investigation into the effects of diet types on bone, as well as interaction of the effect with menopausal status.

The present findings should be considered within context of strengths and weaknesses. To the best of our knowledge, this is the first and the most comprehensive review of the effect of diet-induced weight loss on bone loss, as assessed in clinical trials. The measurement of BMD in individual studies was done with DXA technology, which is considered gold standard for the assessment of bone mass. However, it was not possible to determine the effects of sex or age on the changes in bone mass or turnover with weight loss. This is because the majority of studies investigated female populations only. In studies where both males and females were included (20-22, 24, 29, 42, 44, 71-75), the two sexes were not always reported independently (22, 29, 42, 44, 74). Additionally, although age was reported in all studies, large age ranges prevented us from being able to assign the outcomes to a younger or older population for comparison. Another limitation of this meta-analysis was the widely varying types of dietary interventions. While we were able to categorize interventions according to the levels of prescribed energy intake, this did not take into account differences in macronutrient composition of the diets. A further limitation of this meta-analysis was that only 25% of the included studies involved comparison with a control group (22, 25, 36, 40, 45-47, 73, 74, 80, 82), as discussed above.

This meta-analysis has not only highlighted significant changes that occur in bone physiology in response to diet-induced weight loss, it has also crystallized gaps in the literature where further research is required. First, mathematical modeling and bone structural analyses would be required to determine whether the presently-observed changes in bone in response to diet-induced weight loss represent benign normalization of bone mass to the reduced body weight, or the onset of potentially pathological processes. Secondly, only a minority of studies hereby reviewed

investigated BMD at time points after the end of the dietary intervention (20, 21, 23, 25, 27, 28, 30, 39, 74). This prevented us from being able to assess whether the changes that were observed immediately after finishing the weight loss diet persisted or resolved once the dietary intervention has ceased. Of the studies that did include a follow up time point (21, 23, 25, 27, 28, 30, 39, 74), decreases in BMD of the total hip (23, 30, 74), lumbar spine (25, 28, 30) and total body (21, 27, 28, 30, 39) were still present when measured at 3 to 21 months after completion of the dietary weight loss intervention, raising potential concerns about the long-term impact of repeated weight loss diets on bone. Hence it would be important that future studies investigate the longitudinal effects of dietary weight loss interventions on bone, as in two current trials in our research team (102, 103). An additional outstanding question is the change in fracture risk that may occur as a result of BMD loss subsequent to diet-induced weight loss in overweight or obese people. None of the studies included in this analysis estimated change in fracture risk as a result of the diet, with only one study (23) reporting Z and T scores (which are needed to calculate fracture risk in some algorithms) in conjunction with BMD in  $\text{g/cm}^2$ . Therefore, future research should aim to incorporate BMD values of the lumbar spine and / or hip, Z and T scores, as well as an assessment of fracture risk when investigating the influence of diet-induced weight loss on bone. Further research may also be needed to address the kinds of interventions that could aid in preventing bone loss in response to diet-induced weight loss. Currently there is some, but little evidence from randomized controlled trials indicating that calcium supplementation attenuates the bone loss that occurs with weight loss, and that a higher dietary protein intake and exercise (101) also have similar effects, as highlighted in two reviews (53, 104). Since this meta-analysis included diet-induced weight loss interventions with higher dietary protein intakes (but not those involving calcium supplementation), as well as those that recommended but did not supervise physical activity, the effect of diet-induced weight loss on bone may be even more pronounced than that suggested in the current analysis for individuals on lower protein weight loss diets and / or not engaging in regular physical activity. Additionally, while our sub-group analyses suggested that VLED or LEDs do not seem to have any

worse effect on bone than does MER, there were limited studies available testing VLEDs or LEDs, and only three (22, 28, 70) in direct comparison to less severe energy restriction (i.e. MER). Thus more work is needed in this domain, given the increasing use of severe energy restriction for the management of overweight and obesity.

Obesity is a major health concern that requires treatment to prevent or attenuate associated health issues and diseases. This meta-analysis shows that BMD of the total hip decreases with diet-induced weight loss in overweight or obese people, in conjunction with an early rise in bone turnover, but the change induced by a single intervention is small in comparison to the benefits for metabolic health. Thus, clinicians should continue to recommend weight loss for the treatment of overweight and obesity, with support for weight maintenance after weight loss.

**2.6 References**

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## **Chapter 3: Effect of diet-induced weight loss on muscle strength in overweight or obese adults – a systematic review and meta-analysis of clinical trials**

*Revised manuscript re-submitted to Obesity Reviews on 26<sup>th</sup> February 2016*

### **3.1 Abstract**

We conducted a systematic review and meta-analysis to identify how diet-induced weight loss in adults with overweight or obesity impacts on muscle strength. 27 publications, including 33 interventions, most of which were 8-24 weeks in duration, were included. Meta-analysis of 7 interventions measuring knee extensor strength by isokinetic dynamometry in 108 participants found a significant decrease following diet-induced weight loss (-9.0 [95% confidence interval: -13.8, - 4.1] N/m,  $P < 0.001$ ), representing a 7.5% decrease from baseline values. Meta-analysis of handgrip strength from 10 interventions in 231 participants showed a non-significant decrease (-1.7 [-3.6, 0.1] kg,  $P = 0.070$ ), with significant heterogeneity ( $I^2 = 83.9\%$ ,  $P < 0.001$ ). This heterogeneity may have been due to diet type, because there was a significant decrease in handgrip strength in 7 interventions in 169 participants involving moderate energy restriction (-2.4 [-4.8, -0.0] kg,  $P = 0.046$ ), representing a 4.6% decrease from baseline values, but not in 3 interventions in 62 participants involving very low energy diet (-0.4 [-2.0, 1.2] kg,  $P = 0.610$ ). Due to variability in methodology and muscles tested, no other data could be meta-analyzed, and qualitative assessment of the remaining interventions revealed mixed results. Despite varying methodologies, diets and small sample sizes, these findings suggest a potential adverse effect of diet-

induced weight loss on muscle strength. While these findings should not act as a deterrent against weight loss, due to the known health benefits of losing excess weight, they call for strategies to combat strength loss – such as weight training and other exercises – during diet-induced weight loss.

### **3.2 Introduction**

Overweight and obesity are no longer issues affecting the minority. From 1980 to 2013, the worldwide prevalence of people with a body mass index (BMI) greater than or equal to 25 kg/m<sup>2</sup> rose by 27.5% (1). Excess body weight leads to a number of metabolic health problems (2-4), alongside poor physical performance and musculoskeletal disorders (5).

To help combat the obesity crisis, a plethora of dietary weight loss programs have been devised, varying in macronutrient composition and level of energy restriction. While the aim of weight loss programs is to reduce excess fat, they also result in loss of fat free mass (6), possibly due to changes such as reduced circulating concentrations of insulin like growth factor-1 (IGF-1) or sex steroids (7-10), unless combined with a strength training program (11). Loss of fat free mass is associated with a reduction in muscle mass (12). Moreover, fat free mass is a known predictor of muscle strength (13, 14). Although loss of fat free (and muscle) mass is to be expected when a person is no longer carrying such a high body mass (15), there is no clear consensus about the effect that reductions in muscle mass may have on muscle functions, notably strength.

Muscle strength can be defined as the ability of a muscle to generate maximal force (16, 17). It is a function of both muscle mass and muscle quality, and is an indicator of one's functional capacity (18). Muscle strength declines by 8-10% every decade from the age of 40 onwards (16), in a process known as dynapenia (19) – as opposed to sarcopenia, which is the age-related decline in muscle mass (19, 20). Not only does ageing reduce muscle strength; intentional weight loss may also have adverse effects. Indeed, diet- or diet and exercise-induced weight loss induces hormonal changes that would be expected to have negative effects on muscle strength (9, 10), possibly more so with severe than with moderate energy restriction (9, 21). Such an outcome would be detrimental, as lower muscle strength is associated with reduced mobility and a higher risk of falls and fractures, thereby negatively influencing one's functional ability to contribute to society (14, 22), as well as a higher risk of mortality (22, 23). Therefore, as our population is becoming increasingly aged and obese, and with greater numbers of people of all ages using dietary interventions to lose excess weight (24), it is crucial to identify whether diet-induced weight loss worsens muscle strength.

The purpose of this systematic review and meta-analysis is thus to examine whether diet-induced weight loss in adults that are overweight or obese has an impact on muscle strength. This will help to clarify the need for, or direction of, future research in this area, and identify suitable methodologies for muscle strength assessment in the context of weight reduction programs in overweight or obese populations.

### **3.3 Methods**

#### **3.3.1 Inclusion criteria**

To be included in this systematic review, studies had to involve participants aged 18 years or above who were overweight or obese ( $\text{BMI} \geq 25 \text{ kg/m}^2$ ), but were otherwise healthy. Included studies implemented an energy-restricted diet with the aim of achieving weight loss, with no limits placed on the duration of the dietary intervention period. Included studies were required to have measured muscle strength before and after the energy-restricted diet by one or more of one repetition maximum (1RM) strength testing, isokinetic dynamometry, isometric dynamometry, or handgrip strength testing (a form of isometric dynamometry), as these are the four most commonly applied methods of muscle strength testing. The first 3 methods can be applied to a range of muscle groups, as indicated in Table 1, in the column listing muscles examined and exercises used. 1RM strength testing measures the maximum weight that a person can lift or move in a single maneuver, such as a chest press (25). It uses isoinertial muscle contractions, meaning that the same maximum weight is being lifted (25). Isokinetic (dynamic) dynamometry measures the maximum force generated against a weighted load that is lifted or moved at a constant speed (16). On the other hand, isometric (static) dynamometry measures the maximum force generated in a sustained muscle contraction against an immovable load for a set time, typically 5 seconds (26). Articles were included if they were original research, with any study design accepted.

### **3.3.2 Exclusion criteria**

Studies specifically involving participants with type 2 diabetes mellitus, osteoporosis or arthritis were excluded. Studies were excluded if participants had undergone bariatric surgery, were taking medications designed to induce weight loss, if the intervention involved supervised exercise, or if exercise was the primary means of

eliciting weight loss. Our rationale for excluding interventions involving supervised exercise or exercise as the primary weight loss mediator was that these are not usual in clinical practice, and dietary restriction is the most common means of eliciting weight loss. However, in order to reduce bias, those interventions that recommended exercise as part of a healthier lifestyle regime were included, provided that the exercise was not supervised or was not the primary focus of the intervention. Non-surgical, non-medication or non-exercise arms of any of the above such studies were included in this review if eligible. Reviews, conference papers and abstracts were excluded.

### **3.3.3 Data sources and search strategy**

An electronic search of the following databases was conducted from inception of each database to January 2015: Cinahl, Embase, Medline, Pre-Medline and Sportdiscus. Both medical subject heading (MeSH) terms and free text search strategies were employed, with limitations set for articles published in English and only involving human participants. The following example shows the specific key words (or MeSH terms) that were used for the search of MEDLINE for population, intervention and outcomes.

#### *Population*

exp overweight/ OR obesity/ OR obesity, abdominal/ OR obesity, morbid/ OR overweight.tw OR over weight.tw OR obes\*.tw OR ((abdominal or subcutaneous or intra-abdominal or visceral or retriperitoneal or retro peritoneal) adj3 fat\*).tw

*Intervention*

diet/ OR diet\*.tw OR diet, carbohydrate-restricted/ OR diet, fat-restricted/ OR diet, reducing/ OR diet, protein-restricted/ OR caloric restriction/ OR calor\* restrict\*.tw OR diet therapy/ OR weight loss/ OR weight los\*.tw OR (weight adj5 (los\* or reduc\* or control\* or decreas\*)).tw OR ((low\* or reduc\* or restric\*) adj3 (calori\* or energy or protein or carb\* or fat\*)).tw

*Outcomes*

musc\* function\*.tw. OR muscle strength/ or hand strength/ or pinch strength/ OR musc\* strength.tw. OR musc\* performance.tw. OR Isometric Contraction/ OR (isometric adj2 force).tw. OR (isometric adj2 contraction).tw. OR (isometric adj2 strength).tw. OR (isokinetic adj2 strength).tw. OR strength assess\*.tw. OR 1RM.tw. OR 1 RM.tw. OR rep\* max\*.tw. OR dynamomet\*.tw. OR Muscle Strength Dynamometer/ OR max\* contraction\*.tw. OR (peak adj2 strength).tw. OR (peak adj2 torque).tw. OR (knee adj2 strength).tw. OR handgrip strength.tw. OR hand grip strength.tw. OR grip strength.tw. OR pinch strength.tw. OR (lower adj3 strength).tw. OR (upper adj3 strength).tw. OR extens\* strength.tw. OR flex\* strength.tw.

Our final input command was population AND intervention AND outcomes. The example search strategy for MEDLINE (above) was adapted to suit each database.

### **3.3.4 Data extraction and analysis**

Two independent authors (JZ and MSHH) screened the titles and abstracts of studies identified in the above search strategy. The full text versions of articles that were potentially relevant were retrieved and the inclusion and exclusion criteria were applied. If discrepancies arose, a consensus was reached by discussion with a third author (RVS).

The following data were extracted by JZ (and reviewed by MSHH) from each of the included publications, as summarized in **Table 1**; study details and design (first author, year published, sample size, sex, menopausal status, age and baseline BMI of the participants, duration of the intervention (in weeks), details of the dietary weight loss intervention; whether it was a very low energy diet (VLED, less than 3.4 MJ per day) (27, 28), low energy diet (LED, greater than or equal to 3.4 MJ but less than 5 MJ per day) (27), or a diet involving moderate energy restriction (MER, greater than or equal to 5 MJ per day) (29), as well as the change in weight from baseline until the end of the diet, baseline fat free mass, how it was measured, the direction of any statistically significant change in fat free mass, if any, the method(s) of muscle strength assessment and muscles being examined, baseline muscle strength, the change in muscle strength from baseline until the end of the dietary intervention (both in absolute terms and as a percent of baseline), and the primary outcome of each publication.

Our primary outcome was the change in muscle strength (assessed by 1RM testing, isokinetic dynamometry, isometric dynamometry or handgrip strength testing) in a pre-intervention versus post-intervention manner, rather than comparison to a control

group. This is due to the limited number of publications including a control or weight maintenance group in their protocol (30-36), with many publications comparing different energy restricted interventions or a dietary protocol with and without exercise. Some publications (37-42) had more than one dietary intervention that met the selection criteria, thus in these cases interventions were included in the analysis independently.

Weights were converted to kg, and durations of dietary weight loss interventions were converted to weeks, when data were not already reported or provided in these formats. Conversion to weeks was made assuming 4 weeks in a month. Energy prescriptions for dietary weight loss interventions were tabulated in categories (VLED, LED or MER as described above), with studies reporting energy prescriptions in calories being converted to MJ by multiplying by 0.00418. Two publications using isokinetic dynamometry reported strength in foot/lb (34, 38): these data were converted to N/m by multiplying by 1.35581795.

Separate meta-analyses were performed on data from studies that assessed muscle strength via isokinetic dynamometry (knee extension) and handgrip strength. There was no requirement to contact any authors for additional data. Data from all interventions examining muscle strength using the same assessment technique were pooled and expressed either in actual units, as they were all reported using (or converted to) the same units (N/m for isokinetic dynamometry of knee extension and kg for hand grip strength), or as a percent of baseline values. We used random effects meta-analysis to obtain pooled changes in muscle strength (both in absolute values and as a percent of baseline) for these parameters. We used this same technique to

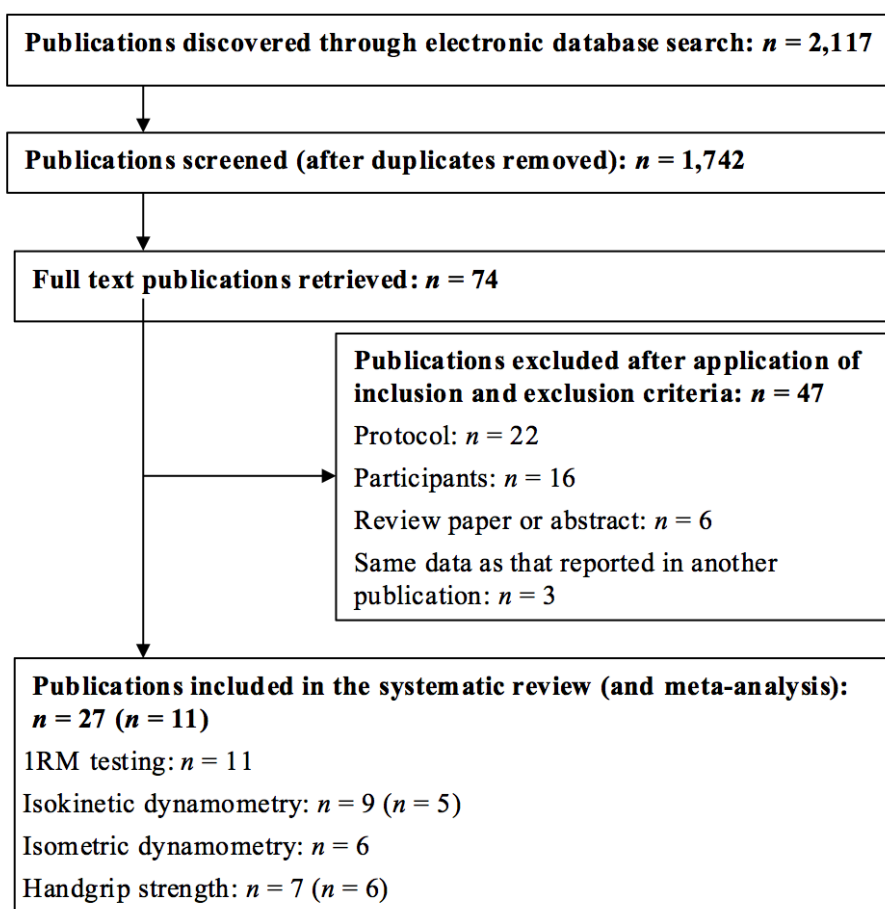
obtain pooled changes in body weight and fat free mass (both in kg). Heterogeneity between studies was assessed using the  $I^2$  statistics. We decided a priori that if significant heterogeneity was detected amongst studies (as indicated by  $I^2$  being greater than 50%), we would undertake sub-group analyses to determine whether variability in muscle strength responses to dietary interventions could be due to differences in the degree of energy restriction. We hypothesized that severe energy restriction via VLED or LED would incur greater negative repercussions on muscle strength than MER. It was not possible to determine the effects of sex or age on the changes in muscle strength with weight loss. This is because in studies where both males and females were included, the two sexes were not always reported independently. Additionally, although age was reported in all studies, large age ranges prevented us from being able to assign the outcomes to a younger or older population for comparison. Statistical analyses were conducted using STATA software version 13.0 (StataCorp, Texas, USA).

Due to the nature of this systematic review, where data were compared before and after an intervention, the usual quality filters that apply to randomized controlled trials or observational studies could not be applied.

### **3.4 Results**

As seen in **Figure 1**, 1,742 unique publications were retrieved from the 5 databases searched, with 74 full text publications being retrieved and assessed for eligibility after title and abstract screening. No further publications were found from screening the reference lists of those 74 articles. We found that 27 publications encompassing 33 interventions matched our selection criteria. Some interventions examined more

than 1 muscle group or used more than 1 muscle strength assessment technique, with the results of these muscle groups or assessments being included as independent interventions. Eleven publications (12 interventions) reported the effect of weight loss on muscle strength as determined by 1RM, 9 publications (11 interventions) examined the effect of weight loss on muscle strength as determined by isokinetic dynamometry, primarily of the knee extensor or quadriceps muscles, 6 publications (10 interventions) examined the effects of weight loss on strength as determined by isometric dynamometry, largely of the leg extensor muscles, and 7 publications (11 interventions) reported on handgrip strength, as shown in **Figure 1**.



**Figure 1. Flow diagram for the process of publication selection, inclusion and exclusion from this systematic review and meta-analysis.**

### **3.4.1 Study Characteristics**

The publications included in this report are listed alphabetically in **Table 1**. Sample size varied from 5 to 71 men and / or women. The lowest mean age reported was 28 years and the greatest was 70 years. Participants had a minimum and maximum baseline mean BMI of 27.8 kg/m<sup>2</sup> and 37.2 kg/m<sup>2</sup>, respectively. Dietary interventions involved either VLED (13 interventions) or MER (20 interventions). No interventions administered a LED. The duration of dietary interventions ranged from 4 to 47 weeks for VLED and 8-24 weeks for MER, with a mode of 8 to 12 weeks for both VLED and MER. Two studies (31, 38) did not have a fixed duration of dietary intervention, rather, participants were prescribed a VLED and were required to lose a fixed percentage of their initial body weight, irrespective of how long it took to achieve.

**Table 1. Study characteristics for interventions investigating muscle strength**

First Author, year, reference	Sample size, and menopausal status of participants (percent female)	Sex	Age – mean (SD) or range	Baseline BMI or weight mean (SD) or selection criterion	Duration and details of dietary weight loss intervention	Weight change from baseline until the end of intervention – mean (SD)	Baseline fat free mass [method of assessment and direction of change baseline]	Method/s of muscle strength assessment	Muscles examined by muscle strength assessment/s (and exercises used) [baseline strength (SD)]	Primary outcome(s)
<b>Armamento-Villareal 2014 (43)</b>	26 Men and women (65.0%)		70.0 (4.0) years	37.2 (4.5) kg/m <sup>2</sup>	24 weeks MER	-9.7 (5.4) kg	61.4 (13.0) kg [DXA ↓]	Isokinetic dynamometry	Knee extensors [71 (24) N/m] Knee flexors [50 (18) N/m]	Muscle strength Body composition Bone mass
<b>Avila 2010 (44)</b>	12 Men and women (58.3%)		67.4 (4.8) years	31.9 (3.4) kg/m <sup>2</sup>	10 weeks MER	-1.7 (0.9) kg	51.9 (9.0) kg [ADP ↓]	1RM	Leg extensors [471 (39) N]	Muscle strength Body composition Physical function
<b>Beavers 2014 (45)</b>	24 Men and women (87.5%)		68.4 (5.5) years	36.0 (6.0) kg/m <sup>2</sup>	12 weeks MER	-7.8 (2.8) kg	51.9 (8.7) kg [DXA ↓]	Isokinetic dynamometry Handgrip strength	Knee extensors [111 (28) N/m] Hand muscles [29.5 (9.0)]	Body composition Physical function Cardiometabolic capacity

<b>Bouchard 2009 (30)</b>	11 Women – post-menopausal	60.7 (4.6) years	31.9 (2.7) kg/m <sup>2</sup>	12 weeks MER	-4.0 (1.0) kg	39.2 (4.7) kg [DXA ↓]	Isometric dynamometry	kg] Leg extensors [104 (40) lb]	Muscle strength
<b>Brinkworth 2009 (37)</b>	32, men and women (71.9%)	48.8 (1.6) years	33.7 (4.0) kg/m <sup>2</sup>	8 weeks MER with low carbohydrate / high fat diet (C)	-8.1 (2.3) kg	51.4 (10.2) kg [DXA ↓]	Isometric dynamometry Handgrip strength	Knee extensors [577 (246) Nm] Hand muscles [39.7 (12.4) kg]	Muscle strength Aerobic capacity Metabolic adaptations
<b>As above</b>	28 Men and women (53.6%)	49.3 (1.7) years	33.7 (4.2) kg/m <sup>2</sup>	8 weeks MER with high carbohydrate / low fat diet (D)	-6.7 (0.5) kg	55.2 (11.8) kg [DXA ↓]	Isometric dynamometry Handgrip strength	Knee extensors [608 (250) Nm] Hand muscles [42.3 (12.7) kg]	As above
<b>Campbell 2009 (46)</b>	8 Women – post-menopausal	67.0 (2.8) years	28.9 (3.4) kg/m <sup>2</sup>	11 weeks MER	-5.7 (0.3) kg	39.3 (3.7) kg [ADP ↓]	1RM	Leg extensors (bilateral leg extension) [125 (23) Nm] Hamstring muscles (bilateral leg curl) [106 (23) Nm]	Body composition Protein metabolism Muscle protein synthesis rate

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												Leg muscles (Bilateral leg press) [909 (178) N] Chest, arm and abdominal muscles (bilateral chest press) 253 (57) N] Chest, arm and abdominal muscles (bilateral arm pull) [315 (37) N]	
<b>Davis (38)</b>	<b>1990</b>	5 Women – pre- menopausal	33.0 (3.1) years	33.5 (3.4) kg/m <sup>2</sup>	12 – 20 weeks VLED with food (G)	-17.2 kg	(2.7)	Not determined		Isokinetic dynamometr y	Knee extensors [130 (5) N/m]	Aerobic capacity	

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<b>As above</b>	5 Women – pre- menopausal	28.0 (5.3) years	31.9 (3.8) kg/m <sup>2</sup>	12 – 20 weeks VLED with meal replacement formula shakes (H)	-18.1 (4.2) kg	Not determined	Isokinetic dynamometr y	Knee extensors [138 (10) N/m]	As above
<b>Eston 1992 (47)</b>	10 Women	23 – 57 years	29.4 (SD could not be calculated ) kg/m <sup>2</sup>	6 weeks VLED	-11.5 (2.0) kg	50.6 (5.4) kg [BIA ↓]	Isokinetic dynamometr y	Quadriceps [138 (19) N/m] Hamstrings [73.4 (14) N/m]	Body composition Cardiometaboli c capacity
<b>Figueroa 2013 (48)</b>	13 Women – post- menopausal	54.0 (3.6) years	34.8 (4.3) kg/m <sup>2</sup>	12 weeks MER	-5.6 (0.7) kg	43.6 (5.8) kg [DXA ↓]	8RM	Leg muscles [118 (29) kg]	Body composition Pulse-wave velocity Arterial stiffness
<b>Frimel 2008 (12)</b>	15 Men and women (60.0%)	70.3 (4.8) years	36.9 (4.9) kg/m <sup>2</sup>	24 weeks VLED	-10.7 (4.5) kg	59.8 (13.2) kg [DXA ↓]	1RM	Shoulder and biceps (biceps curl) [Data not available] Back, chest and arm muscles (seated row) [Data not available] Chest, arm	Muscle strength

											and abdominal muscles (bench press) [Data not available]	
											Leg muscles (leg press) [Data not available]	
											Knee flexors [Data not available]	
											Knee extensors [Data not available]	
<b>Geliebter 1997 (49)</b>	22 Men and women (63.6%)	36.0 (8.0) years	$\geq$ 25.0 kg/m <sup>2</sup>	8 weeks MER	-9.5 (3.1) kg	57.0 (12.2) kg [BIA ↓]			Handgrip strength	Hand muscles [34.5 (12.0) kg]	Body composition Metabolic capacity	
<b>Hue 2008 (31)</b>	17 Men	36.9 (7.7) years	34.0 (4.7) kg/m <sup>2</sup>	15 – 47 weeks VLED	-11.8 (0.8) kg	Not determined			Isometric dynamometry	Quadriceps [795 (175) N]	Muscle strength	
<b>Kraemer 1997 (32)</b>	8 Women	34.6 (10.2) years	27.3 (3.1) kg/m <sup>2</sup>	12 weeks MER	-6.2 (SD not reported) kg	43.8 (5.3) kg [Hydrodensitometry ↔]			1RM	Chest, arm and abdominal muscles (bench press) [38 (5) kg]	Physical capacity Physiological adaptations	
										Leg and		

<b>Krotkiewski 1990 (50)</b>	25 Women	40.1 (14.6 ) years	36.9 (6.0) kg/m <sup>2</sup>	4 weeks VLED	-8.6 (4.5) kg	60.2 (10.0) kg [Total body potassium content ↓]	Isokinetic dynamometry	gluteal muscles (squat) [72 (30) kg] Knee extensors [data not available]	Muscle strength Muscle morphology
<b>Larson-Meyer 2009 (34)</b>	12 Men and women (50.0%)	39.0 (5.0) years	27.8 (1.4) kg/m <sup>2</sup>	24 weeks MER	-8.3 (2.8) kg	5.8 (2.1) kg [DXA ↓]	Isokinetic dynamometry	Knee extensors [152 (16) N/m] Knee flexors [67 (11) N/m]	Body composition Cardiometabolic capacity
<b>Marks 1994 (35)</b>	10 Women	38.3 (7.8) years	30.1 (3.2) kg/m <sup>2</sup>	20 weeks MER	-3.7 (4.4) kg	47.4 (5.9) % [Hydrodensitometry ↔]	1RM	Chest, arm and abdominal muscles (chest press) [42 (13) kg] Leg extensor [56 (13) kg] Shoulder and biceps muscles (arm curl) [13 (4) kg] Shoulder and triceps muscles (triceps extension)	Muscle strength Body composition Physical capacity

<b>Messier 2009 (51)</b>	71 Women – post-menopausal	58.0 (4.7) years	32.2 (4.6) kg/m <sup>2</sup>	24 weeks VLED	-5.2 (19.4) kg	45.4 (7.2) kg [DXA ↓]	1RM	[9 (2) kg] Lower body [147 (39) kg]	Psychosocial function
<b>Napoli 2014 (52)</b>	26 Men and women (65.0%)	70.0 (4.0) years	37.2 (4.5) kg/m <sup>2</sup>	24 weeks MER	-9.7 (5.4) kg	61.4 (13.0) kg [DXA ↓]	1RM	Upper body [106 (45) kg] Lower body [170 (55) kg]	Psychological function Health quality
<b>Pargman 1967 (53)</b>	25 Women	30 – ≥ 50 years	30.0 kg/m <sup>2</sup>	12 weeks VLED	-16.3 (SD not reported) kg	Not determined	Isokinetic dynamometry Handgrip strength	Knee extensors [41 (9) lb] Elbow extensors [25 (7) lb] Hand muscles [36.8 (5.2) lb]	Muscle strength
<b>Pavlou 1985 (54)</b>	41 Men	46.1 (8.3) years	100.8 (14.1) kg	8 weeks VLED	-9.2 (1.9) kg	62.2 (9.6) kg Total body potassium	Isokinetic dynamometry	Quadriceps [Data not available]	Muscle strength Body composition Metabolic capacity
<b>Pronk 1992 (55)</b>	40 Women	42.6 (10.5) years	104.7 (18.2) kg	22 weeks VLED	-20.8 (5.5) kg	55.0 (7.4) kg [Hydrodensitometry ↓]	1RM	Chest, arm and abdominal muscles (bench press) [28 (6) kg] Chest, arm	Muscle strength

												and abdominal muscles (lateral pull down) [34 (7) kg] Knee extensors [29 (11) kg] Knee flexors [18 (6) kg]	
<b>Scott (39)</b>	<b>1992</b>	19 Women – pre- menopausal	38.0 (6.0) years	28.9 (2.3) kg/m <sup>2</sup>	8 weeks MER with high fat diet (E)	-7.4 kg	(10.9)	49.7 (4.9) kg	[Hydrodensitometr y ↔]	1RM Isokinetic dynamometr y	Chest, arm and abdominal muscles (bench press) [36 (5) kg] Leg muscles (leg press) [93 (15) kg] Knee extensors [135 (23) N/m] Knee flexors [77 (14) N/m]	Muscle strength Physical capacity Metabolic capacity	
<b>As above</b>		17 Women – pre- menopausal	37.0 (5.0) years	30.7 (2.5) kg/m <sup>2</sup>	8 weeks MER with high carbohydrat e diet (F)	-6.5 kg	(11.1)	50.9 (5.0) kg	[Hydrodensitometr y ↔]	1RM Isokinetic dynamometr y	Chest, arm and abdominal muscles (bench press) [39	As above	

											(7) kg Leg muscles (leg press) [98 (14) kg] Knee extensors [137 (18) N/m] Knee flexors [80 (12) N/m]	
<b>Siervo 2012 (56)</b>	21 Men and women (80.9%)	56.9 (9.7) years	36.4 (5.7) kg/m <sup>2</sup>	15 weeks MER	-9.0 (22.5) kg	54.5 (13.8) kg [BIA ↓]	Handgrip strength	Hand muscles [28.2 (11.4) kg]	Psychological function			
<b>Uusi-Rasi 2009 (41)</b>	37 Women – pre- menopausal	42.0 (7.0) years	35.2 (5.2) kg/m <sup>2</sup>	12 weeks MER	-4.3 (4.5) kg	49.2 (8.6) kg [DXA ↔]	Isometric dynamometr y	Leg extensors [24 (5) N/kg]	Muscle strength Body composition Bone mass			
<b>Uusi-Rasi 2010 (40)</b>	20 Women – pre- menopausal	42.1 (3.7) years	33.3 (3.3) kg/m <sup>2</sup>	12 weeks VLED with large weight loss (-19.2 to -13.5% of body weight) (I)	-14.3 (3.7) kg	47.9 (6.1) kg [DXA ↔]	Isometric dynamometr y Handgrip strength	Leg extensors [28 (12) N/kg] Hand muscles [36.8 (4.3) kg]	Muscle strength Body composition Bone mass Bone biomarkers			
<b>As above</b>	21 Women – pre- menopausal	39.2 (5.6) years	33.1 (4.5) kg/m <sup>2</sup>	12 weeks VLED with medium weight loss (-13.4 to -	-9.7 (4.8) kg	45.5 (7.0) kg [DXA ↔]	Isometric dynamometr y Handgrip strength	Leg extensors [24 (4) N/kg] Hand	As above			

<b>As above</b>	21 Women – pre- menopausal	39.3 (5.7) years	34.4 (5.5) kg/m <sup>2</sup>	8.2% of body weight (J) 12 weeks VLED with low weight loss (-8.2 to -2.1% of body weight) (K)	-5.6 (4.5) kg	47.3 (5.5) kg [DXA ↔]	Isometric dynamometr y Handgrip strength	muscles [34.8 (4.5) kg] Leg extensors [26 (6) N/kg] Hand muscles [36.3 (4.7) kg]	As above
<b>Villareal 2011 (36)</b>	26 Men and women (65.0%)	70.0 (4.0) years	37.2 (4.5) kg/m <sup>2</sup>	24 weeks MER	-9.0 (5.4) kg	61.4 (13.0) kg [DXA ↓]	1RM	Shoulder and biceps muscles (bicep curls) Chest, arm and abdominal muscles (bench press) Back, chest and arm muscles (seated row) Knee extensors Knee flexors Leg muscles (leg press) – Reported as total 1RM [607 (213) lb]	Physical capacity

<b>Wycherley 2013 (42)</b>	21 Men	47.7 (8.0) years	27 - kg/m <sup>2</sup>	40	12 weeks MER with high protein diet (A)	-10.7 kg	(5.3)	70.5 (7.0) kg [DXA ↓]	Isometric dynamometr y Handgrip strength	Knee extensors [210 (52) Nm] Hand muscles [50.0 (12.0) kg]	Muscle strength Aerobic capacity
<b>As above</b>	21 Men	45.9 (8.1) years	27 - kg/m <sup>2</sup>	40	12 weeks MER with normal protein diet (B)	-8.7 (3.5) kg		68.1 (8.5) kg [DXA ↓]	Isometric dynamometr y Handgrip strength	Knee extensors [188 (41) Nm] Hand muscles [55.1 (6.6) kg]	As above

↓: fat free mass is statistically significantly decreased; ↑: statistically significantly increased; or ↔: not statistically significantly different from baseline. ADP, Air displacement plethysmography; BIA, bioelectrical impedance analysis; BMI, body mass index; DXA, dual energy X-ray absorptiometry; MER, moderate energy restriction; 1RM, one repetition max; 8RM, eight repetition maximum (rather than a weight being moved a maximum of once (as in 1RM), it is moved a maximum of 8 times); SD, standard deviation; VLED, very low energy diet.

### **3.4.2 One repetition maximum strength testing**

Of the 11 publications (12 interventions) that measured muscle strength via 1RM testing, a variety of muscle groups from both the upper and lower body were tested in response to a variety of dietary interventions, as shown in **Table 1**. Because 8 out of 11 publications (12, 32, 35, 36, 39, 46, 52, 55) investigated more than one particular muscle group through varying exercise movements, such as knee extension, and because different muscle groups may have opposing responses to a particular intervention, it was not appropriate to combine all interventions into a meta-analysis and so the results were analyzed qualitatively.

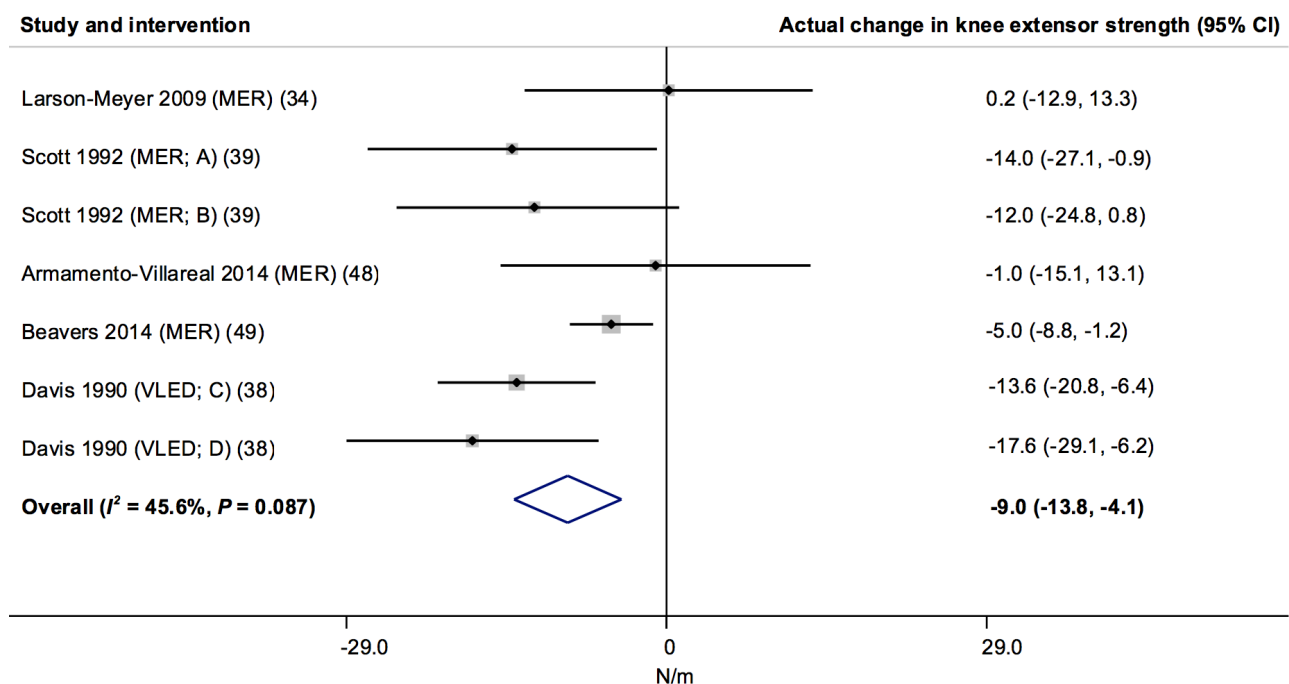
Despite variations in muscle groups examined and dietary interventions, the results were consistent, in that weight loss did not induce significant changes in muscle strength as assessed by 1RM, with the exception of 1 intervention (52) involving a diet of MER which found that upper body strength was significantly decreased, whilst lower body strength was significantly increased relative to baseline, albeit muscle strength was not a primary outcome in that study. Some studies reported non-significant trends in changes in muscle strength with diet-induced weight loss, as assessed by 1RM testing. Specifically, two interventions, both of which prescribed MER, reported non-significant decreases in chest, arm (35) and lower body (35, 51) strength. In contrast, non-significant increases in triceps (35) and total body (36) strength were noted in 2 interventions with participants also on a diet involving MER. Only 1 intervention (55) investigating muscle strength through 1RM administered a VLED, but no trends or significant changes in muscle strength were found in that study. Taken together, it is evident that diet-induced weight loss does not affect muscle strength as assessed by 1RM testing, despite muscle strength being the primary outcome in 7 of the 11 publications that measured muscle strength with this method (12, 32, 35, 36, 39, 44, 55).

### 3.4.3 Isokinetic dynamometry

Of the 9 publications (11 interventions) that measured muscle strength via isokinetic dynamometry, 7 investigated knee extensor muscles (34, 38, 39, 43, 45, 50, 53), 3 of these also investigated knee flexor muscles (34, 39, 43), and 3 publications examined the effect of weight loss on quadriceps muscles (47, 54), hamstring muscles (47) or elbow flexion (53).

A meta-analysis was conducted on 5 of the 7 publications that examined knee extensor muscle strength as determined by isokinetic dynamometry (34, 38, 39, 43, 45). The 2 remaining publications (2 interventions) that examined knee extensor strength (50, 53) were not included in the meta-analysis because the units of measurement – Nm (50) and tensiometer units (53) – were different from that of the meta-analyzed studies (N/m), and conversion was not possible. These studies were analyzed qualitatively as described below. The 5 meta-analyzed studies were conducted in a total of 108 participants, in which body weight was significantly decreased by the intervention (-9.2 [95% confidence interval: -13.6, -4.7] kg,  $P < 0.001$ ). As shown in **Figure 2**, this weight loss intervention resulted in a significant decrease in knee extensor strength (-9.0 [-13.8, -4.1] N/m,  $P < 0.001$ ), with no significant heterogeneity present ( $P = 0.087$ ). This decrease in knee extensor strength represents a 7.5% decrease from the mean baseline value of  $125 \pm 18$  N/m, as calculated using random effects meta-analysis to obtain pooled changes in muscle strength as a percent of baseline. When interventions were analyzed according to the degree of energy restriction, significant decreases in body weight and isokinetic knee extensor strength were apparent for both MER and VLED interventions. Indeed, for the 98 participants on the 5 MER interventions from 4 publications (34, 39, 43, 45), significant decreases in body weight (-6.2 [-9.0, -3.5] kg,  $P < 0.001$ ) and isokinetic knee extensor strength (-5.5 [-8.7, -2.2] N/m,  $P < 0.001$ ) were apparent, representing a pooled 5.2% decrease from the baseline strength value of  $121 \pm 22$  N/m. For the 10 participants on 2 VLED interventions from 1 publication (38), significant reductions in body weight (-17.4 [-18.3, -16.6] kg,  $P < 0.001$ ) and isokinetic knee extensor strength (-14.7 [-20.8, -8.7] N/m,  $P <$

0.001) were also apparent, representing a pooled 13.1% decrease from the baseline strength value of  $134 \pm 8$  N/m. Of the 2 publications that were excluded from the meta-analysis and analyzed qualitatively instead, knee extensor strength significantly decreased in 1 publication, which administered a 4-week VLED (50), while there was no significant change reported in the other publication, which also administered a VLED (12 weeks in this instance) (53). This discrepancy may be due to the latter publication (53) being published in 1967 and using different equipment.



**Figure 2. Forest plot of change in knee extensor strength (as determined by isokinetic dynamometry) from baseline until the end of dietary weight loss interventions.** The letters in parentheses to the right of each study are used to distinguish whether the intervention involved moderate energy restriction (MER) or a very low energy diet (VLED), and to distinguish different dietary interventions from the same publication, with details of all corresponding dietary interventions listed in **Table 1**. Plotted values (and the numbers at right) represent the actual change in knee extensor strength in N/m, with 95% confidence intervals (CI) illustrated by the error bars (or the numbers in parentheses at right). Mean  $\pm$  SD of knee extensor strength at baseline was  $125 \pm 18$  N/m for all studies overall,  $121 \pm 22$  N/m for MER and  $134 \pm 8$  N/m for VLED.  $I^2$  indicates the

percentage heterogeneity.

Those publications that used isokinetic dynamometry but did not examine knee extensor strength were analyzed qualitatively. Four publications (5 interventions) administering a diet involving MER showed no significant changes in knee flexion (34, 39, 43) or quadriceps strength (54) as examined via isokinetic dynamometry, which is different from our findings in the meta-analysis of knee extensor strength. A total of 2 publications investigated isokinetic muscle strength after a VLED intervention (47, 53). One publication involving VLED showed a significant decrease in quadriceps strength (47), albeit there were no significant changes in elbow flexion (53) or, as measured in a separate publication, hamstring strength (47), in response to VLED and as assessed via isokinetic dynamometry.

Taking the results of our quantitative and qualitative analyses together, diets involving both MER and VLED have a negative effect on knee extensor strength, and VLED may also have a negative effect on quadriceps strength as examined by isokinetic dynamometry, whilst neither diet seems to have any significant effect on knee or elbow flexion strength despite significant weight loss.

#### **3.4.4 Isometric dynamometry**

Muscle strength was assessed via isometric dynamometry in 6 publications (30, 31, 37, 40-42), which involved 10 interventions. As isometric dynamometry data from these interventions were collected using different techniques (involving differences in measurement parameters such as extension angles), actual changes could not be pooled using meta-analysis. Thus, these interventions were examined qualitatively.

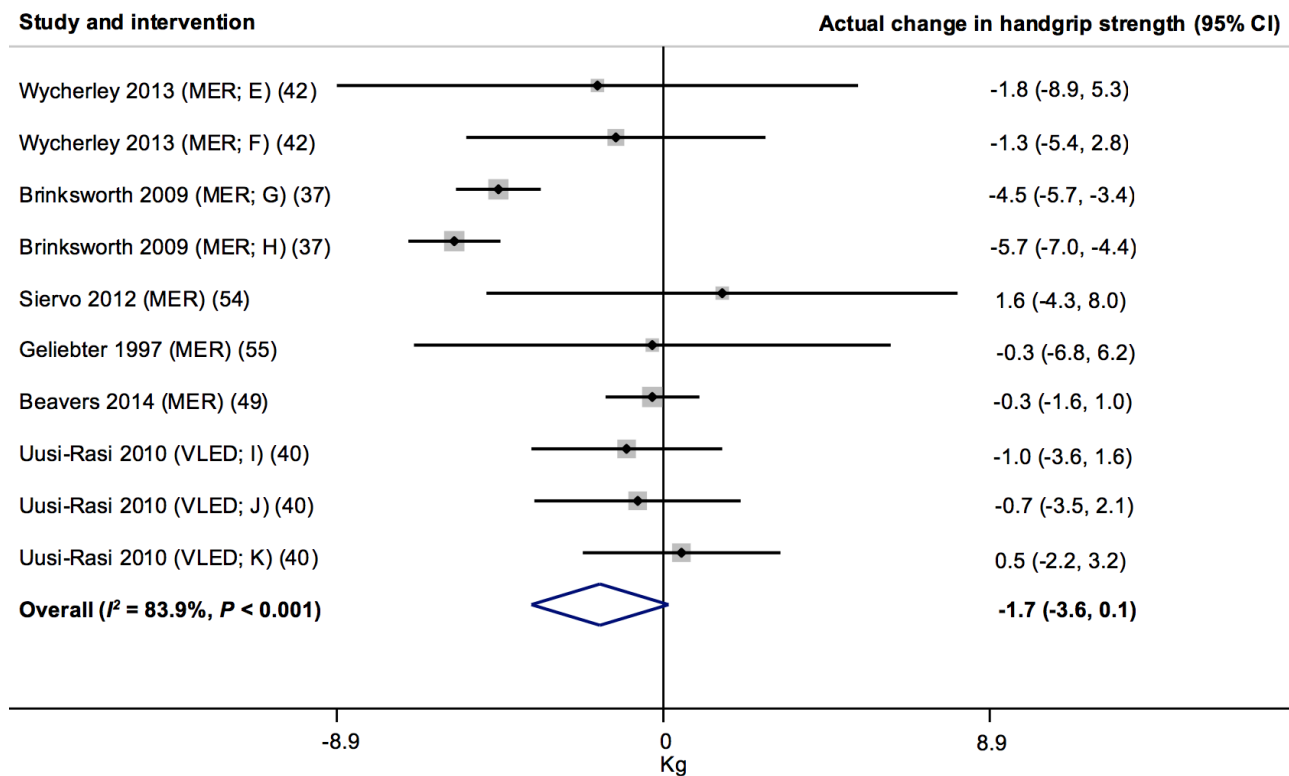
We separated interventions according to the severity of energy restriction. Six of the 10 interventions from 4 publications (30, 37, 41, 42) involved MER, whilst the 4 remaining

interventions from 2 publications (31, 40) assessed the effect of VLED on muscle strength as determined by isometric dynamometry. Of the 6 MER interventions, a significant decrease in leg extension strength was found with 1 such intervention (30), while another one induced a significant increase in leg extension strength (41). The other 4 MER interventions examined isometric knee extensor strength. Two such interventions (37) found a tendency to increase, whilst the other 2 interventions from 1 publication (42) found significant increases after MER in isometric knee extensor strength. Similar to MER, mixed results were found in the 4 interventions inducing weight loss via VLED. One intervention investigating quadriceps strength in participants on a VLED found a significant decrease in strength as assessed by isometric dynamometry (31), consistent with the results reported above for knee extensor strength and quadriceps strength as assessed using isokinetic dynamometry. In contrast, 2 interventions involving VLED found no significant differences in leg extension strength (40), whilst a further VLED intervention from the same publication found a significant increase in leg extension strength (40). Interestingly, this latter VLED intervention also resulted in a greater percentage body weight loss compared to the other 2 VLED interventions where no significant change in leg extension strength was observed. From these overall results (2 interventions inducing significant decreases in muscle strength, 4 inducing significant increases, and 4 inducing non-significant changes), both MER diets and VLED induce unclear effects on muscle strength as assessed by isometric dynamometry of various leg muscles, with no sound conclusion possible from the available data.

### **3.4.5 Handgrip strength**

There were 7 publications (11 interventions) that investigated changes in handgrip strength in response to diet-induced weight loss (37, 40, 42, 45, 49, 53, 56). Of these, 1 publication (1 intervention) (53) was excluded from the meta-analysis, because it used a cable tension dynamometer rather than the hydraulic dynamometer that was used in the other publications, and the unit of measurement (tensiometer units) could not be converted into kg, so that intervention was

analyzed qualitatively. Our meta-analysis was thus conducted on 10 interventions in a total of 231 participants. In these, body weight was significantly decreased by the intervention (-8.8 [-10.2, -7.4] kg,  $P < 0.001$ ), and overall handgrip strength decreased, but this latter effect was not statistically significant (-1.7 [-3.6, 0.1] kg,  $P = 0.070$ ) (**Figure 3**). This change in handgrip strength represents a -3.6% change from the mean baseline value  $39 \pm 9$  kg, as calculated using random effects meta-analysis to obtain pooled changes in muscle strength as a percent of baseline. Significant heterogeneity was found ( $I^2 = 83.9\%$ ,  $P < 0.001$ ), and so we questioned whether differences in results occurred due to differences in dietary interventions. Of the 10 interventions included in our meta-analysis of handgrip strength, 7 prescribed MER in a total of 169 participants (with a duration of 8 weeks in 3 interventions, 12 weeks in 3 interventions, and 15 weeks in 1 intervention), and 3 prescribed VLED in a total of 62 participants (all with a duration of 12 weeks). MER resulted in a significant decrease in handgrip strength (-2.4 [-4.8, -0.0] kg,  $P = 0.046$ ), representing a pooled 4.6% decrease from the mean baseline value of  $40 \pm 8$  kg, in conjunction with significant weight loss (-7.6 [-8.3, -6.9] kg,  $P < 0.001$ ). In contrast, VLED induced no significant change from baseline in handgrip strength (-0.4 [-2.0, 1.2] kg,  $P = 0.610$ ), representing a pooled -1.1% change from the mean baseline value of  $36 \pm 4$  kg, despite significant weight loss (-9.0 [-15.0, -3.0] kg,  $P = 0.003$ ). This difference between MER and VLED in terms of effects on handgrip strength is unlikely due to differences in diet duration, given the similar duration of interventions. These findings are in contrast to the results from our meta-analysis of knee extensor strength as determined by isokinetic dynamometry, where significant decreases were found with both MER and VLED interventions. The publication that was not included in our meta-analysis of handgrip strength administered a VLED in 25 participants (53). The results of that publication agree with the results of this meta-analysis, in that no significant effect of VLED on handgrip strength was detected.



**Figure 3. Forest plot of change in handgrip strength from baseline until the end of dietary weight loss interventions.** The letter in parentheses to the right of each study is used to distinguish whether the intervention involved moderate energy restriction (MER) or a very low energy diet (VLED), and to distinguish different dietary interventions from the same publication, with details of all corresponding dietary interventions listed in **Table 1**. Plotted values (and the numbers at right) represent the actual change in handgrip strength in kg with 95% confidence intervals (CI) illustrated by the error bars (or the numbers in parentheses at right). Mean  $\pm$  SD of handgrip strength at baseline was  $39 \pm 9$  kg for all studies overall,  $40 \pm 8$  kg for MER and  $36 \pm 4$  kg for VLED.  $I^2$  indicates the percentage heterogeneity.

### 3.4.6 Relationship between changes in muscle strength with changes in fat free mass

As fat free mass is a known predictor of muscle strength (13, 14), we hypothesized that a decrease in fat free mass may contribute to reduced muscle strength with diet-induced weight loss. Thus, changes from baseline in fat free mass were meta-analysed in those studies that investigated knee extensor strength (via isokinetic dynamometry) and handgrip strength.

For studies investigating knee extensor strength via isokinetic dynamometry, fat free mass was only available for meta-analysis from participants that underwent MER. This is because the 2 interventions from the 1 study (38) that examined effects of VLED did not investigate fat free mass. In these studies, MER resulted in a significant decrease from baseline in fat free mass (-2.0 [-3.6, -0.4] kg,  $P < 0.05$ ), echoing the significant decrease in knee extensor strength determined by isokinetic dynamometry, as reported above.

For participants undergoing handgrip strength testing for studies included in our meta-analysis, fat free mass was significantly decreased from baseline after diet-induced weight loss (-1.9 [-3.3, -0.5] kg,  $P < 0.01$ ). The degree of energy restriction influenced whether the decrease in fat free mass was significant. Indeed, participants on MER lost a significant amount of fat free mass (-2.2 [-4.0, -0.4] kg,  $P < 0.05$ ), in contrast to those on VLED that did not (-1.5 [-3.6, 0.6] kg,  $P = 0.153$ ). These findings for fat free mass echo the significant decrease in handgrip strength seen with MER, and the non-significant change in handgrip strength seen with VLED, reported above.

### 3.5 Discussion

This systematic review and meta-analysis showed that diet-induced weight loss in adults that are overweight or obese resulted in a significant decrease from baseline in knee extensor strength as assessed by isokinetic dynamometry, with significant decreases found for participants on either MER or VLED, and a non-significant decrease in handgrip strength, with significant heterogeneity in results. This heterogeneity may have been due to diet type, because there were significant decreases in handgrip strength after MER but not VLED. No significant effects of diet-induced weight loss were detected with 1RM strength testing, including 3 interventions involving a VLED (12, 51, 55), with the exception of 1 MER intervention (52) that observed a significant decrease in upper body strength and a significant increase in lower body strength. Qualitative assessment of the

7 and 10 interventions that assessed strength of other muscles by isokinetic and isometric dynamometry, respectively, revealed mixed results (decreases, no significant changes or increases from baseline), with no clear distinction between effects of MER versus VLED. Indeed, there were studies involving MER inducing a significant decrease (30) or increase (41) in leg extension strength and a significant increase in knee extensor strength (42), with studies administering VLED inducing a significant decrease in quadriceps strength (31) or a significant increase in leg extension strength (40). This lack of clear, statistically significant effect of diet induced weight loss on muscle strength may be due to a lack of evidence either way, rather than lack of a true underlying effect. Given the findings from our meta-analyses, we thus conclude that diet-induced weight loss may reduce muscle strength, but further research would be required to clarify any such effect and the impact of different degrees of energy restriction.

Our results suggest that the loss of knee extensor and handgrip strength with diet-induced weight loss occurs at 16-33 times the rate of normal age-associated loss of muscle strength. Knee extensor strength, as assessed by isokinetic dynamometry, was reduced by 9.0 N/m (7.5%) from baseline over a 12-24-week period in all diets combined (5.2% with MER, 13.1% with VLED), whilst handgrip strength was reduced by 2.4 kg (4.6%) from baseline over an 8-15-week period with MER but not VLED. Considering that muscle strength is reduced by 8-10% per decade beyond the age of 40 (16), it appears that a diet of less than 6 months in duration induces strength losses equivalent to 4.6-16.4 years of normal ageing. A 5 kg reduction in handgrip strength over a 4-year period was associated with an elevated hazard ratio of all-cause mortality, cardiovascular mortality, non-cardiovascular mortality, myocardial infarction and stroke (hazard ratio 1.07 to 1.17) (23). Therefore, our observed decreases in muscle strength are a potential cause for concern, especially when considering that many individuals undergo dietary weight loss interventions of 6 months in duration or longer, or undergo repeated dietary weight loss interventions as a means of long-term weight management. However, given the consistent evidence for health benefits of losing at least 3-

15% of body weight (57), the current findings should not be used to discourage weight loss in people with overweight or obesity. Rather, they indicate a need for interventions that attenuate possible strength reductions during diet-induced weight loss. Such interventions would likely include weight training or other forms of exercise that have been shown to increase muscle strength despite concurrent weight loss (12, 33, 44, 48, 55).

From our results, we believe that isokinetic dynamometry and handgrip strength testing may provide more informative results about changes in muscle strength in response to diet-induced weight loss in comparison to 1RM testing and isometric dynamometry. Only 1 of 12 interventions from 11 publications using the 1RM technique showed significant changes in muscle strength, and that study showed contrasting results depending on the muscle group being tested, and moreover, muscle strength was not a primary outcome in that study (52). Contrastingly, 5 of the 11 publications that investigated 1RM had muscle strength as the primary outcome (12, 35, 39, 44, 55), and those studies did not find statistically significant changes in muscle strength. Reasons for the lack of significant results from 1RM testing may be due to an apparent lack of set speed of contraction or positioning of the subject when performing the tests, with both of these factors influencing the results (58). Studies that used isometric dynamometry to measure muscle strength showed inconsistent results. Therefore, 1RM and isometric dynamometry may not be sensitive enough to determine potential changes in muscle strength in response to diet-induced weight loss, in contrast to isokinetic dynamometry and handgrip strength testing. Different techniques vary in their ability to detect changes in strength in response to a 12-week strength-training program in men (59), with 1RM testing registering the largest increase, followed by isokinetic dynamometry and lastly, with the least difference from baseline detected, isometric dynamometry. Despite the apparent sensitivity of 1RM testing for detecting increases in muscle strength, isokinetic dynamometry has been considered as the reference method for muscle strength testing (25, 60-63), providing valid and reliable results compared to other forms of muscle strength assessment (64-66). However,

recent studies have revealed that handgrip strength, as measured with handheld dynamometers, provides a greater validity and reliability of results when compared with 1RM or isokinetic dynamometry (67, 68). Moreover, handgrip strength testing is a more feasible method of measuring muscle strength than isokinetic dynamometry, due to cost and logistics. Given these practical considerations, and given that handgrip strength can be representative of upper body strength (69) and lower extremity muscle power (70, 71), as well as being associated with nutritional status and a predictor of health outcomes (23, 72, 73), handgrip strength testing will likely be a useful and clinically relevant technique for assessing changes in muscle strength in response to diet-induced weight loss.

This systematic review has highlighted numerous limitations in studies conducted to date. 14 of the 33 interventions included in this review had very small sample sizes of less than 20 participants (12, 30-32, 34, 35, 38, 39, 44, 46-48), with a further 14 interventions having small sample sizes of only 20-30 participants (36, 37, 40, 42, 43, 45, 49, 50, 52, 53, 56). 4 interventions had moderate sample sizes of 30-41 participants (37, 41, 54, 55), and only 1 intervention (51) investigating muscle strength via 1RM testing had a large sample size (71 participants), albeit that study found no significant results. Additionally, muscle strength was a primary outcome in only 15 (12, 30, 31, 35, 37, 39-44, 50, 53-55) of the 27 publications included in this review. Of these 15 publications, 8 found significant changes in muscle strength in varying muscle groups (30, 31, 37, 39-42, 50); 9 muscle groups showed significant decreases in strength (30, 37, 39, 40, 42, 50, 74), 30 showed no significant change (12, 35, 37, 40, 43, 44, 53-55), and 3 muscle groups showed significant increases in strength (41, 42). Therefore, the lack of clarity of results in the reviewed work may be a consequence of small cohorts, as well as muscle strength not being a primary outcome in approximately half of the 27 publications. An additional limitation of the reviewed publications was that only 4 publications (36, 43, 47, 52) investigated muscle strength at a time point subsequent to the end of the dietary intervention, one of which was only 1 week post VLED (47), whilst the other

3 publications (36, 43, 52) investigated muscle strength 6 months after completion of the dietary intervention. Long-term follow up studies are required to determine whether any changes in muscle strength in response to severe or moderate energy restriction are temporary, as in two current trials by our team (74, 75), as well as the possible long-term implications for balance and performing activities of daily living (46).

In summary, isokinetic dynamometry of knee extensors revealed significant decreases in muscle strength in adults that were overweight or obese after interventions involving either MER or VLED, and handgrip strength testing showed overall trends for decreases in muscle strength, with significant reductions being seen with interventions involving MER but not VLED. Neither 1RM testing nor isometric dynamometry showed any clear effect of diet-induced weight loss on muscle strength, regardless of whether a VLED or MER was prescribed. While sample sizes were small, and approximately half of the reviewed publications were not powered to detect changes in muscle strength, these findings nonetheless indicate that diet-induced weight loss could have adverse effects on muscle strength. Given that muscle strength is an important predictor of mobility and mortality (14, 22, 23), and given that our population is getting heavier, with diet-induced weight loss being an important treatment option that helps to lessen numerous co-morbidities (2-4, 57, 76), further research is warranted to ensure that different types of diet-induced weight loss do not inadvertently lead to reduced muscle strength in a way that adversely impacts on long-term health.

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## **Chapter 4: General Discussion**

Due to the increasingly obese population and consequently an increase in the number of individuals undergoing dietary interventions for weight loss, two systematic reviews and meta-analyses were conducted in this thesis in order to elucidate the effects of diet-induced weight loss on bone and muscle strength, markers of disease and health status.

In summary, Chapter 2 illustrated that BMD decreased with diet-induced weight loss in overweight and obese individuals. Total Hip BMD significantly decreased from baseline in interventions of durations 6 to 24 months, as well as in an analysis of all interventions of varying durations. Whilst lumbar spine and total body BMD showed no significant changes from baseline with weight loss when all durations of dietary interventions were grouped, a significant decrease in total body BMD was found after dietary weight loss interventions of 6 months in duration. However, the lumbar spine and total body are not the gold standard sites when translating changes in BMD to risk of osteoporosis and fractures (1, 2). The reason for this, as outlined in Chapter 2, is because the lumbar spine is prone to greater measurement error with DXA (3). Moreover, the spine is susceptible to calcification that can lead to inaccurate BMD readings (4, 5). Thus, total hip BMD measurements are more reliable and accurate than the lumbar spine or total body BMD at estimating osteoporosis and fracture risk (6-8). Bone formation markers, serum P1NP and serum osteocalcin concentrations, showed no overall change in response to diet-induced weight loss, except for a significant increase in serum osteocalcin concentration with dietary interventions lasting 3 months. Bone markers of resorption, serum CTX as well as serum and

urinary NTX concentrations, showed overall tendencies to increase in response to diet-induced weight loss. Serum CTX, when reported in nmol/L, showed significant increases whilst, when reported in  $\mu\text{g/L}$ , a significant increase was found at 3 months, with no significant changes from baseline at 2, 6, 12 or 24 months. However, the more commonly reported unit for serum-CTX is  $\mu\text{g/L}$ , with reference ranges for serum CTX being reported in  $\mu\text{g/L}$  rather than mmol/L (9, 10), and thus, those results reported in  $\mu\text{g/L}$  are of greater interest. Future measurements of serum CTX should be reported in  $\mu\text{g/L}$  so that comparisons between interventions can be drawn. Serum and urinary concentrations of NTX, a marker of bone resorption, showed an overall trend to increase with diet-induced weight loss, with a significant increase only occurring with dietary interventions of 2 months for serum NTX. Taken together, these changes in bone turnover markers (increases in markers of bone formation and resorption) compliment the negative change seen in total hip BMD, suggesting an overall catabolic effect of diet-induced weight loss on bone. These results further support the need for bone turnover marker measurements, as they are able to detect changes occurring in bone in the short term; 2-3 months compared to BMD measurements, where significant changes were registered only after 6 months. Detecting changes in bone earlier rather than later is important, as with the screening for other diseases, because it allows for prompt treatment to be initiated as required.

Changes in muscle strength following a dietary weight loss intervention in overweight and obese populations were analyzed in Chapter 3 using one of 4 different techniques: 1RM, isokinetic dynamometry, isometric dynamometry and handgrip strength testing. Muscle strength as assessed by 1RM and isometric dynamometry were analysed qualitatively and showed mixed findings for both of prescribed dietary interventions.

Only one study, administering an intervention involving MER, found a significant change in muscle strength as measured by 1RM in an overweight and obese cohort. A significant decrease in upper body strength and a significant increase in lower body strength as assessed by 1RM was shown in that study. On the other hand, isometric dynamometry demonstrated inconsistent results with 4 significant increases in muscle strength being reported, 2 significant decreases, 2 tendencies to increase and 2 non significant changes in muscle strength being found across varying muscle groups, with no clear distinction between the effects of MER or VLED dietary interventions. Isokinetic knee extensor strength and handgrip strength changes were analysed quantitatively through meta-analyses. Irrespective of the degree of energy restriction administered (MER or VLED), isokinetic knee extensor strength showed significant decreases in response to diet-induced weight loss compared to baseline, whilst non-significant decreases in handgrip strength compared to baseline were found with all types of diets combined. Due to significant heterogeneity in results, we quantitatively analysed the effect of an intervention involving MER or VLED on isokinetic knee extensor strength and handgrip strength. MER interventions resulted in significant decreases on isokinetic knee extensor strength and handgrip strength, whilst VLED intervention only resulted in a reduction in isokinetic knee extensor strength and no change in handgrip strength. In summation, these results show that dietary interventions prescribed for weight loss in overweight and obese populations may incur negative changes in muscle strength. However, in order to accurately compare changes in muscle strength in response to dietary interventions, there is a need to identify a standard technique to assess muscle strength as well as a group of muscles that are comparable across overweight and obese populations.

When comparing the effects of the degree of energy restriction on the parameters under examination in both meta-analyses described in Chapters 2 and 3, that is, bone and muscle strength respectively, mixed results were found. A total of 6 parameters were analysed quantitatively through meta-analyses; total hip BMD, lumbar spine BMD, total body BMD, osteocalcin, isokinetic knee extensor strength and handgrip strength. The significant results that were found with both MER and VLED interventions favoured negative changes on bone and muscle strength following dietary weight loss intervention. Those studies administering MER interventions revealed significant decreases in total hip BMD, isokinetic knee extensor strength and handgrip strength. A significant increase was found in the bone formation marker osteocalcin, indicating a change in bone homeostasis, and no significant changes were found with lumbar spine BMD and total body BMD when overweight and obese individuals were prescribed a MER intervention. Similarly, VLED interventions induced significant decreases in lumbar spine BMD and isokinetic knee extensor strength, but also induced a significant increase in total body BMD and no significant changes in total hip BMD, osteocalcin or handgrip strength. Taken together, these findings highlight that diet-induced weight loss, regardless of the type of dietary intervention prescribed, may contribute to negative changes in bone and muscle strength in an overweight and obese population. Thus, due to no clear delineation between which degree of energy restriction, MER or VLED, affects bone and muscle strength more detrimentally, there appears to be no reason not to prescribe fast weight loss interventions such as VLED over MER in terms of the potential adverse effects on bone or muscle strength. However, more research is needed in order to gain a clearer understanding as to the effects of varying energy restriction on bone and muscle strength, and this is currently being undertaken in two clinical trials in our

team: the PREVIEW Study Australia (PREVention of diabetes through lifestyle Intervention and population studies in Europe and around the World) and the TEMPO Diet Trial (Type of Energy Manipulation for Promoting optimum metabolic health and body composition in Obesity)(11, 12).

#### **4.1 Relationship between bone and muscle strength**

Although bone and muscle strength were examined independently in Chapters 2 and 3, they do have a strong relationship with one another, with both organs being derived from a shared mesenchymal origin (13). Parallel changes occur in bone and muscle in response to exercise, disuse or aging (13). An example of the bone-muscle relationship is a study that prescribed resistance training to older men, which resulted in increases in both BMD and strength (14). Furthermore, in a clinical trial where the primary aim of the intervention was to increase BMD, muscle strength was found to concomitantly increase (15). Similarly, in another clinical trial that aimed to increase muscle strength as the primary outcome, BMD was also found to increase (16). In keeping with these findings, bone strength is proportional to muscle strength, due to its inherent mechanosensitive properties (17). Indeed, bone cells, in particular osteocytes, are specialized cells that are able to sense and subsequently respond to mechanical stimuli (18). The ‘mechanostat’ theory as proposed by Frost states that bone properties can be improved through high strain physical activity (19), and this would also increase muscle strength. These correlations are reflected in our findings, where diet-induced weight loss stimulated negative changes in BMD, bone turnover markers as well as decreases in muscle strength.

##### **4.1.1 Mechanisms contributing to bone loss**

Mechanisms as to why bone loss may occur with weight loss have been proposed, relating to the changes in circulating concentrations of cytokines, hormones as well as the mechanical unloading effect of weight loss on bone. One such proposed mechanism is that with weight loss there is loss of fat mass, which results in a decrease in circulating concentrations of oestrogen (20, 21). Normally, oestrogen plays a protective role in bone integrity, by decreasing osteoclast differentiation and increasing factors that enhance osteoblastic maturation (22). Decreased levels of oestrogen, as seen in post-menopausal women, is known to contribute to bone loss through increased rates of bone resorption, via alterations in osteoblastic-osteoclastic homeostasis (23, 24). Thus, decreases in oestrogen that occur with diet-induced weight loss (21) may contribute to the decline in BMD. Another potentially contributing factor to the reduction in BMD with weight loss is the suppression of IGF-1 (25). Although there is little evidence to clarify whether decreases in IGF-1 occurs with diet-induced weight loss in obese individuals, reductions in circulating IGF-1 with diet-induced weight loss have been shown in both rodents (26-28), and lean men and women (29). IGF-1 is normally responsible for the differentiation of osteoblasts (30), for the promotion of osteoblastic activity, that is the building of the bone matrix (22). For instance, with advancing age, growth hormone production by the pituitary gland is decreased, leading to a decrease in IGF-1 produced by the liver (22). This then has been shown to cause an imbalance in bone homeostasis with a decrease in the rate of bone formation and an increase in the rate of bone resorption (31, 32). Thus, despite a lack of substantial evidence of the effect of diet-induced weight loss in obese individuals on IGF-1, from the current information it could be suggested that a decrease in IGF-1 may occur. Therefore, diet-induced weight loss, like ageing (33), results in decreases in oestrogen (20, 21) and potential decreases in

circulating IGF-1 levels (34). As such, reductions in these hormones may contribute to the bone loss that occurs following dietary weight loss interventions.

As foreshadowed, another factor that may influence the reduction of bone during energy restriction is the reduction in mechanical loading as a consequence of a reduction in body weight (3). Mechanical loading is an integral part of skeletal development as well as playing a role in increasing the strength of bone (35). Osteocytes are mechanosensing bone cells, which, when stimulated by a mechanical force, modulate the growth and maintenance of bone (35). When a mechanical load is placed on bone, the secretion by osteocytes of sclerostin, a protein that inhibits bone formation, is suppressed. However, when mechanical unloading occurs, sclerostin is secreted (35). Studies that have examined the changes in sclerostin levels in response to diet-induced weight loss or in states of unloading have found that sclerostin levels increase when patients are prescribed a dietary intervention without specific addition of, or supervision, of physical activity (35-37). Interestingly, a weight loss diet, when combined with both aerobic and resistance exercise, prevents the increase in circulating sclerostin levels otherwise associated with dietary restriction, suggesting a potential protective mechanism induced by exercise (36). Mechanical unloading differentially affects the two types of bone tissue (trabecular and cortical, as described in Chapter 1). That is, trabecular bone, due to its high turnover and metabolic activity (38, 39) appears to have a heightened sensitivity to mechanical unloading (40). This is relevant to this thesis, as the hip and spine bones both contain a high proportion of trabecular bone (2), which may explain why significant decreases in BMD were evident in the total hip and not total body BMD, as described in Chapter 2. Taken together, mechanical unloading and the resultant changes in sclerostin secretion seems to be a potential mechanism for the bone loss that occurs with diet-induced weight

loss, however more evidence is needed in order to determine a cause and effect relationship.

#### **4.1.2 Mechanisms contributing to muscle strength loss**

Like bone, muscle is affected by numerous neuroendocrine changes and physiological adaptations that occur with diet-induced weight loss. Muscle mass is associated with fat free mass, where a reduction in one is associated with a reduction in the other (41). For clarification, muscle mass is a subset of fat free mass that incorporates bone, extracellular fluids as well as non-bone fat free mass, including muscle, organs and connective tissue (42). This association has been shown in states of energy deficit, where both decreases in fat free mass and catabolism of skeletal muscle has been concurrently observed (43, 44). As a consequence of a loss of muscle mass, muscle strength also is reduced (45). Changes in the body's neuroendocrine system during states of negative energy balance have been proposed to contribute to the loss of fat free mass (26). One particular neuroendocrine pathway that is affected by energy restriction is the hypothalamic-pituitary-gonadal axis, responsible for the body's secretion of sex hormones such as oestrogen. A recent review has illustrated the beneficial influence of oestrogen on muscle strength (46). In post-menopausal women as well as in ovariectomized rodents, muscle strength was observed to be less compared to their oestrogen positive counterparts (46). Furthermore, a meta-analysis has found that muscle strength was greater in post-menopausal women on hormone replacement therapy than in those without treatment (47). However, the mechanism for the detrimental effect of oestrogen on muscle strength is unknown, with one study highlighting various potential factors including a decrease in motor units and an increase in oxidative stress markers that may lead to this observed outcome (48). As

mentioned above, circulating oestrogen concentrations have been shown to decrease in states of negative energy balance such as in diet-induced weight loss (21, 26), and therefore may contribute to the decreases in muscle strength revealed in Chapter 3. Additionally, as described earlier, during states of energy deficit, reductions in circulating IGF-1 concentrations, due to reduced activity of the somatotrophic axis, have been noted, and this may confer reductions in muscle strength (26, 49). IGF-1 has anabolic activity on muscle (50). It plays a central role in muscle protein synthesis, muscle cell activity and the proliferation of muscle progenitor cells, all of which influence muscle strength (22). In support of this, studies have found that in growth hormone deficient patients, and therefore IGF-1 deficient patients, muscle strength was reduced (51-53). Despite little evidence available surrounding changes in IGF-1 in obese individuals in response to diet-induced weight loss as mentioned previously, present data suggests that low levels of circulating IGF-1, or greater circulating concentrations of IGF-1 binding proteins – which bind IGF-1 and thereby reduce the amount of bioactive IGF-1 available (29, 54, 55) – occurs with weight loss (21). A decrease in bioavailability of IGF-1 results in reductions in the anabolic activity of muscle as well as reductions in the proliferation of myoblasts, all of which probably decrease muscle strength (56). Thus, hormonal changes in oestrogen and IGF-1 exerted on muscle appear to negatively affect bone integrity and can also negatively affect muscle function.

Another point of consideration, as explored with bone, is the effect of mechanical unloading on muscle. Muscle is a highly plastic tissue that is able to alter its structure according to changing functional requirements (50). Normally, physical activity or increased mechanical load results in anabolic effects on muscle by inducing muscle

hypertrophy or an increase in muscle mass (13, 50). Therefore, the contrary occurs, that is, reducing the normal weight bearing load or decreasing the amount of physical activity has been shown to induce muscle atrophy and cause a reduction in muscle mass (40, 50, 57, 58). Studies conducted in rodents undergoing mechanical unloading through hind-limb suspension (40, 57), as well as in studies on humans in simulated space flight or microgravity (59, 60), showed that these interventions induced weight loss with associated reductions in muscle mass and muscle strength. A proposed explanation for the decrease in muscle strength was a reduction in protein synthesis due to the decrease in mechanical force (40). The aim of diet-induced weight loss is a reduction of body weight. Decreasing the normal weight load of the body as mentioned above decreases muscle mass and strength. These negative shifts in muscle may be explained by the reduced protein synthesis as seen in rodents and humans during mechanical unloading (40, 57, 59, 60). Therefore, a reduction in normal weight bearing forces exerted on the body may be a contributing factor to the reduction in muscle strength with diet-induced weight loss.

Bone and muscle are two organ systems that are intertwined and heavily influence the other, with changes occurring in muscle due to unloading forces preceding bone changes (13, 40). The interrelated properties of bone and muscle are highlighted by the overlap in mechanisms that cause reductions in BMD and strength, respectively. The relationship between bone and muscle is also seen in a clinical context. Sarcopenia has been thought to contribute to reductions in BMD, leading to the pre-disease state of osteopenia (40). Moreover, it has been found that individuals with sarcopenia are more likely to also have the bone disease of osteoporosis (61). These implications have important clinical relevance to our overweight and obese

population undergoing diet-induced weight loss interventions. Low muscle strength is correlated with an increased risk of fractures (62) and therefore, if not only muscle strength but also BMD are reduced in individuals during dietary intervention, this could contribute to an increase in the incidence of fractures and osteoporosis. Indeed, studies have found that weight loss, whether intentional or unintentional, is associated with significant increases in the risk of a hip fracture (63, 64). In the meta-analysis looking at the effect of weight loss on bone in Chapter 2, the decrease in BMD corresponded to an estimated 10-15% increase in fracture risk, with the maximum duration of dietary intervention administered equaling 24 months. Therefore, the findings from other studies as well as from the current meta-analyses suggest that there may be a need for dietary weight loss interventions to include muscle-strengthening exercises, due to the known anabolic effects of exercise on bone and muscle (13).

#### **4.2 Effect of exercise on bone and muscle strength**

The particular exercise regime of exercise that produces the greatest yields for both bone and muscle strength has yet to be determined, however resistance training has shown to be preferable for both bone and muscle strength. Muscle strengthening exercises have been shown to promote the development and maintenance of healthy bone matter (65). Meta-analyses examining the effect of different exercise protocols on BMD have been conducted in post-menopausal women (66, 67) and in pre-menopausal women (68). All meta-analyses (66-68) conclusively reported that resistance training lasting 12 months has significant impacts on preserving BMD compared to other forms of exercise, including jogging mixed with walking or stair climbing or agility exercises. Moreover, studies have found that resistance training

without the addition of aerobic exercise results in a more significant increase in muscle strength than when a combination of resistance and aerobic training are used (69-71). Muscle adapts differently according to the type of physical activity being undertaken. Resistance training induces an increase in muscle strength and muscle hypertrophy (72, 73), whilst aerobic or endurance regimes result in an increase in the muscle content of mitochondria, thereby increasing its maximal oxygen uptake ability (71, 74). A justification for why resistance training alone exerts greater benefits on muscle strength compared with protocols that encompass the same level of resistance training, but with the addition of aerobic training, is that aerobic exercise is thought to interfere or antagonize further increases in strength, due to changes in neural mechanisms or attenuation of muscle hypertrophy (75-77). However, a meta-analysis would be required to investigate the true overall effect of varying exercise modalities on both bone and muscle.

### **4.3 Effect of weight regain on bone**

Overweight and obese individuals often will attempt to lose weight multiple times over the course of many years, with these periods of dietary restriction usually followed by weight regain (78, 79). During diet-induced weight loss, 6-month interventions resulted in a loss of BMD from the hip or lumbar spine (80, 81). When weight was measured at 12- and 18-month time points, weight regain had occurred but BMD regains had not (80, 81). Therefore, weight cycling – weight loss followed by regain, occurring 1 or more times – places a detrimental risk on bone, with this being an additive risk in those people who are inactive, or in post-menopausal women (78, 79). A large population-based study of 20,745 females and males over 15 years of age (82) demonstrated that females who lost 11 kg or more, or who recalled having

dieted over 11 times during their life, had an adjusted hazard ratio of 1.48 (95% confidence interval: 1.13-1.94) and 1.73 (1.11-2.68) for osteoporotic fractures, respectively (82). These findings suggest that the loss in BMD that occurs with weight loss both in a single diet-induced weight loss attempt, or several weight loss attempts, has a negative impact on the integrity of bone and thus measures need to be taken to ensure that the benefits of diet-induced weight loss outweigh any potential negative side effects.

#### **4.4 Future directions**

From the two studies presented, future research into the effect of diet-induced weight loss on the body should implement exercise regimes in order to find the optimal intervention for not only reducing obesity, but also preventing other areas of the body from deteriorating as a by-product. Given the numerous other benefits of physical activity (83), clinicians should recommend exercise regimes in conjunction with dietary interventions to curb the potential of a concomitant decrease in bone and muscle strength. The guidelines for preventing osteoporosis from the Australian Medical Association already suggest an exercise regime should be implemented in general (84). Stronger suggestions to include exercise in a weight loss regime should be made, especially to any aged cohorts due to the early onset of muscle deterioration from the age of 40 onwards (85), and because bone begins to decline (86, 87) by the age of 30, as part of the natural aging process.

Another direction for future research is to determine the potential impact that this loss of bone and muscle strength with diet-induced weight loss could have on long term

health, and how this compares to the long term known metabolic benefits of weight loss. The studies in both Chapter 2 and 3 highlighted the negative changes that occur with diet-induced weight loss on bone and muscle strength respectively, in the short term. Future investigations are paramount in order to crystalize gaps in the literature surrounding whether weight loss causes permanent negative long-term effects on bone and muscle strength, or whether these changes dissipate with time. As alluded to in Chapter 2, only a handful of studies have measured BMD at time point beyond the completion of the dietary intervention. In those studies with a follow up time point, decreases in BMD of the total hip (88-90), lumbar spine (88, 91, 92) and total body (88, 92-95) were still evident 3 to 21 months after the conclusion of the diet-induced weight loss intervention. Fewer studies examining the affect of diet-induced weight loss on muscle strength had a follow up time point (90, 96-98), and in those studies that did investigate muscle strength at follow up, inconsistent results were found. Despite these potentially harmful adverse affects that may eventuate with diet-induced weight loss, a meta-analysis has found that in the long term, weight loss has a positive impact on all-cause mortality relating to improvements in peak  $\text{VO}_2$ , blood pressure, glucose and circulating concentrations of interleukin-6 (99), which is a pro-inflammatory marker (100). Exactly when the benefits of weight loss for all-cause mortality manifest themselves is yet to be determined, with some studies noting benefits 4-5 years post intervention (101, 102), whilst others noticed improvements throughout the course of the post-intervention follow up period (103). As such, not only should future research be aimed at identifying what interventions are optimal for both promoting weight loss without excessive loss of bone or muscle strength, but long term studies are essential so that the benefits versus risks of weight loss can be clarified.

**4.5 Conclusion**

In conclusion, from both meta-analyses presented in this thesis, the importance of combating the health problem of obesity through dietary interventions, including measures to prevent reductions in bone and muscle strength, has been highlighted. In brief, dietary interventions alone without exercise may potentially cause deleterious effects on the body. Thus, a holistic approach is needed when attempting to attenuate negative changes that occur in bone and muscle during diet-induced weight loss.

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## **Supporting Information**

### **Supporting Methods**

The following example shows the specific key words (or MeSH terms) that were used for the search of MEDLINE for population, intervention and outcomes.

#### *Population*

1. exp overweight/ OR obesity/ OR obesity, abdominal/ OR obesity, morbid/ OR overweight.tw OR over weight.tw OR obes\*.tw OR ((abdominal or subcutaneous or intra-abdominal or visceral or retriperitoneal or retro peritoneal) adj3 fat\*).tw

#### *Intervention*

2. diet/ OR diet\*.tw OR diet, carbohydrate-restricted/ OR diet, fat-restricted/ OR diet, reducing/ OR diet, protein-restricted/ OR caloric restriction/ OR calor\* restrict\*.tw OR diet therapy/ OR weight loss/ OR weight los\*.tw OR (weight adj5 (los\* or reduc\* or control\* or decreas\*)).tw OR ((low\* or reduc\* or restric\*) adj3 (calori\* or energy or protein or carb\* or fat\*)).tw

#### *Outcomes*

Bone mineral density/homeostasis

3. (“Bone and Bones”/ AND homeostasis/) OR bone homeostasis.tw OR bone mass.tw OR bone turnover marker\*.tw OR bone density/ OR (bone mineral adj3 density.tw OR BMD.tw OR (bone\* adj2 (los\* or degrade\* or deminerali\*)).tw OR (bone density adj2 (reduc\* or decreas\*)).tw OR bone resorption/ OR bone resorption.tw OR osteolysis/ or osteolysis.tw OR bone remodeling/ OR bone remodel?ing OR bone strength.tw

*Supporting information*

Bone markers

4. (Procollagen type 1 adj3 propeptide).mp OR (Procollagen type I adj3 propeptide).mp OR P1NP.mp OR osteocalcin/ OR osteocalcin.mp OR (C-telopeptide adj3 type-1 collagen).mp OR (C-telopeptide adj3 type-I collagen).mp OR CTX.mp OR CTX1.mp OR CTXI.mp OR sCTX.mp OR OR (N-telopeptide adj3 type 1 collagen).mp OR (N-telopeptide adj3 type I collagen).mp OR NTX.mp or sNTX.mp.

Our final input command was population AND intervention AND outcome (bone mineral density/homeostasis OR bone markers). The example search strategy for MEDLINE (above) was adapted to suit each database.

Supporting information

**Supporting Table 1. Study characteristics for interventions investigating bone mineral density (BMD) and serum or urinary concentrations of bone turnover markers.**

First author, year, reference	Sample size	Sex and menopausal status of participants (percent female)	Age in years – mean $\pm$ SE and / or range	Baseline BMI in $\text{kg/m}^2$ – mean $\pm$ SE or range	Duration of dietary weight loss intervention (additional time points reported and used in this review)	Details of dietary weight loss intervention	Baseline weight (and weight change from baseline at end of intervention) in kg – mean $\pm$ SE	BMD outcome measurement sites relevant to this review (baseline mean $\pm$ SE in $\text{g/cm}^2$ )	Bone turnover outcome measurements relevant to this review, all measured in serum unless specified as urinary (baseline mean $\pm$ SE)
Andersen (1997) <sup>(41)</sup>	9	Women	38.1 $\pm$ 2.5	33.6 $\pm$ 0.6	6 months	LED for the first 20 weeks, then MER for the final 4 weeks	91.3 $\pm$ 3.0 (-19.4 $\pm$ 4.5)	Lumbar spine (L2 – L4) (1.29 $\pm$ 0.07) Total body (1.22 $\pm$ 0.03)	
Avenell (1994) <sup>(25)</sup>	16	Women – post-menopausal	60.1 $\pm$ 1.3	31.4 $\pm$ 0.8	6 months	MER with a high fibre, low fat diet	81.1 $\pm$ 2.3 (-2.8 $\pm$ 2.4)	Lumbar spine (L2 – L4) (0.97 $\pm$ 0.05)	
Bosy-Westphal (2011) <sup>(20)</sup>	72	Men and women – pre-menopausal (76%)	36.2 $\pm$ 0.7	34.6 $\pm$ 0.5	3 months	LED	103.5 $\pm$ 2.0 (- 9.2 $\pm$ 0.5)	Lumbar spine (region not specified) (1.07 $\pm$ 0.02) Total body (1.00 $\pm$ 0.01)	
Bowen (2004) <sup>(42)</sup>	25	Men and women (40%)	45.7 $\pm$ 2.8	34.6 $\pm$ 4.2	3 months	MER with a high dairy protein diet (K)	98.5 $\pm$ 3.1 (-9.0 $\pm$ 4.4)	Total body (1.09 $\pm$ 0.02)	
	25	Men and women (60%)	47.4 $\pm$ 3.4	32.2 $\pm$ 0.8	3 months	MER with a high mixed protein diet (L)	90.7 $\pm$ 2.8 (-9.3 $\pm$ 3.9)	Total body (1.09 $\pm$ 0.03)	
Campbell (2010) <sup>(26)</sup>	15	Women – post-menopausal	60.0 $\pm$ 3.0	30.0 $\pm$ 0.9	3 months	MER with a normal protein diet (M)	80.0 $\pm$ 2.9 (-9.0 $\pm$ 3.9)	Total body (1.17 $\pm$ 0.03)	
	15	Women – post-menopausal	51.0 $\pm$ 2.0	30.8 $\pm$ 1.1	3 months	MER with a high protein diet (N)	82.5 $\pm$ 4.2 (-8.2 $\pm$ 5.8)	Total body (1.18 $\pm$ 0.03)	
	14	Women – post-menopausal	59.0 $\pm$ 2.0	28.4 $\pm$ 0.9	9 weeks – included in 3 month category	MER with a high carbohydrate diet (O)	75.9 $\pm$ 2.4 (-5.6 $\pm$ 3.4)	Total body (1.11 $\pm$ 0.03)	Osteocalcin (1.3 $\pm$ 1.2 nmol/L) Urinary NTX (33.0 $\pm$ 4.2 nmol/L BCE / nmol/L creatine)

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	15	Women – post-menopausal	60.0 ± 2.0	29.1 ± 1.1	9 weeks – included in 3 month category	MER with a high chicken protein diet (P)	76.2 ± 2.8 (-8.9 ± 3.8)	Total body (1.14 ± 0.02)	Osteocalcin (1.1 ± 0.7 nmol/L) Urinary NTX (45.1 ± 4.2 nmol/L BCE / nmol/L creatine)
	14	Women – post-menopausal	58.0 ± 2.0	30.1 ± 0.8	9 weeks – included in 3 month category	MER with a high beef protein diet (Q)	81.0 ± 2.5 (-6.6 ± 3.6)	Total body (1.14 ± 0.03)	Osteocalcin (1.3 ± 1.5 nmol/L) Urinary NTX (36.5 ± 4.3 nmol/L BCE / nmol/L creatine)
Chao (2000) <sup>(40)</sup>	27	Women – post-menopausal	66.3 ± 1.1 60 – 80	≥ 27.3	12 months (6 months)	MER	81.6 ± 2.0 (-7.7 ± 2.0)	Lumbar spine (L2 – L4) (1.02 ± 0.03) Total body (1.10 ± 0.02)	Osteocalcin (3.9 ± 0.3 nmol/L) Urinary NTX (32.6 ± 5.6 nmol/L BCE / nmol/L creatine)
Cifuentes (2004) <sup>(80)</sup>	16	Women – post-menopausal	62.3 ± 1.3	27.3 ± 0.4	6 weeks – included in 2 month category	MER	74.5 ± 1.8 (-3.3 ± 2.5)		Osteocalcin (3.0 ± 0.2 nmol/L) NTX (10.8 ± 0.8 nmol/L BCE )
Clifton (2008) <sup>(43)</sup>	51	Women	20 – 65	27.0 – 40.0	12 months	MER with a high protein diet (R)	87.5 ± 1.7 (-5.7 ± 2.4)	Total body (1.02 ± 0.02)	
	48	Women	20 – 65	27.0 – 40.0	12 months	MER with high carbohydrate diet (S)	86.2 ± 1.8 (-5.7 ± 2.6)	Total body (1.02 ± 0.02)	
Compston (1992) <sup>(27)</sup>	13	Women	47.2 ± 1.6	32.2	3 months	VLED	93.6 ± 3.2 (-15.6 ± 4.2)	Total body (1.21 ± 0.02)	
Fogelholm (2001) <sup>(28)</sup>	74	Women – pre-menopausal	40.0 ± 0.5	34.0 ± 0.3	3 months	VLED (I)	92.0 ± 1.1 (-13.2 ± 0.4)	Lumbar spine (L2 – L4) (1.13 ± 0.01) Total body (1.12 ± 0.01)	
	28	Women – pre-menopausal	39.7 ± 0.6	≥ 30	3 months	MER (J)	93.2 ± 1.6 (-13.5 ± 2.4)	Lumbar spine (L2 – L4) (1.14 ± 0.02) Total body (1.12 ± 0.01)	

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Foster (2010) (29)	154	Men and women (68%)	44.9 ± 0.8	36.1 ± 0.3	24 months (6 months, 12 months)	MER with low fat diet (E)	103.5 ± 1.2 (-7.4 ± 0.9)	Total hip (1.10 ± 0.01) Lumbar spine (L1 – L4) (1.10 ± 0.01)	
	153	Men and women (67%)	46.2 ± 0.7	36.1 ± 0.3	24 months (6 months, 12 months)	MER with a low carbohydrate diet (F)	103.3 ± 1.3 (-6.3 ± 0.9)	Total hip (1.10 ± 0.01) Lumbar spine (L1 – L4) (1.10 ± 0.01)	
Gossain (1999) (30)	11	Women			6 months	VLED	103.8 ± 4.8 (-20.6 ± 6.0)	Total hip (0.87 ± 0.02) Lumbar spine (L1 – L4) (1.02 ± 0.04) Total body (1.12 ± 0.02)	
Hamilton (2013) (23)	115	Women – pre- menopausal	34.4 ± 0.6	28.2 ± 0.1	6 months	VLED	77.1 ± 0.6 (-12.1 ± 0.9)	Total hip (1.08 ± 0.02)	
Hendel (1996) (44)	31	Men and women (81%)	42.0 ± 1.8	37.1 ± 0.2	12 months	MER	105.5 ± 2.8 (-10.6 ± 1.2)	Total body (1.11 ± 0.02)	
Hinton (2009) (71)	37	Men and women (65%)	50.7 ± 1.6	39.3 ± 0.9	3 months	VLED	111.6 ± 2.9 (-21.5 ± 3.8)		Osteocalcin (0.9 ± 0.4 nmol/L) CTX (0.72 ± 0.02 µg/L)
Hinton (2010) (21)	113	Men and women (57%)	40.8 ± 0.9 19 – 50	34.3 ± 0.4	12 months	MER	101.9 ± 1.2 (-12.4 ± 0.2)	Total body (1.30 ± 0.00)	
Hosny (2012) (31)	20	Women – pre- menopausal	35.2 ± 0.6	32.9 ± 0.3	3 months	LED	77.1 ± 1.2 (-8.2 ± 1.5)	Total hip (0.96 ± 0.02) Lumbar spine (L2 – L4) (1.15 ± 0.02)	
Hylstrup (1993) (75) (Data analysed qualitatively)	24 (44)	Men and women (84%)	20 – 53	38.8 ± 1.4	8 months (2 months)	VLED	(-29.7 ± 1.2)		Osteocalcin
Jensen (2001) (105)	27	Women	19 – 70	34.0 ± 1.0	3 months	LED	93.8 ± 2.7 (-6.2 ± 3.9)		Osteocalcin (1.3 ± 0.3 nmol/L)
Jesudason	164	Women – post-	40 – 70	34.0 ± 0.4	24 months	MER with a high	88.6 ± 1.1 (-9.0 ± -	Total hip (0.99	Osteocalcin (0.6 ± 0.2

Supporting information

(2013) <sup>(32)</sup>		menopausal			(12 months)	protein diet (G)	2.0	± 0.01) Lumbar spine (L2 – L4) (1.17 ± 0.01)	nmol/L) CTX (0.39 ± 0.02 µg/L)
	159	Women – post- menopausal	40 – 70	33.4 ± 0.4	24 months (12 months)	MER with a normal protein diet (H)	88.6 ± 1.1 (-10.3 ± 2.1)	Total hip (1.00 ± 0.01) Lumbar spine (L2 – L4) (1.19 ± 0.02)	Osteocalcin (0.6 ± 0.2 nmol/L) CTX (0.39 ± 0.01 µg/L)
Lucey (2008) <sup>(72)</sup>	66	Men and women (64%)	66.0 ± 0.6	30.0 ± 0.2	2 months	MER	86.7 ± 1.0 (-4.4 ± 0.3)		Osteocalcin (1.5 ± 0.2 nmol/L) CTX (0.46 ± 0.03 µg/L) Urinary NTX (41.9 ± 3.5 nmol/L BCE / nmol/L creatine)
Nakata (2008) <sup>(33)</sup>	21	Women – pre- menopausal	40.3 ± 1.4	27.4 ± 0.6	3.5 months – included in 3 month category	MER	67.2 ± 2.1 (-6.2 ± 1.1)	Lumbar spine (L2 – L4) (1.60 ± 0.02) Total body (1.20 ± 0.01)	Osteocalcin (1.8 ± 0.2 nmol/L) Urinary NTX (35.2 ± 3.0 nmol/L BCE / nmol/L creatine)
Noakes (2005) <sup>(68)</sup>	52	Women	50 ± 1.4	32.0 ± 0.8	3 months	MER with a high protein diet (T)	87.0 ± 1.6 (-7.6 ± 0.1)		Osteocalcin (1.2 ± 0.1 nmol/L)
	48	Women	49.0 ± 1.3	33.0 ± 0.6	3 months	MER with a high carbohydrate diet (U)	86.0 ± 1.7 (-6.9 ± 0.1)		Osteocalcin (0.9 ± 0.1 nmol/L)
Ramsdale (1994) <sup>(34)</sup>	45	Women – pre- menopausal	33.7 ± 1.0	25.6 ± 0.3	6 months	MER	67.3 ± 1.0 (-3.3 ± 1.4)	Lumbar spine (L1 – L4) (1.22 ± 0.02) Total body (1.19 ± 0.02)	
Rector (2009) <sup>(81)</sup>	11	Women – pre- menopausal	18 – 35	27.9 ± 0.8	6 weeks – included in 2 months category	LED	76.6 ± 2.7 (-3.9 ± 7.9)		Osteocalcin (1.9 ± 1.6 nmol/L) CTX (0.73 ± 0.09 µg/L)

Supporting information

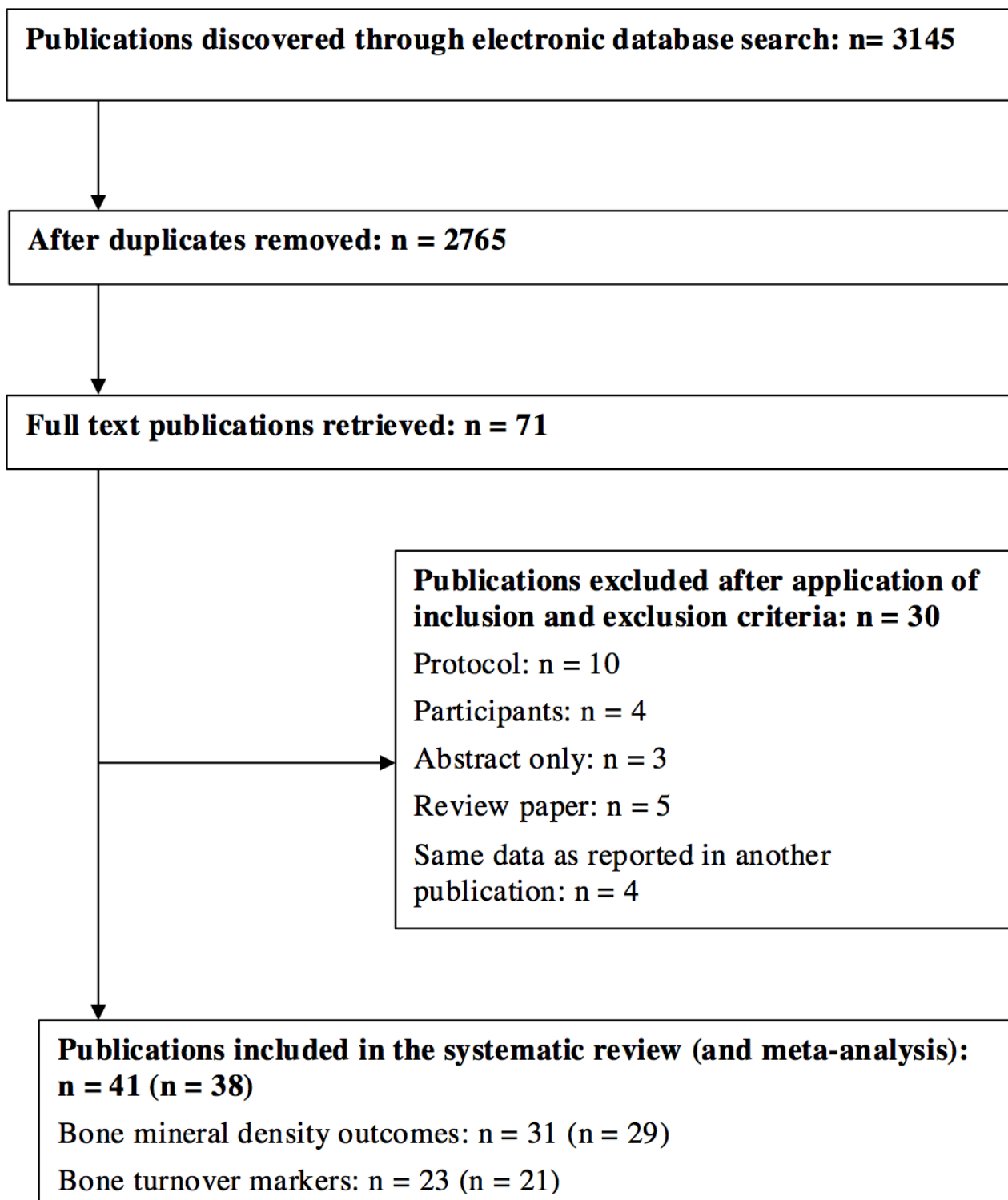
Redman (2008) <sup>(22)</sup>	12	Men and women (50%)	39.0 ± 2.0	27.3 ± 0.5	6 months (3 months)	MER (A)	81.2 ± 3.3 (-8.4 ± 4.5)	Total hip (1.07 ± 0.06) Total body (1.15 ± 0.03)	Osteocalcin (2.5 ± 1.0 nmol/L) CTX (0.60 ± 0.40 µg/L) NTX (24.7 ± 1.6 nmol/L BCE)
	11	Men and women (64%)	39.0 ± 2.0	27.8 ± 0.5	6 months (3 months)	LED (B)	81.1 ± 3.2 (-11.2 ± 4.4)	Total hip (0.98 ± 0.05) Total body (1.12 ± 0.02)	Osteocalcin (2.4 ± 1.1 nmol/L) CTX (0.50 ± 0.10 µg/L) NTX (23.7 ± 1.7 nmol/L BCE)
Ricci (1998) <sup>(35)</sup>	16	Women – post-menopausal	55.0 ± 2.1	32.9 ± 1.1	6 months	MER	88.2 ± 3.5 (0.3 ± 5.1)	Total body (1.15 ± 0.02)	Osteocalcin (0.7 ± 0.1 nmol/L)
Ricci (2001) <sup>(45)</sup>	14	Women – post-menopausal	55.9 ± 1.5	32.7 ± 1.0	6 months	MER	87.3 ± 2.6 (-8.2 ± 3.8)	Total body (1.16 ± 0.02)	Osteocalcin (0.7 ± 0.1 nmol/L)
Riedt (2005) <sup>(36)</sup>	24	Women – post-menopausal	61.6 ± 1.3	27.2 ± 0.4	6 months	MER	73.8 ± 1.4 (-7.3 ± 2.0)	Total body (1.12 ± 0.01)	Osteocalcin (2.5 ± 0.2 nmol/L) NTX (12.9 ± 1.0 nmol/L BCE)
Riedt (2007) <sup>(46)</sup>	17	Women – pre-menopausal	24 – 49	27.7 ± 0.5	6 months	MER	71.4 ± 1.6 (-4.7 ± 2.2)	Total hip (1.03 ± 0.02) Lumbar spine (L2 – L4) (1.20 ± 0.03) Total body (1.17 ± 0.02)	Osteocalcin (1.3 ± 0.00 nmol/L) NTX (14.6 ± 1.0 nmol/L BCE)
Shah (2011) <sup>(73)</sup>	26	Men and women (65%)	70.0 ± 0.8	37.2 ± 0.9	6 months	MER	104.0 ± 2.9 (-9.0 ± 1.1)		PINP (41.5 ± 2.4 µg/L) Osteocalcin (2.0 ± 0.7 nmol/L) CTX (0.31 ± 0.02 µg/L)
Shapses (2001) <sup>(47)</sup>	14	Women – pre-menopausal	42.1 ± 0.8	34.5 ± 1.0	6 months	MER	93.8 ± 3.5 (-8.1 ± 4.6)	Lumbar spine (L2 – L4) (1.27 ± 0.02) Total body (1.21 ± 0.01)	Osteocalcin (0.9 ± 0.1 nmol/L)

Supporting information

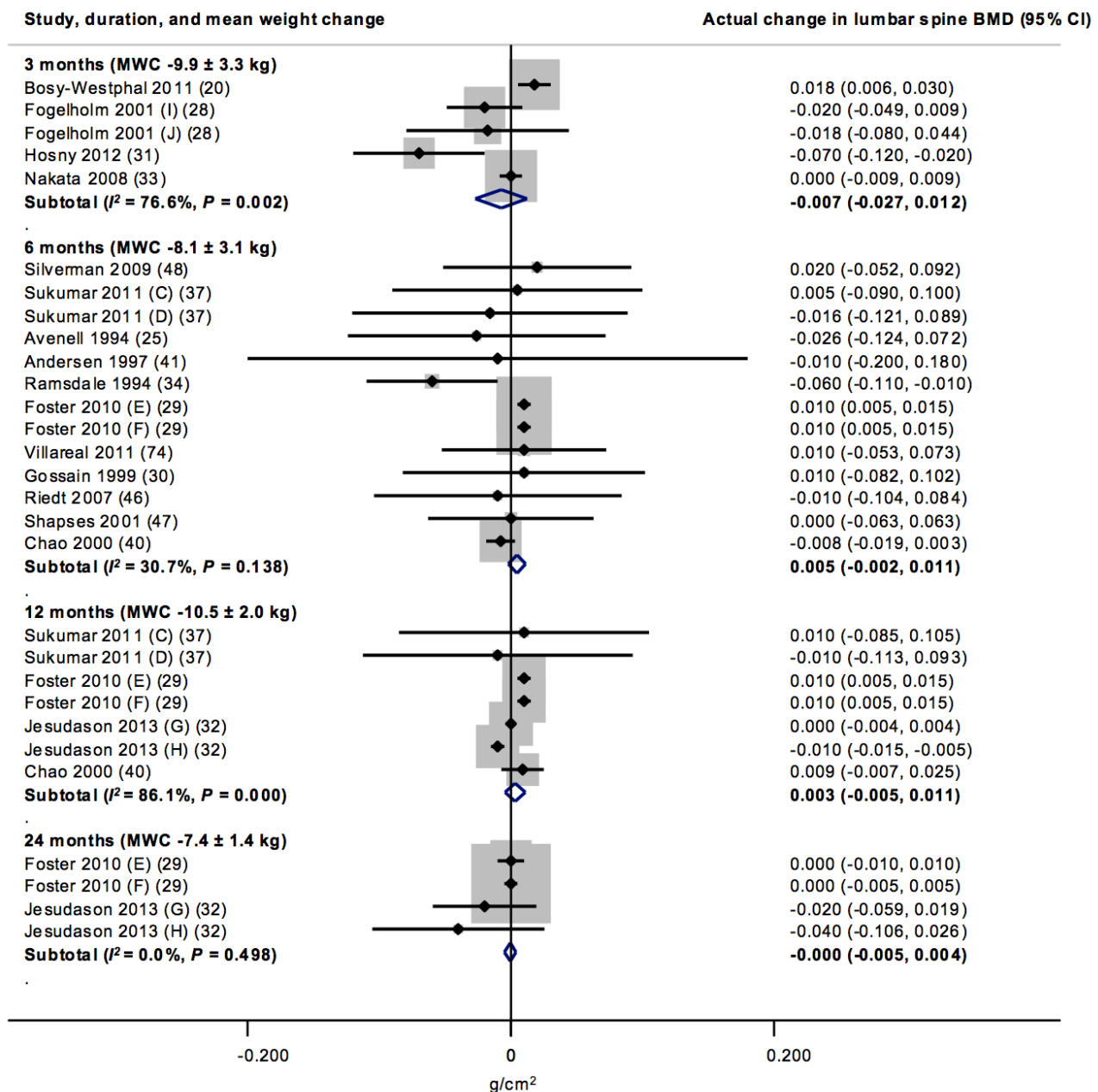
Silverman (2009) <sup>(48)</sup>	40	Women – post-menopausal	58.0 ± 0.8	32.4 ± 0.7	6 months	MER	87.4 ± 2.3 (-6.4 ± 3.3)	Lumbar spine (L2 – L4) (1.12 ± 0.03)	
Sukumar (2011) <sup>(37)</sup>	26	Women – post-menopausal	58.5 ± 0.8	32.1 ± 0.7	12 months (6 months)	MER with a high protein diet (C)	88.5 ± 3.0 (-5.7 ± 4.2)	Total hip (1.02 ± 0.02) Lumbar spine (L2 – L4) (1.24 ± 0.03) Total body (1.20 ± 0.02)	P1NP (48.2 ± 3.2 µg/L) NTX (12.5 ± 0.7 nmol/L BCE)
	21	Women – post-menopausal	57.4 ± 1.0	32.1 ± 0.7	12 months (6 months)	MER with a normal protein diet (D)	82.7 ± 2.7 (-6.1 ± 3.7)	Total hip (0.94 ± 0.02) Lumbar spine (L2 – L4) (1.13 ± 0.04) Total body (1.14 ± 0.02)	P1NP (56.6 ± 3.4 µg/L) NTX (13 ± 1.4 nmol/L BCE)
Svensden (1993) <sup>(49)</sup> (Data analysed qualitatively)	51	Women	45 – 54	≥ 25.0	3 months	MER	(-9.5 ± 0.4)	Lumbar Spine Total body	Osteocalcin
Thorpe (2008) <sup>(24)</sup> (Data analysed qualitatively)	64	Men and women (56%)	45.5	30.9	12 months	MER with a high protein diet	Data not available	Total hip Lumbar spine (L1 – L4) Total body	
		Men and women (53%)	47.0	31.9	12 months	MER with a high carbohydrate diet	Data not available	Total hip Lumbar spine (L1- L4) Total body	
Uusi-Rasi (2009) <sup>(70)</sup>	26	Women – pre-menopausal	43.1 ± 1.2	35.9 ± 2.6	3 months	MER (V)	95.6 ± 4.9 (-2.0 ± 5.1)		P1NP (33.3 ± 4.5 µg/L) CTX (11.0 ± 2.4 nmol/L)
	11	Women – pre-menopausal	40.6 ± 2.4	34.0 ± 1.2	3 months	VLED (W)	98.1 ± 3.7 (-9.5 ± 6.6)		P1NP (36.6 ± 3.2 µg/L) CTX (10.2 ± 1.5 nmol/L)

Supporting information

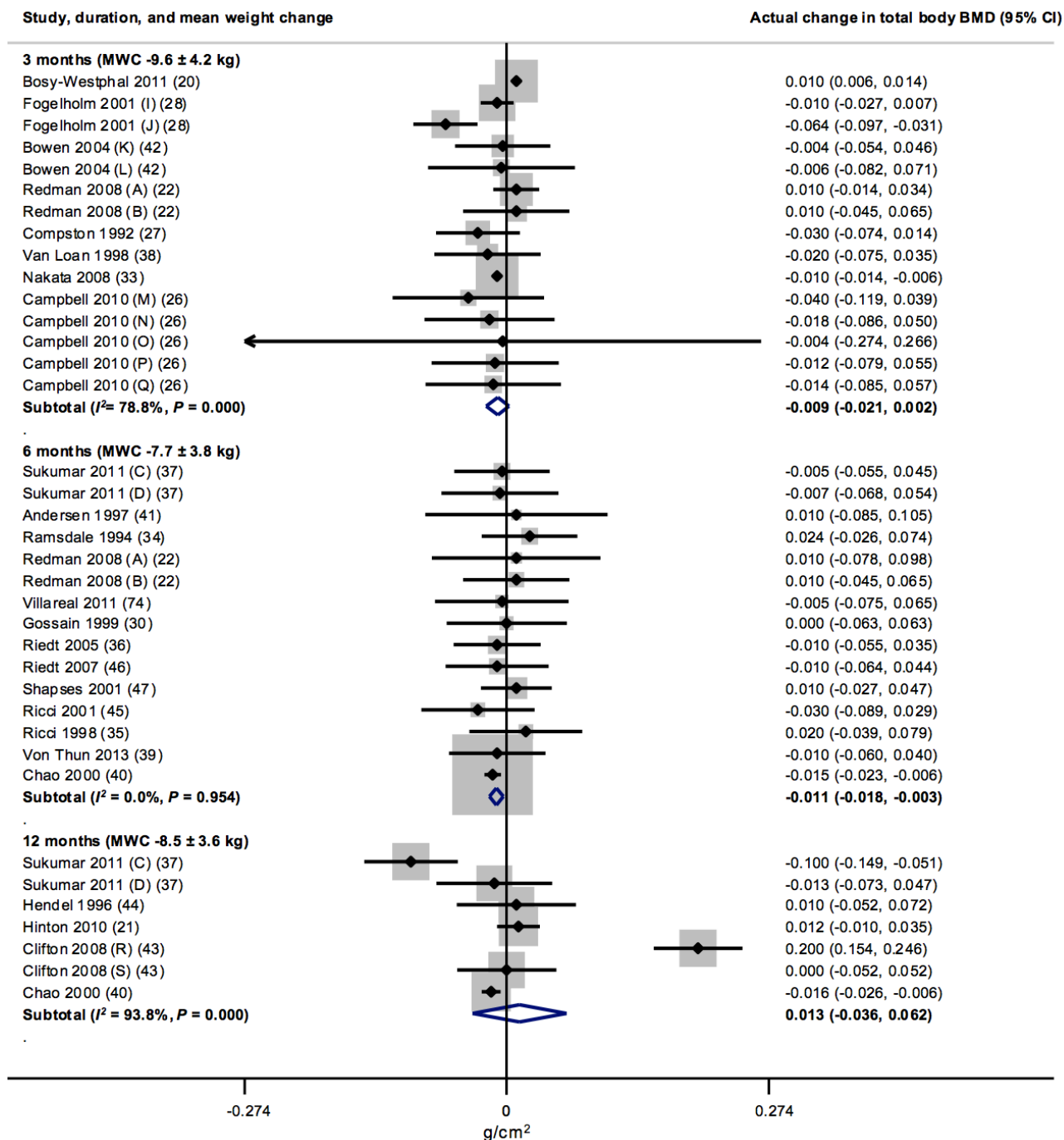
Uusi-Rasi (2010) <sup>(69)</sup>	22	Women – pre-menopausal	41.4 ± 0.8	33.3 ± 0.7	3 months	VLED with large weight loss (-19.2 to -13.5% of body weight) (X)	97.1 ± 3.2 (-14.5 ± 4.4)		P1NP (40.1 ± 3.7 µg/L) CTX (13.6 ± 1.0 nmol/L)
	22	Women – pre-menopausal	40.2 ± 1.0	33.1 ± 1.0	3 months	VLED with medium weight loss (-13.4 to -8.23% of body weight) (Y)	93.1 ± 3.6 (-9.5 ± 5.1)		P1NP (33.0 ± 2.8 µg/L) CTX (13.4 ± 1.2 nmol/L)
	22	Women – pre-menopausal	37.7 ± 1.4	34.4 ± 1.20	3 months	VLED with low weight loss (-8.21 to -2.1% of body weight) (Z)	88.6 ± 2.3 (-4.7 ± 3.3)		P1NP (37.5 ± 3.2 µg/L) CTX (14.2 ± 1.3 nmol/L)
Van Loan (1998) <sup>(38)</sup>	14	Women	25 – 42	32.9 ± 0.2	4 months – included in 3 month category	MER	89.7 ± 3.6 (-9.3 ± 5.0)	Total body (1.22 ± 0.02)	
Villareal (2011) <sup>(74)</sup>	26	Men and women (65%)	70.0 ± 0.8	37.2 ± 0.9	6 months	MER	104.0 ± 2.9 (-9.0 ± 1.1)	Total hip (1.02 ± 0.03) Lumbar spine (L1 – L4) (1.12 ± 0.03) Total body (1.25 ± 0.03)	
Von Thun (2013) <sup>(39)</sup>	22	Women – post-menopausal	60.7 ± 0.8	28.3 ± 0.4	6 months	MER	73.1 ± 1.5 (-7.6 ± 2.0)	Total body (1.11 ± 0.02)	Osteocalcin (3.0 ± 1.3 nmol/L) NTX (11.6 ± 1.8 nmol/L BCE)



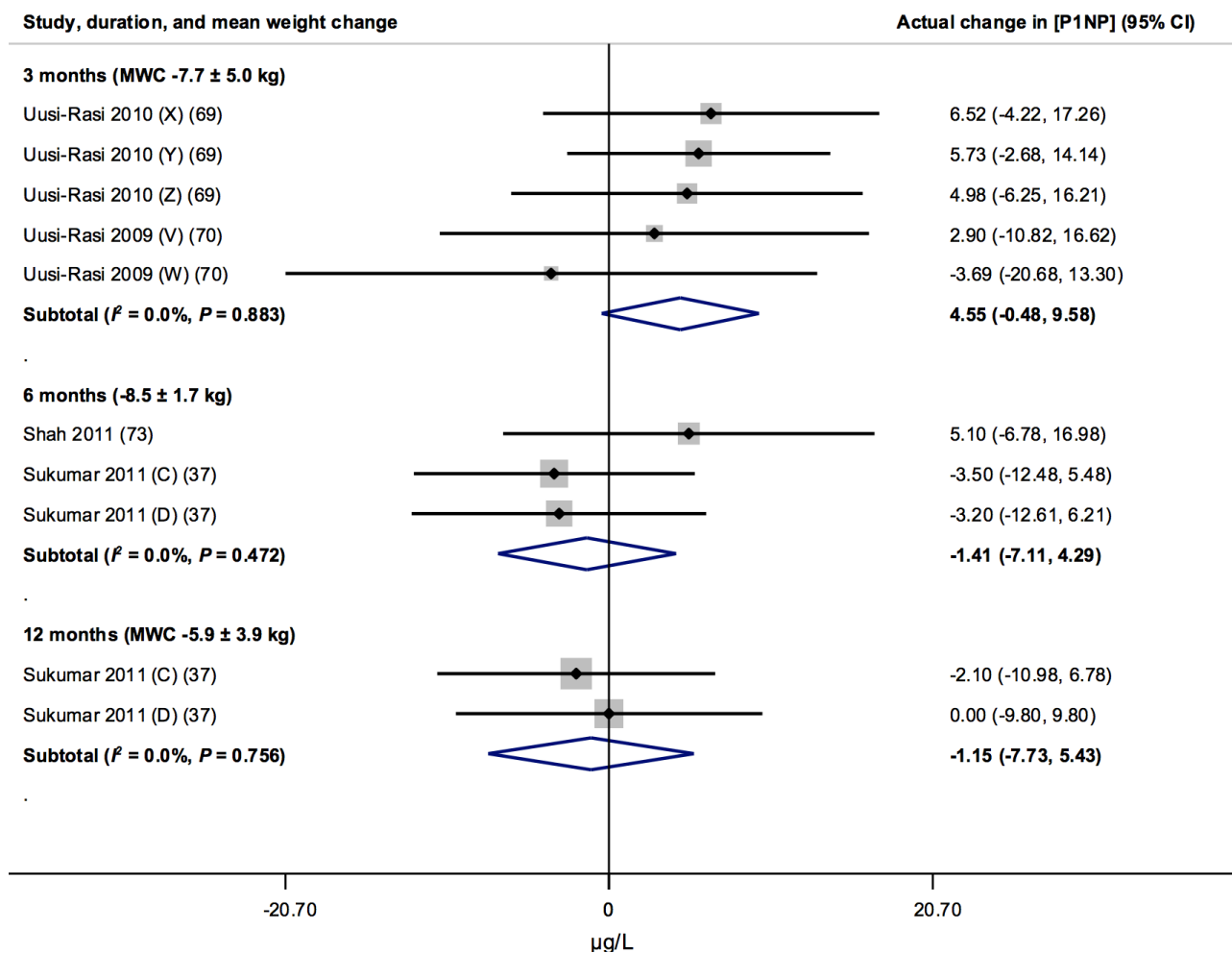
Supporting Figure 1. Flow diagram for the process of publication selection, inclusion and exclusion from this systematic review and meta-analysis.



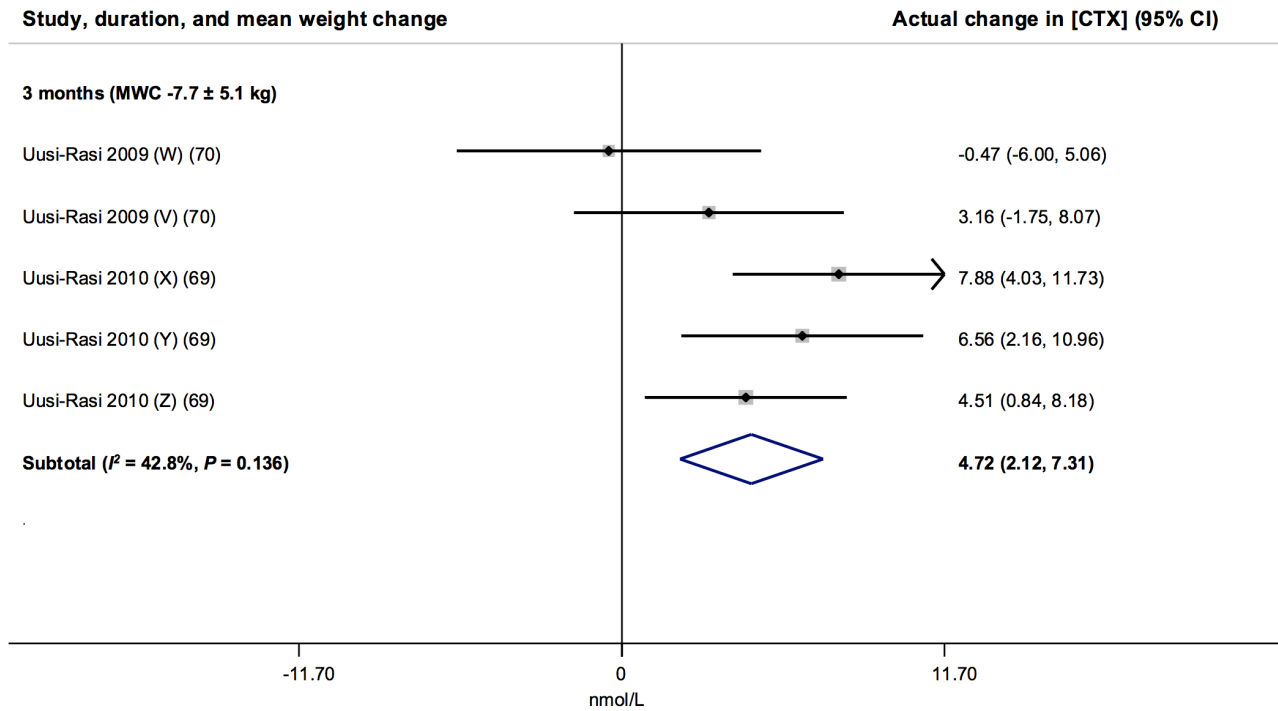
**Supporting Figure 2. Forest plot of change in lumbar spine BMD (bone mineral density) from baseline until the end of dietary weight loss interventions of varying durations.** Mean weight changes (MWC) during the dietary interventions are recorded next to the corresponding duration,  $\pm$  standard deviations. The letter in parentheses to the right of each study is used to distinguish different dietary interventions from the same publication, with details of all corresponding dietary interventions listed in **Supporting Table 1**. Plotted values (and the numbers at right) represent the absolute changes in lumbar spine BMD (in  $\text{g}/\text{cm}^2$ ), with 95% confidence intervals (CI) illustrated by the error bars (or the numbers in parentheses at right). Mean  $\pm$  SE of lumbar spine BMD at baseline was  $1.16 \pm 0.03 \text{ g}/\text{cm}^2$ .  $I^2$  indicates the percentage of heterogeneity for the dietary interventions of each duration.



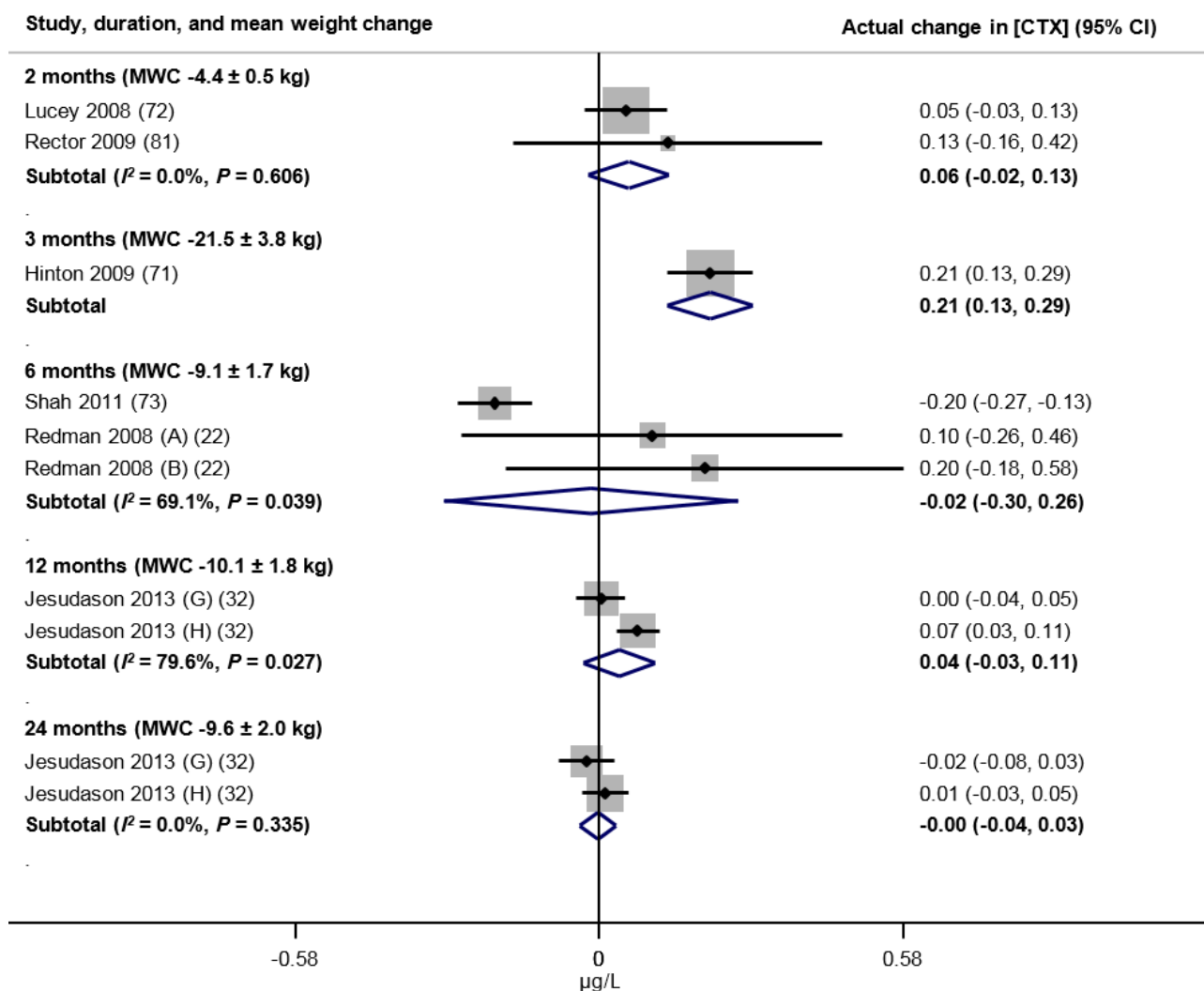
**Supporting Figure 3. Forest plot of change in total body BMD (bone mineral density) from baseline until the end of dietary weight loss interventions of varying durations.** Mean weight changes (MWC) during the dietary interventions are recorded next to the corresponding duration,  $\pm$  standard deviations. The letter in parentheses to the right of each study is used to distinguish different dietary interventions from the same publication, with details of all corresponding dietary interventions listed in **Supporting Table 1**. Plotted values (and the numbers at right) represent the absolute changes in total body BMD (in g/cm<sup>2</sup>), with 95% confidence intervals (CI) illustrated by the error bars (or the numbers in parentheses at right). Mean  $\pm$  SE of total body BMD at baseline was  $1.17 \pm 0.02$  g/cm<sup>2</sup>.  $I^2$  indicates the percentage of heterogeneity for the dietary interventions of each duration.



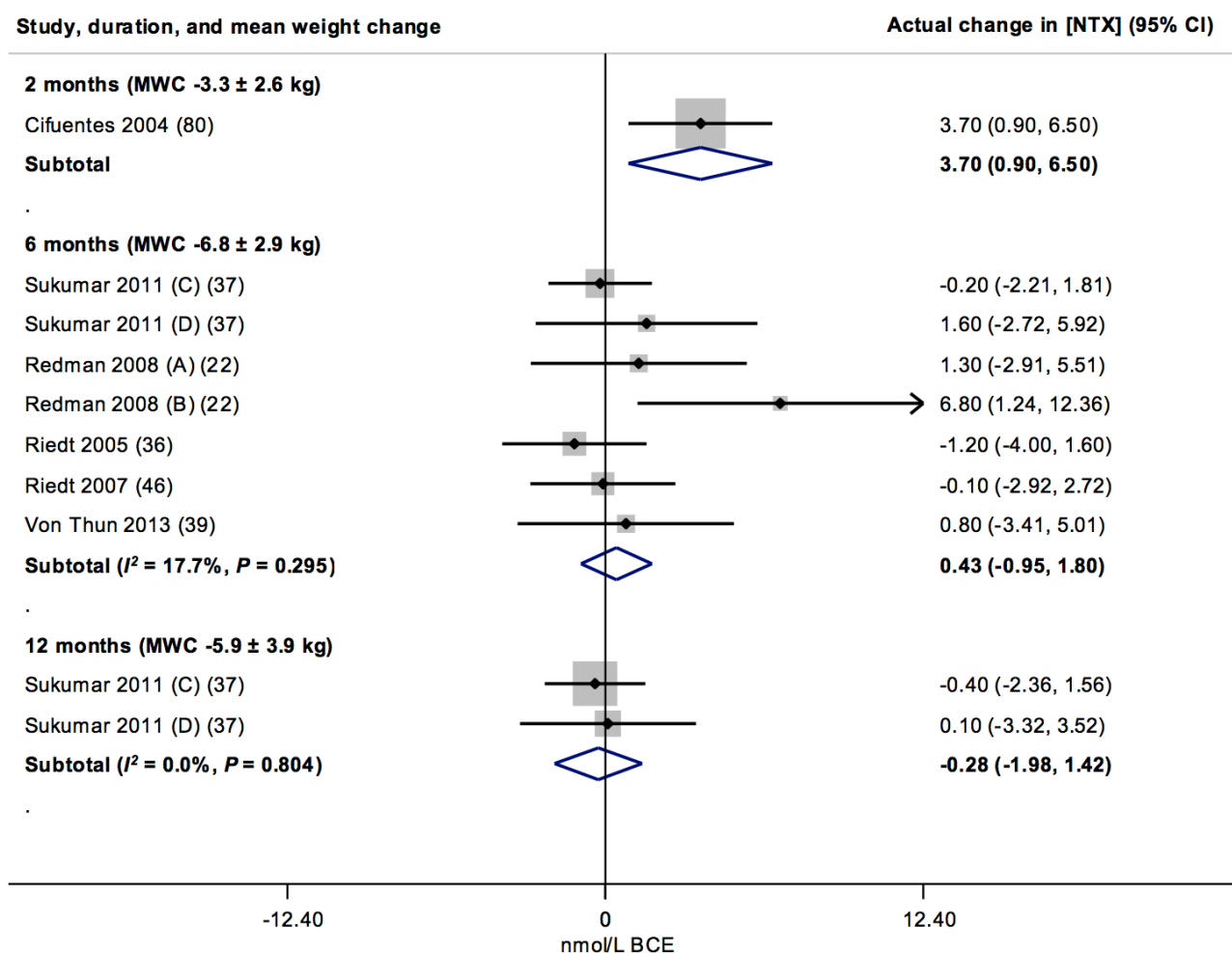
**Supporting Figure 4. Forest plot of change in serum N-terminal propeptide of type I procollagen (P1NP) from baseline until the end of dietary weight loss interventions of varying durations.** Mean weight changes (MWC) during the dietary interventions are recorded next to the corresponding duration,  $\pm$  standard deviations. The letter in parentheses to the right of each study is used to distinguish different dietary interventions from the same publication, with details of all corresponding dietary interventions listed in **Supporting Table 1**. Plotted values (and the numbers at right) represent the absolute changes in serum P1NP concentrations (in  $\mu\text{g/L}$ ), with 95% confidence intervals (CI) illustrated by the error bars (or the numbers in parentheses at right). Mean  $\pm$  SE of serum P1NP concentrations at baseline was  $40.8 \pm 3.3 \mu\text{g/L}$ .  $I^2$  indicates the percentage of heterogeneity for the dietary interventions of each duration.



**Supporting Figure 5. Forest plot of change in serum C-terminal telopeptide of type I collagen (CTX, in nmol/L) from baseline until the end of dietary weight loss interventions of varying durations.** Mean weight changes (MWC) during the dietary interventions are recorded next to the corresponding duration,  $\pm$  standard deviations. The letter in parentheses to the right of each study is used to distinguish different dietary interventions from the same publication, with details of all corresponding dietary interventions listed in **Supporting Table 1**. Plotted values (and the numbers at right) represent the absolute changes in serum CTX concentrations (in nmol/L), with 95% confidence intervals (CI) illustrated by the error bars (or the numbers in parentheses at right). Mean  $\pm$  SE of serum CTX concentrations at baseline was  $12.5 \pm 1.5$  nmol/L.  $I^2$  indicates the percentage of heterogeneity for the dietary interventions of each duration.



**Supporting Figure 6. Forest plot of change in serum C-terminal telopeptide of type I collagen (CTX, in  $\mu\text{g/L}$ ) from baseline until the end of dietary weight loss interventions of varying durations.** Mean weight changes (MWC) during the dietary interventions are recorded next to the corresponding duration,  $\pm$  standard deviations. The letter in parentheses to the right of each study is used to distinguish different dietary interventions from the same publication, with details of all corresponding dietary interventions listed in **Supporting Table 1**. Plotted values (and the numbers at right) represent the absolute changes in serum CTX concentrations (in  $\mu\text{g/L}$ ), with 95% confidence intervals (CI) illustrated by the error bars (or the numbers in parentheses at right). Mean  $\pm$  SE of serum CTX concentrations at baseline was  $0.51 \pm 0.09$   $\mu\text{g/L}$ .  $I^2$  indicates the percentage of heterogeneity for the dietary interventions of each duration.



**Supporting Figure 7. Forest plot of change in serum N-terminal telopeptide of type I collagen (NTX) from baseline until the end of dietary weight loss interventions of varying durations.** Mean weight changes (MWC) during the dietary interventions are recorded next to the corresponding duration,  $\pm$  standard deviations. The letter in parentheses to the right of each study is used to distinguish different dietary interventions from the same publication, with details of all corresponding dietary interventions listed in **Supporting Table 1**. Plotted values (and the numbers at right) represent the absolute changes in serum NTX concentrations (in nmol/L bone collagen equivalents, BCE), with 95% confidence intervals (CI) illustrated by the error bars (or the numbers in parentheses at right). Mean  $\pm$  SE of serum NTX concentrations at baseline was  $15.5 \pm 1.2$  nmol/L BCE.  $I^2$  indicates the percentage of heterogeneity for the dietary interventions of each duration.



## **List of Abbreviations**

ADP	Air displacement plethysmography
BCE	Bone collagen equivalents
BIA	Bioelectrical impedance analysis
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
CTX	C-terminal telopeptide of type I collagen
DXA	Dual energy x-ray absorptiometry
IGF-1	Insulin-like growth factor 1
LED	Low energy diet
MER	Moderate energy restriction
NTX	N-terminal telopeptide of type I collagen
P1NP	N-terminal propeptide of type I procollagen
1RM	One repetition maximum
8RM	Eight repetition maximum
VLED	Very low energy diet