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Villareal DT, Fontana L, Das SK, Redman L, Smith SR, Saltzman E, Bales C, Rochon J, Pieper C, Huang M, Lewis M, Schwartz AV; CALERIE Study Group. Effect of Two-Year Caloric Restriction on Bone Metabolism and Bone Mineral Density in Non-Obese Younger Adults: A Randomized Clinical Trial. J Bone Miner Res. 2016 Jan;31(1):40-51.

What We Know, Think We Know, or Are Starting to Know

The human skeleton is not a biological inert structure; it is a live organ tissue comprised of minerals and proteins, and is in a constant state of flux throughout the lifespan [(1)]. This flux, which is characterised by bone formation and bone remodelling processes, can be broadly divided into three distinct phases:

1. The period of bone growth through infancy and adolescence, primarily as the size and length of bone [up to ~20yrs of age].
2. The period of bone consolidation, where bone length growth has ceased but bone continues to acquire mass and change shape, in early adulthood [from ~20–34yrs of age].
3. The period of bone remodelling, where the rate of bone breakdown [known as "resorption"] begins to exceed the rate of bone formation [from ~34yrs of age onwards].

While ~60–80% of bone mass is genetically determined, 20–40% is influenced by lifestyle factors, with nutrition and physical activity the two most influential factors [(1)]. Thus, the ability to reach one's full genetic potential for bone development is dependent on appropriate nutrition and physical activity levels [a concept nicely illustrated in the **figure**, below] [(1)].

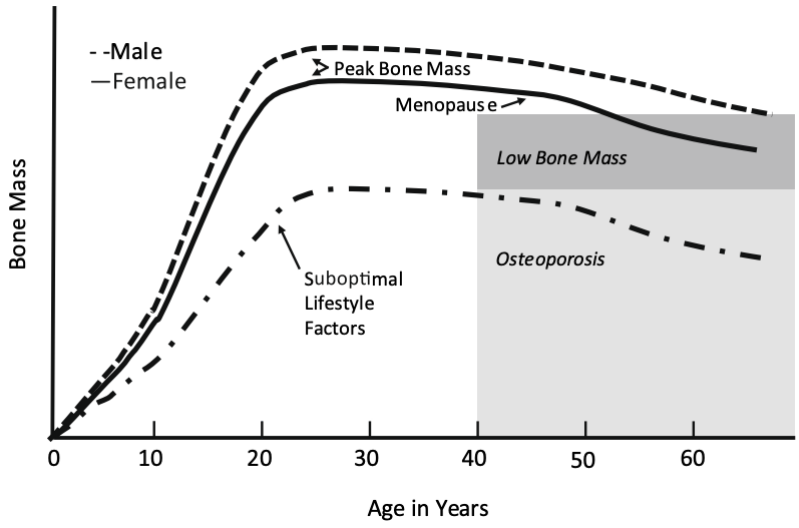


***Figure** from [(1)] illustrating bone mass over the course of the lifespan; the curve of the solid black line [**men**] and dashed black line [**women**] represent the full potential for bone mass given optimal lifestyle factors additional to genetic predisposition. The mixed dashed/dotted black line indicates the trajectory of bone mass development with suboptimal lifestyle factors, with osteoporosis risk as early as 40yrs of age.*

Nutrients are critical to the processes of bone formation and resorption throughout the lifecycle [1,2]. The two most abundant minerals in bone are calcium and phosphorous, both of which are required for mineralisation, i.e., the hardening of bones, through formation of a structure known as hydroxyapatite. Magnesium, potassium, and sodium bind to and support this structure. Bone formation, and the uptake of calcium and phosphorous into bone, are mediated by vitamins D and K. Thus, adequate intake of multiple micronutrients is required for optimal bone health.

However, as nutrient intakes are themselves a function of total daily energy intake, it is surprising that energy intake is often an afterthought for bone health. Indeed, two reviews of the role of nutrition in bone health make not a single reference to total energy intake [2,3]. Nevertheless, cross-sectional studies have demonstrated positive relationships between total daily energy intake and bone mineral density [BMD], i.e., higher energy intakes associated with greater BMD [4,5].

To date, there are no randomised controlled trials [RCTs] examining the effects of deliberate energy restriction on bone markers beyond 1yr. The present study was a secondary analysis of bone health markers from a 2yr RCT of sustained energy restriction.

The Study

The CALERIE [Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy] trial was a 2yr RCT that investigated the effects of sustained energy restriction [ER] in otherwise healthy individuals. Healthy adults aged between 20–50yrs with either a normal weight or overweight Body Mass Index [BMI, ≤28.0kg/m^2] were recruited, and randomised in 2:1 order [i.e., two to intervention group, one to control] to one of two groups:

- **Intervention – Energy Restriction**: A 25% reduction in energy intake from maintenance levels of individually-calculated daily energy requirements. No type of diet or macronutrient prescription was given to participants in the intervention group. Participants were provided with an intensive behavioural intervention, including individual and group counselling, and self-monitoring of dietary intake.
- **Control – Ad Libitum Energy Intake**: Participants in the control group were advised to continue with their current diet with no restrictions on energy intake. Similarly, no type of diet or macronutrient prescription was given to participants in the control group.

All participants were supplemented with 1,000IU/d vitamin D3 and 1,000mg/d calcium. Participants in the intervention group were provided with all meals for the first 27-days of the study to maximise adherence and become accustomed to portion sizes.

Change in BMD and bone turnover markers were the main outcomes for the present study. Other outcomes included body composition, diet and nutrient intakes, grip strength, and physical activity.

Results: 118 participants completed the full 2yr study; 117 in the intervention group and 71 in the control group. Under the principle of "intention-to-treat" [ITT]* [see ***Geek Box** below for further details], the final analysis included 218 participants: 143 and 75 in the intervention and control groups, respectively.

Participants were ~37yrs of age at baseline, of 70% of participants were female. The average baseline BMI was 25.1kg/m^2. There were no differences in baseline characteristics between the intervention and control groups, which were matched for age, sex, ethnicity, education level, BMI, body composition, and BMD. The achieved energy restriction in the intervention group was 19.5% at 6-months, 10.8% at 1yr, and 8.3% at 2yrs.

***Change in BMD*:** At both 1yr and 2yrs of the study, BMD at each site [lumbar spine, total hip, femoral neck, and intertrochanter] decreased significantly by 1.2–1.7% in the energy restriction intervention group compared to the *ad libitum* control group. This effect did not differ between men and women. The analysis demonstrated that the decrease in BMD was predicted by weight loss.

***Change in Bone Turnover Markers*:** Measured levels of bone resorption [i.e., breakdown] markers increased by ~28% at 1yr and remained elevated by 20% at 2yrs. This was accompanied by a ~7% decrease in measured bone formation markers.

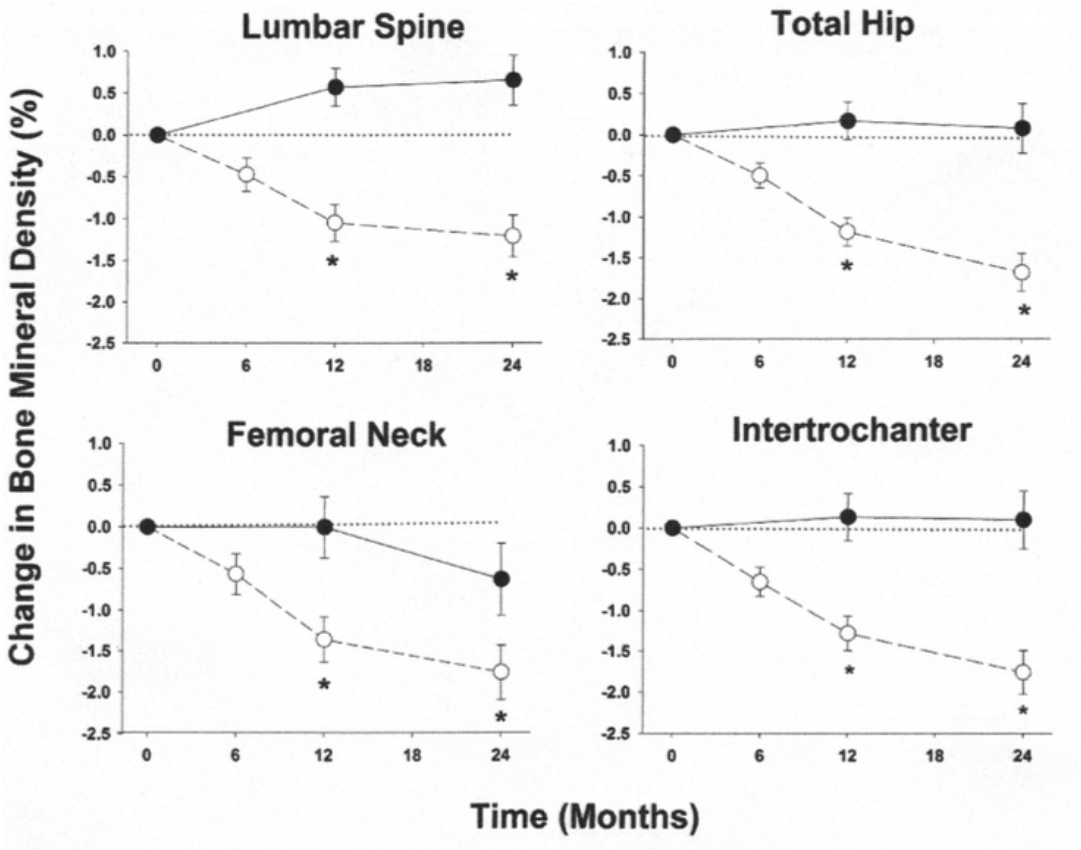


***Figure** from the paper illustrating the change in BMD [as a percentage from baseline] in each of the major skeletal sites measured in the study. The open-circle/dashed line represents the intervention group; the solid circle/solid line represents the control group. BMD declined as a function of weight loss in the intervention group.*

*Geek Box: Intention-to-Treat Analysis

In a randomised controlled trial, it is important to match both arms of the trial to ensure that one side doesn't influence [i.e., bias] the results more than the other. This can be a problem if there is, for example, a high drop-out rate in one arm of the trial; the other arm will then have more statistical power, and it may over-inflate the effect of that arm vs. the comparative arm [or the control, if it is a control arm]. Intention to treat [ITT] is where the researchers will conduct analysis as if all subjects randomised in the trial completed it, irrespective of whether they dropped out, or didn't comply with the protocol. Drop-out and noncompliance are two issues which face many trials, in particular nutrition and weight loss interventions. True intention to treat analysis requires complete data to be available for all subjects who didn't complete the trial according to protocol. However, that data is not always available, and so often researchers will make assumptions based on, e.g., a last data point or a baseline measurement. Let's say a participant dropped out of a 1yr weight loss trial after 6-months; the researchers would use their baseline weight and weight at 6-months, and the change in weight, as the data included in the statistical analysis, as if the participant had completed the full study. Intention to treat is a positive because it maintains the study sample size, and it assumes a real-world practicality – because in the real world, not everyone is compliant with a protocol.

The Critical Breakdown

Pros: The study had clearly presented aims and is one of the few studies to investigate the effects of deliberate energy restriction in otherwise healthy, lean, young adults. Randomisation was stratified for factors like sex and BMI to ensure balance between groups. Energy expenditure and requirements were assessed using doubly-labelled water, the gold standard method of measurement. Bone was comprehensively assessed with both BMD measures using DEXA scans and markers of bone turnover. One month of meals were provided to participants to aid compliance with the targeted energy restriction. Personnel conducting the outcome assessments were blinded to the group allocation of participants. The statistical analysis used ITT, a positive given there were more dropouts from the intervention group. The statistical analysis also sought to identify the primary factors related to the loss of BMD, potentially beyond the effects of weight loss alone. Vitamin D and calcium were supplemented at sufficient doses and maintained levels throughout the study [more under ***Key Characteristic***, below].

Cons: The target level of 25% energy restriction was never achieved in the intervention group, highlighting the difficulties of adherence to such prescribed levels of energy. The rationale for the unequal randomisation ratio of 2:1 was not provided; we could presume this reflected an anticipation that more participants would drop-out of the intervention group. Indeed, an expected high dropout rate in one study arm is one of the justifications for an unequal randomisation ratio, and 26 participants dropped-out of the intervention group compared to 4 in the control group. This analysis was a secondary analysis of data from the overall CALERIE trial, which was designed to investigate the effects of energy restriction on ageing. Thus, bone related outcomes were not primary intended outcomes of the parent RCT and are best considered as exploratory findings.

Key Characteristic

As we noted in the ***What We Know...*** section, micronutrients take centre-stage in the literature on nutrition and bone health, and total energy intake is often an afterthought. However, interactions between total energy, macronutrients – protein in particular – and critical bone micronutrients such as calcium and vitamin D, have been demonstrated in this area of research [4,6,7].

An important aspect of any RCT is isolating the independent effects of the exposure, or treatment, in the intervention group. In the case of the present study, the exposure of interest was clearly stated as energy restriction, and the effects of energy restriction and weight loss on bone. Isolating any potential effects of energy restriction and weight loss required that factors like vitamin D status, calcium intake, and protein, were maintained at adequate levels for the duration of the study.

And the key characteristic of this trial is that this was achieved; vitamin D levels [as measured 25(OH)D3] were ~75nmol/L, which is the range at which maximal intestinal absorption of calcium is observed, and where parathyroid hormone levels – which is the principal hormone influencing bone resorption and release of calcium from bone – are prevented from increasing [8].

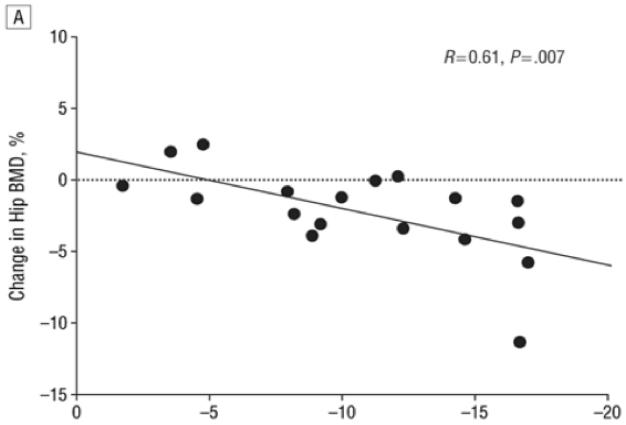
In addition, calcium supplementation at 1,000mg, and dietary protein intake of ~1.1g per kg bodyweight would be expected to further prevent calcium losses, which have been shown along with increased parathyroid hormone levels at protein intakes <0.8g/kg [9].

Thus, the study was well-executed in controlling for these crucial nutrient factors, thereby isolating effects of the weight loss consequent on energy restriction in the intervention group.

Interesting Finding

Following on from the previous section, the interesting finding from the present study is its main finding of the decrease in BMD over 2yrs of energy restriction and sustained weight loss. The relationship between energy intake, calcium, and BMD was demonstrated in a 2003 cross-sectional study of elderly adults [average age 68.6yrs], where higher intakes of both energy and calcium were associated with higher BMD independent of each other [4]. Thus, both energy and calcium were independently associated with BMD, again highlighting the importance of maintaining sufficient calcium intake in the present study.

However, evidence from weight loss interventions and total energy intake have yielded some conflicting findings that may relate to two important factors that influence bone: age and time. A 2006 RCT by Villareal *et al*. [10] in older adults [average age 57yrs] undergoing a ~15% energy restriction showed decreases in BMD following energy restriction and consequent weight loss. The **figure** below illustrates this correlation, as BMD decreased as the magnitude of weight loss increased with a moderate strength of correlation [$R = 0.61$].



However, there is also evidence from a 2008 RCT that BMD may be preserved in younger adults [average age ~37yrs] undergoing energy restriction, once adequate calcium intakes are maintained [11]. But what is noteworthy is that this previous trial was only 6-months in duration; the present study is 2yrs in duration and the differences in BMD between groups were only significant after 1yr. Thus, it is likely that the prior study suggesting that energy restriction and weight loss may not result in BMD losses in healthy younger adults was too short to detect any real changes.

Relevance

Given the 2yr duration of the present study and the population of healthy, lean, young adults, the present study supersedes prior research in this area and represents the most up-to-date evidence on this question. The findings indicate that energy restriction and related weight loss sustained over a 2yr period increases bone resorption and decreases bone formation, resulting in loss of BMD.

However, context is always important. First, it is important to remind ourselves that changes in biomarkers do not necessarily mean that a "hard" clinical endpoint, which in the case of bone is fractures, is inevitable. The authors of the present study, to their credit, put their findings into proper context; although there were "statistically significant" decreases in BMD, the absolute magnitude of these changes was miniscule and would translate to <0.5% increase in risk of fractures over 10yrs in a women ~50yrs of age.

The second important piece of context relates to the potential impact of the other primary lifestyle factor influencing bone health, namely physical activity. In their 2006 RCT, Villareal *et al*. [10] compared methods of achieving weight loss with either energy restriction alone or exercise; while both groups lost the same amount of weight [~8–10% from baseline], the change in bodyweight only correlated with loss of BMD in the energy restriction group, while there were no significant changes in BMD in the exercise group. Thus, physical activity may attenuate or prevent any potential adverse effects of energy restriction and weight loss on bone.

The final important piece of context relates to the intended aims of the CALERIE trial: sustaining an energy deficit of ~25% constantly over years. As the lack of achieving the targeted energy deficit, and the ~6:1 drop-out ratio between the intervention and control group, both demonstrate, few people are about that life.

Application to Practice

There are several factors that emerge from this literature. The first is, of course, the importance of maintaining appropriate intakes of micronutrients crucial to bone; calcium, phosphorous, magnesium, and potassium, in addition to vitamin D3 levels and other co-factors, in particular vitamin K. As stated, these nutrients take much of the focus in the research on nutrition and bone health.

However, it is also clear that there is a relationship between energy intake, weight loss and BMD, and the present study suggests this modest decline in BMD, although perhaps not one that would be expected to lead to significant increases in fracture risk, is related to energy restriction and weight loss independent of the critical bone health micronutrients.

However, the wider research also suggests that physical activity and exercise may attenuate any potential decreases in BMD related to weight loss. And let's think about the generalisability of the present study; we are not likely to be recommending 2yrs of sustained energy restriction to anyone.

Ultimately, it is important to remember that the two main lifestyle factors that influence bone mass – nutrition and physical activity – are modifiable factors, and that in population subgroups prone to BMD loss, i.e., postmenopausal women and the elderly, the boring but solid advice applies: good nutrition and exercise are what someone can look to aside from picking their parents for genes.

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