

## 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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### 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 bone mineral density (BMD), as assessed by dual-energy X-ray absorptiometry (DXA). Secondary outcomes were markers of bone turnover. Diet-induced weight loss was associated with significant decreases of 0.010 to 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 [CIs], –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 g/cm<sup>2</sup>; 95% CI, –0.018 to –0.003 g/cm<sup>2</sup>) after 6 months. Although 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' duration) induced significant increases in serum concentrations of osteocalcin (0.26 nmol/L; 95% CI, 0.13 to 0.39 nmol/L), C-terminal telopeptide of type I collagen (CTX) (4.72 nmol/L; 95% CI, 2.12 to 7.30 nmol/L) or N-terminal telopeptide of type I collagen (NTX) (3.70 nmol/L; 95% CI, 0.90 to 6.50 nmol/L bone collagen equivalents [BCEs]), 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. © 2015 American Society for Bone and Mineral Research

### 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 years, 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 threefold 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 (BMI  $\geq$  35 kg/m<sup>2</sup>) 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 BMI  $\geq$  30 kg/m<sup>2</sup> 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 that are maintained for up to 10 to 14 years postsurgery[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 that 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 to 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 to 3 months compared to a minimum of 6 months required before significant changes in BMD can be detected.[54, 55]

## Materials and Methods

### *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  $\geq$  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, because of 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 nonsurgical, nonmedication, nonsupplementation, or nonexercise 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 because of its correlation with fracture risk.[60-62] Both of these sites, because of 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 abovementioned bone turnover markers because although there is currently no standardized 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 standardized 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 showing 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 (ie, before commencement of the dietary weight-loss regime) and a time point immediately upon completion of the dietary regime.

#### *Search strategy*

MEDLINE, PreMEDLINE, EMBASE, CINAHL, and SPORTDiscus 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 Supporting 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.

#### *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 Supporting 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 meters (m) and kilograms (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 Supporting 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 extracted in megajoules (MJ), with studies reporting energy prescriptions in calories being converted to MJ by multiplying by 0.00418. 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 5.0 MJ per day.[76] A moderately energy-restricted diet (MER) was defined as a diet providing greater or equal to 5.0 MJ per day.[78] BMD in all studies was reported as grams per square centimeter (g/cm<sup>2</sup>). Data for lumbar spine BMD measured between L1 and L4 were pooled with data measured between L2 and L4. In all studies, serum P1NP concentrations were reported in micrograms per liter (μg/L). Serum concentrations of osteocalcin were reported by studies in either nanomoles per liter (nmol/L) or μg/L. For this analysis we converted all data for osteocalcin to nmol/L using the molecular weight of osteocalcin as 5800.[79] The included studies reported serum concentrations of CTX in nmol/L or μg/L, depending on which manufacturer's assay was used. Because 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 μg/L). NTX concentrations were also analyzed in two distinct data sets because measurements had been made either in serum or urine, both of which also have their own units of measurement and noncomparable concentrations; nmol/L bone collagen equivalents (BCE) for serum, and nmol/L BCE/nmol/L creatinine 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 analyzed qualitatively rather than quantitatively.

#### *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 (ie, 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 analyzed.

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  $\mu$  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  $\sigma^2$ . The random-effects model recognizes the possibility of heterogeneity of between-study variation (ie, that  $\sigma^2$  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 subgroup analyses for the type of dietary weight loss intervention under study (VLED or LED versus MER) and menopausal status (premenopausal versus postmenopausal). In order to avoid duplication of data in these subgroup 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 subgroup 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 (ie, 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, College Station, TX, USA).

## Results

### *Characteristics of clinical trials*

As seen in Supporting Fig. 1, 3145 publications were retrieved from the five databases searched, equating to 2765 unique publications. Following screening of titles and abstracts, the full texts of 71 publications were then retrieved and analyzed against the inclusion and exclusion criteria, resulting in the exclusion of 30 publications for the reasons shown in Supporting Fig. 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 three[24, 49, 75] being analyzed 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) (Supporting Fig. 1).

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

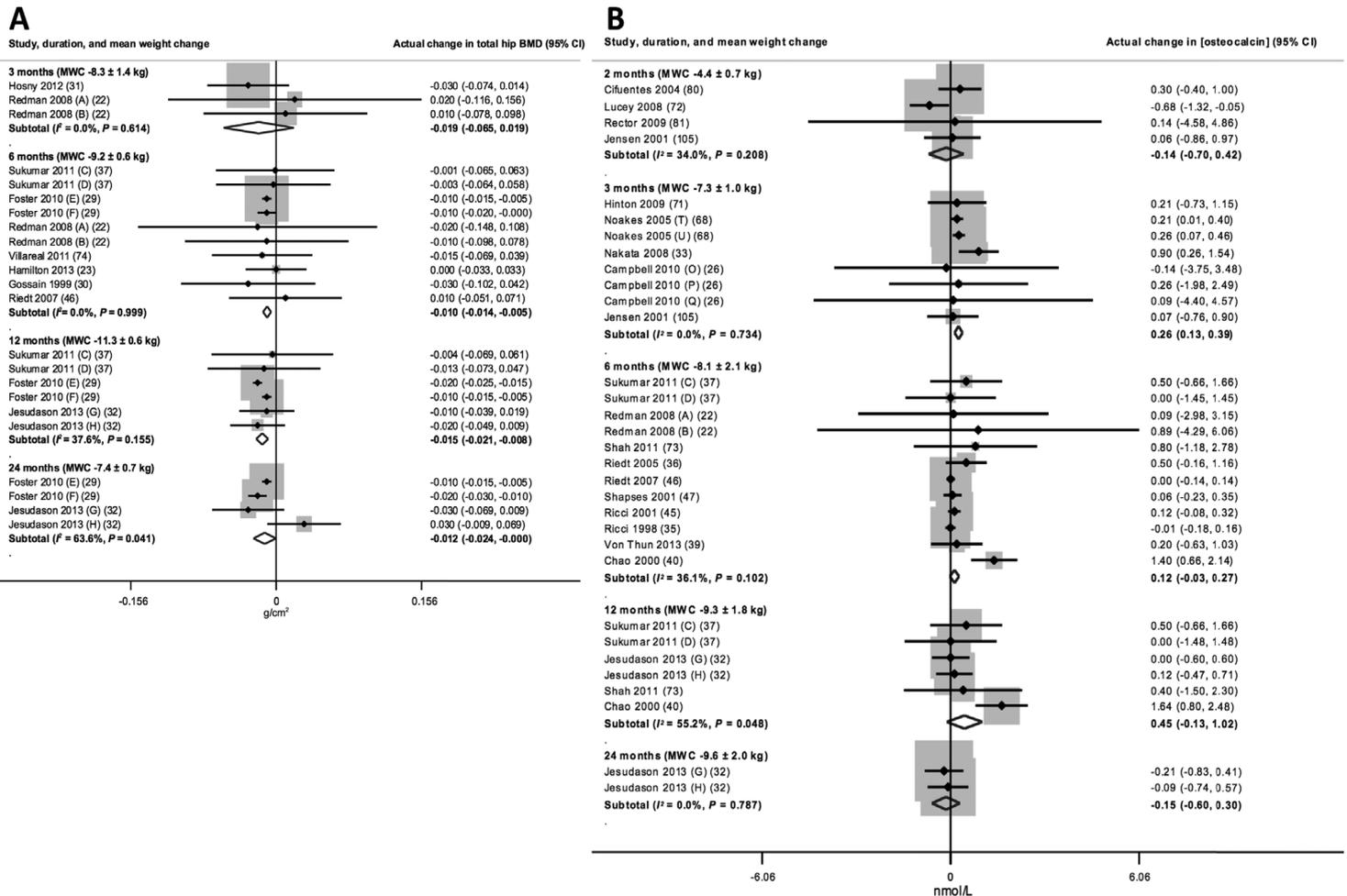
and high protein diets, so the total number of interventions reported in the 38 publications used for this meta-analysis was 53. Dietary weight-loss interventions differed among studies with respect to both the duration and details of intervention (Supporting Table 1). A number of publications measured outcomes for each intervention at more than one time point (eg, 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.

### *Meta-analysis*

#### Total hip BMD

In summary, this meta-analysis shows significant reductions by 0.010 to 0.015 g/cm<sup>2</sup> in total hip BMD after dietary weight loss interventions of 6 to 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 (Fig. 1A), depending on the duration. Mean  $\pm$  SE of total hip BMD at baseline was  $1.01 \pm 0.02$  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 ( $-0.010$  g/cm<sup>2</sup>; 95% CI,  $-0.014$  to  $-0.005$  g/cm<sup>2</sup>;  $p=0.001$ ), 12 months ( $-0.015$  g/cm<sup>2</sup>; 95% CI,  $-0.021$  to  $-0.008$  g/cm<sup>2</sup>;  $p=0.001$ ), and 24 months ( $-0.012$  g/cm<sup>2</sup>; 95% CI,  $-0.024$  to  $0.000$  g/cm<sup>2</sup>;  $p=0.047$ ), as shown in Fig. 1A. Significant heterogeneity was found among interventions lasting 12 months and 24 months, but not among those of 3-month or 6-month duration, as seen from the  $I^2$  statistics in Fig. 1A.



**Figure 1.** Forest plot of change in (A) total hip BMD and (B) serum osteocalcin from baseline until the end of dietary weight loss interventions of varying durations. MWC during the dietary interventions are recorded next to the corresponding duration,  $\pm$ SDs. 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 (A) total hip BMD (in g/cm<sup>2</sup>) and (B) serum osteocalcin concentrations (in nmol/L), with 95% CIs illustrated by the error bars (or the numbers in parentheses at right). Mean  $\pm$  SE of total hip BMD and serum osteocalcin concentrations at baseline were  $1.01 \pm 0.02$  g/cm<sup>2</sup> and  $1.6 \pm 0.5$  nmol/L, respectively.  $I^2$  indicates the percentage of heterogeneity for the dietary interventions of each duration. BMD = bone mineral density; MWC = mean weight changes; CI = confidence interval.

Because of this significant heterogeneity in the data at 12 and 24 months, subgroup 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 Materials and Methods), regardless of the duration of the dietary intervention. Weight loss was greater with VLED or LED ( $-11.1$  kg; 95% CI,  $-14.4$  to  $-7.8$  kg;  $p=0.001$  versus baseline), than with MER ( $-9.6$  kg; 95% CI,  $-10.8$  to  $-8.6$  kg;  $p=0.001$  versus baseline).

Although total hip BMD decreased with both types of dietary intervention, the decrease was significant only with MER ( $-0.013$  g/cm<sup>2</sup>; 95% CI,  $-0.018$  to  $-0.008$  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 because of 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 ( $p=0.492$ ).

#### *Lumbar spine BMD*

To summarize, 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 premenopausal but not postmenopausal 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  $\pm$  SE values of  $1.16 \pm 0.03$  g/cm<sup>2</sup> in a total of 20 interventions and 29 observations in 1097 participants, with no overall significant differences being identified by this meta-analysis at any time point (Supporting Fig. 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 ( $-0.031$  g/cm<sup>2</sup>; 95% CI,  $-0.062$  to  $0.000$  g/cm<sup>2</sup>;  $p=0.05$ ), with no significant change in this parameter in response to MER ( $0.002$  g/cm<sup>2</sup>; 95% CI,  $-0.003$  to  $0.007$  g/cm<sup>2</sup>;  $p=0.497$ ). This result was opposite to that found for the total hip as described in the previous section, 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 premenopausal women ( $-0.023$  g/cm<sup>2</sup>; 95% CI,  $-0.005$  to  $0.000$  g/cm<sup>2</sup>;  $p=0.05$ ) and not in postmenopausal women ( $0.002$  g/cm<sup>2</sup>; 95% CI,  $-0.011$  to  $0.016$  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 analyzed according to the diet type and menopausal status of the participants. No evidence of publication bias was found.

#### *Total body BMD*

In 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 postmenopausal but not premenopausal women).

Supporting Fig. 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  $\pm$  SE 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 ( $-0.011$  g/cm<sup>2</sup>; 95% CI,  $-0.018$  to  $-0.003$  g/cm<sup>2</sup>;  $p=0.004$ ). 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 nonsignificant increase in this

parameter in response to MER ( $p=0.59$ ). Although both premenopausal and postmenopausal women lost a similar amount of body weight, and although BMD of the total body decreased in both premenopausal and postmenopausal women, the effect of diet on total body BMD was significant only in the postmenopausal women ( $p=0.006$ ). This finding is different to that identified in the results for total hip and lumbar spine BMD, where no change in total hip BMD was found in either premenopausal or postmenopausal women, and a significant decrease in lumbar spine BMD was only detected in the premenopausal cohort. No publication bias was detected.

#### *P1NP*

There were eight 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  $\pm$  SE at baseline of  $40.8 \pm 3.3 \mu\text{g/L}$ . Serum P1NP concentrations showed no significant change in response to diet at any time point (Supporting Fig. 4). No heterogeneity was observed for serum P1NP.

#### *Serum osteocalcin*

To summarize, 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  $\pm$  SE at baseline  $1.6 \pm 0.5 \text{ nmol/L}$ . Overall, serum osteocalcin concentrations tended to increase with diet-induced weight loss (Fig. 1B), with a significant increase occurring in response to dietary interventions of 3 months' duration ( $0.26 \text{ nmol/L}$ ; 95% CI, 0.13 to 0.39 nmol/L;  $p=0.001$ ). Heterogeneity was present only at 12 months. Weight reduction was greater with VLED or LED ( $-13.7 \text{ kg}$ ; 95% CI,  $-23.3$  to  $-4.2 \text{ kg}$ ;  $p=0.005$  versus baseline) than with MER ( $-6.8 \text{ kg}$ ; 95% CI,  $-23.3$  to  $-6.1 \text{ kg}$ ;  $p=0.001$  versus baseline). However, a significant increase in osteocalcin was only found in response to the MER ( $0.10 \text{ nmol/L}$ ; 95% CI, 0.01 to 0.19 nmol/L;  $p=0.025$  versus baseline) and not in response to the VLED or LED ( $0.23 \text{ nmol/L}$ ; 95% CI,  $-0.68$  to  $1.13 \text{ nmol/L}$ ;  $p=0.624$  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 premenopausal or postmenopausal status (premenopausal:  $0.17 \text{ nmol/L}$ ; 95% CI,  $-0.14$  to  $0.48 \text{ nmol/L}$ ;  $p=0.290$ ; postmenopausal:  $0.06 \text{ nmol/L}$ ; 95% CI,  $-0.06$  to  $-0.18 \text{ nmol/L}$ ;  $p=0.320$ ). There was no publication bias.

#### *Serum CTX*

Serum CTX was investigated and reported in nmol/L units in five interventions and five observations with a total of 103 participants, all with a 3-month duration and a mean  $\pm$  SE at baseline of  $12.5 \pm 1.5 \text{ nmol/L}$  (Supporting Fig. 5). A significant increase in this parameter was observed ( $4.72 \text{ nmol/L}$ ; 95% CI, 2.12 to 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 eight interventions and 10 observations involving 486 participants overall and a mean  $\pm$  SE at baseline of  $0.51 \pm 0.09 \mu\text{g/L}$  (Supporting Fig. 6). There was a nonsignificant trend for an increase in this parameter for interventions of 2 months in duration, and a significant increase for interventions of 3 months in duration ( $0.21 \mu\text{g/L}$ ; 95% CI, 0.13 to  $0.29 \mu\text{g/L}$ ;  $p=0.001$  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  $\mu\text{g/L}$  (Supporting Fig. 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 because of the limited number of

studies available. Indeed, only one study that investigated serum CTX in  $\mu\text{g/L}$  used an LED, and only one study investigated a distinctly premenopausal population.

#### *Serum and urinary NTX*

There were 14 interventions and 17 observations that investigated serum or urinary NTX concentrations in a total of 306 participants. For both serum (Supporting Fig. 7) and urinary (Supporting Fig. 8) concentrations of the bone resorption marker NTX, there was an overall trend for an increase from baseline mean  $\pm$  SE values of  $15.5 \pm 1.2$  nmol/L BCE and  $37.4 \pm 4.1$  nmol/L BCE / 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] ( $3.70$  nmol/L BCE; 95% CI, 0.90 to 6.50 nmol/L BCE;  $p=0.010$  versus baseline). No heterogeneity was observed for serum or urinary NTX.

Qualitative analysis of studies that could not be included in the meta-analysis For the three 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 and colleagues[49] placed 50 overweight postmenopausal 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 nonsignificant decreases being observed in both parameters when measured at 3 months. The second study, by Thorpe and colleagues,[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 nonsignificant trend toward decreased BMD at all three sites (total hip, lumbar spine, and total body). Hyldstrup and colleagues[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.

## **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 shown 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 onward. 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. Although 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 four studies[36, 40, 45, 74] investigating postmenopausal women or people older than 65 years. Second, 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, because 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. Third, 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, given that 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. It would therefore appear unlikely that a single dietary weight-loss intervention of 6 to 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 6785 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 nonvertebral 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, although 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, 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-month 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 subregions of whole-body DXA scans, such as the pelvis, are used to measure BMD rather than the total hip or proximal femur, underdiagnosis 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 versus 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 postmenopausal women would exhibit more detrimental changes in bone mass and turnover in response to diet-induced weight loss than premenopausal women, because they are already predisposed to detrimental changes in bone. However, our subgroup 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 the 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 the 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. Although 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 mentioned in the second paragraph of this Discussion.

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. Second, only a minority of studies reviewed herein investigated BMD at time points after the end of the dietary intervention. 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 had 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 by 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 g/cm<sup>2</sup>. 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 previously.[53, 104, 105] Because 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, although our subgroup analyses suggested that VLEDs or LEDs do not seem to have any worse effect on bone than do MERs, there were limited studies available testing VLEDs or LEDs, and only three [22, 28, 70] in direct comparison to less severe energy restriction (ie, 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.

### Disclosures

AS has received payment from Eli Lilly, the Pharmacy Guild of Australia, Novo Nordisk, and the Dietitians Association of Australia for seminar presentation at conferences. She is also the author of *The Don't Go Hungry Diet* (Bantam, Australia and New Zealand, 2007) and *Don't Go Hungry For Life* (Bantam, Australia and New Zealand, 2011).

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Authors' roles: JZ, RVS, CMYL, AAG, and AS designed the study and interpreted the data. JZ and MSHH acquired the data, in consultation with RVS. JZ analyzed the data, under the supervision of RVS, CMYL, and AAG. SAS provided extensive original data and discussion that helped to shape the manuscript. JZ drafted and AS edited the manuscript. TVN provided critical interpretation of the data and wrote some parts of the manuscript. All authors revised the manuscript critically for important intellectual content, approved the final version of the submitted manuscript, and are accountable for all aspects of the accuracy and integrity of the work.

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