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Continuing Education for Nutrition Professionals

Diet, Body Composition, and Disease on Bone Health

Assessment and clinical management of bone disease in adults with eating disorders: a review

Abstract

Aim: To review current medical literature regarding the causes and clinical management options for low bone mineral density (BMD) in adult patients with eating disorders.

Background: Low bone mineral density is a common complication of eating disorders with potentially lifelong debilitating consequences. Definitive, rigorous guidelines for screening, prevention and management are lacking. This article intends to provide a review of the literature to date and current options for prevention and treatment.

Methods: Current, peer-reviewed literature was reviewed, interpreted and summarized.

Conclusion: Any patient with lower than average BMD should weight restore and in premenopausal females, spontaneous menses should resume. Adequate vitamin D and calcium supplementation is important. Weight-bearing exercise should be avoided unless cautiously monitored by a treatment team in the setting of weight restoration. If a patient has a Z-score less than expected for age with a high fracture risk or likelihood of ongoing BMD loss, physiologic transdermal estrogen plus oral progesterone, bisphosphonates (alendronate or risedronate) or teriparatide could be considered. Other agents, such as denosumab and testosterone in men, have not been tested in eating-disordered populations and should only be trialed on an empiric basis if there is a high clinical concern for fractures or worsening bone mineral density. A rigorous peer-based approach to establish guidelines for evaluation and management of low bone mineral density is needed in this neglected subspecialty of eating disorders.

Keywords: Bone mineral density, Osteopenia, Osteoporosis, Anorexia nervosa, Malnutrition, Premenopausal

Plain English summary

Young adults with eating disorders can develop osteoporosis, or fragile bones, which can cause lifelong debilitating consequences. Despite its high prevalence, general guidelines for diagnosis and treatment are lacking and further collaboration is needed. Some current osteoporosis medications may have severe side effects or cause birth defects in pregnant women and thus require special scrutiny. Currently, weight restoration, resumption of a regular menstrual period in women and ensuring adequate vitamin D and calcium levels are the mainstays of therapy. This review summarizes the current literature, outlines best practice recommendations and suggests areas for improvement in the field to better help these patients in the future.

Background

Eating disorders are becoming more common in the United States and currently affect approximately 20 million women and 10 million men [1]. They are defined by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM–V) as "a persistent disturbance of eating or eating-related behavior that results in altered consumption or absorption of food and that significantly impairs physical health or psychosocial functioning" [2]. Eating disorders represent a unique intersection of brain-based disorders that are associated with catastrophic physical consequences. The lifetime prevalence of anorexia nervosa (AN) is estimated to be 0.9%, for bulimia nervosa 1.5%, and for binge eating disorder

3.5% among women; with a prevalence of 0.3%, 0.5%, and 2.0% respectively among men [3–5]. AN is associated with an extraordinarily elevated premature mortality rate estimated between 4.1–5.86 per 1000 person years [6, 7], most commonly due to suicide or sudden cardiovascular complications [6, 8].

Many consequences of AN can be reversed with weight restoration and resumption of normal eating behaviors. However, other complications, such as low bone mineral density (BMD), can persist for decades after disease reso-lution [9, 10] and cause lifelong debilitation. Deterioration of bone health can be seen with AN-induced malnutrition, affecting over 90% of malnourished inpatients [11]. It is an insidious consequence of AN given its lack of clinical symptoms, but is associated with prolonged increased fracture risk [9, 10, 12–14]. Fragility fractures in the malnourished patient with AN can prove detrimental to young individuals and can lead to permanent disability. Prompt evaluation and management of low BMD is crucial in preventing fractures in this susceptible population.

Despite the prevalence of low BMD in eating disorders, clear definitions and treatment guidelines are lacking. This is primarily due to an unclear approach to diagnosis and management of low BMD in young patients in general, but also due to limited data in the eating disorder population. A rigorous peer-based approach to establishing guidelines is further needed in this neglected area of eating disorders.

Methodology

Studies published in the English language between 1996 and 2016 were searched in PubMed. We used keywords for the search including "anorexia nervosa" and "bone density" and then manually selected relevant papers based on the number of included subjects, journal impact factor and participant age range. Treatment-specific articles for this patient population were searched using additional keywords such as "premenopausal" and "treatment" and randomized controlled trials were favored for review. Several articles were referenced prior to 1996 that provided essential baseline data for this population that were not found in the abovementioned search criteria.

Definition

The World Health Organization (WHO) and International Society of Clinical Densitometry (ISCD) clearly define "osteopenia" and "osteoporosis" in postmenopausal women and men over the age of 50 [15, 16]. However, given the general lack of longitudinal data, these definitions, treatment guidelines or use of the Fracture Risk Assessment Tool (FRAX) cannot be applied to the typically younger patients with AN [17]. Bone mineral density scores, based on dual-energy X-ray absorptiometry (DXA) scans, are classified as osteoporosis if the T-score is below or equal to 2.5 standard deviations below the norm (T-score ≤ −2.5). Osteopenia is defined as a T-score between 1.0 and 2.5 standard deviations below the norm (T-score − 1.0 to −2.5). In the premenopausal population and in men under age 50, Z-scores are preferred. T-scores compare a patient's BMD to that of a healthy young adult at peak bone mass, whereas Z-scores use age and sex-matched comparisons [15, 18]. Z-scores less than 2 standard deviations below the norm (≤ −2.0) are defined as "below the expected range for age" and Z-scores greater than −2.0 meet the "expected range for age" [15, 16]. Patients with Z-scores less than −2.0, with a fracture risk or a continued cause of bone loss may be classified as having osteoporosis.

In contrast, the International Osteoporosis Foundation (IOF), recommends the use of T-scores in patients aged 20 to 50 years old and classifies osteoporosis as a T-score less than −2.5 [18]. At the current time, clinicians must ultimately use the nomenclature with which they are most familiar and make consistent therapeutic decisions based on their best clinical judgment and available data.

Prevalence

Reduced BMD is frequently seen in patients with eating disorders [9, 11, 15, 19–22]. The data consistently report evidence of low BMD in patients with AN, which includes anorexia nervosa restricting subtype (AN-R) and anorexia nervosa binge/purge subtype (AN-BP), but the data are varied in regards to its prevalence in bulimia nervosa (BN) and avoidant restrictive food intake disorder (ARFID) previously known as eating disorder not otherwise specified (ED-NOS per DSM-IV) [11, 15, 19, 23]. In one study, greater than 90% of patients with AN had a T-score less than −1.0 and 38% had a T-score less than −2.5 [11]. There are two recent meta-analyses which evaluated the association between eating disorder subtype and bone density [19, 21]. In the meta-analysis by Robinson et al., 27 studies were reviewed that reported significantly lower BMD of the spine in AN and BN patients compared to healthy controls. The meta-analysis by Solmi et al. reviewed 57 studies to find that a significantly lower BMD existed between AN and healthy controls but not between BN and controls. Fracture risk in patients with ARFID shows mixed results in the literature [10, 21]. In a study reviewing all subtypes of severely malnourished eating disorder patients requiring inpatient hospitalizations (average body mass index 13.0), 83% of all patients had low BMD. However, this study did not differentiate the prevalence of bone disease by eating disorder subtype [24].

Pathophysiology, bone abnormalities and fracture risk in anorexia nervosa

The etiology of decreased bone strength in AN is multifactorial and still under evaluation. Some patients with AN have well-established risk factors for low BMD, such as cigarette smoking or alcohol consumption [15]. Other

comorbidities including gastrointestinal malabsorption, hyperthyroidism, renal or liver disease can be seen in patients with AN, which places them at an increased risk for low bone density and may warrant a referral to an appropriate specialist [25]. Additionally, medications such as corticosteroids, some diuretics, proton pump inhibitors (PPIs), selective serotonin reuptake inhibitors (SSRIs) or antiepileptic drugs (AEDs) such as valproic acid and carbamazepine [26] may also contribute to low BMD. However, as many patients with AN have a clinical benefit from the abovementioned medications, the clinician must use caution and weigh the risks and benefits of discon-tinuing or altering doses.

Many pathophysiologic changes leading to low BMD in patients with AN are closely linked to decreased body mass index (BMI) and malnutrition [9–11, 15, 19–22]. Broadly speaking, a decrease in BMI alters body composition and leads to multiple physiologic and adaptive hormonal changes. Downregulation in the hypothalamic-pituitary-gonadal axis leads to decreased estrogen and testosterone levels [27–30]. Women and adolescent girls with AN have decreased levels of estrogen compared to healthy controls, and a study in adolescent boys demon-strated decreased serum testosterone [29–31]. This can cause clinical symptoms such as oligo-amenorrhea in females and decreased libido in males. It should be noted that not all women with AN have ovulatory dysfunction or experience oligo-amenorrhea and women can ovulate without menstruation. Amenorrhea is, therefore, no longer required for the diagnosis of AN per the new DSM-V criteria [2]. Estrogen and testosterone affect bone turnover either directly or indirectly via inflammatory cytokines and stimulation of osteoclast formation, function and survival via the receptor activator of nuclear factor kappa-B ligand pathway (RANKL).

Hypercortisolism is also seen in patients with AN, likely due to many physiologic changes including an increased basal cortisol secretion, increased steroid pulse frequency and reduced renal cortisol clearance [27, 32]. Similar to patients taking exogenous corticosteroids, hypercortisolism can lead to lower lean mass of the extremities and low BMD via decreased bone formation and increased bone resorption [33, 34].

Other hormonal shifts affected by malnutrition include a decrease in insulin-like growth factor-1 (IGF-1), leptin, insulin and oxytocin along with growth hormone (GH) resistance [35–38]. An increase in peptide YY and adiponectin, plus ghrelin resistance, is also noted and their exact relationships to bone disease are currently being studied [37, 39].

Changes in body composition in AN include a decrease in lean mass and brown adipose tissue (BAT) and an increase in bone marrow adipose tissue [40, 41]. In a small study in 2012, the presence of BAT was associated with higher BMD and only 20% of patients with AN had BAT compared to 80% of healthy comparisons [41]. Muscular pull on attached bone has an anabolic effect on bone growth and a decrease in lean mass is known to cause low BMD in AN [31, 42]. Similar to the long-term effects of malnutrition on bone, AN may also cause longstanding muscular impairment [43].

Bone strength is most frequently reflected by bone mineral density measurements but is also influenced by other factors such as bone geometry, microarchitecture, bone turnover and degree of mineralization. A DXA scan is the most common and accepted method of determining bone density given its ease of use and relatively low cost. Low bone mineral density is a common consequence of AN and has been seen repeatedly in the literature [13, 30, 44]. In a study of 75 women with active AN, an annual BMD decline of 2.6% at the spine and 2.4% at the hip was observed [45].

Bone geometry refers to the size and shape of a bone and can be analyzed with hip structural analysis on DXA. Several studies have noted impaired bone geometry in patients with AN which is associated with a higher hip fracture risk [13, 14]. With aging there is a normal, physiologic decrease in bone microarchitecture, depicted by loss and widening of vertical and horizontal trabeculae, that leads to increased bone fragility. In a study of 57 girls with AN (mean age of 15.1), bone microarchitecture (calculated via trabecular bone score) was decreased in 40% of participants when compared to a healthy cohort [46]. Interestingly, the average BMI of these individuals was relatively high at 18.9 kg/m^2 and was thought to be representative of a less severe AN population.

In multiple studies, bone turnover was found to be altered in patients with AN [30, 47, 48]. Bone turnover refers to the balance between bone resorption and bone formation as measured by markers from the peripheral blood or urine. Studies have found that bone formation markers are low and bone resorption markers are elevated in patients with AN including an increase in pyridinoline, deoxypyridinoline, N-telopeptide and C-telopeptide and a decrease in osteocalcin and total and bone-specific alkaline phosphatase [30, 47, 48]. Elevated turnover markers can still be detected during the initial 6–12 months of recovery from malnutrition but they do eventually normalize with continued nutrition and weight restoration [30, 47].

Finally, bone mineralization refers to the establishment of a mineral matrix within the bone after formation is complete. It includes the development of a collagen matrix as well as calcium and phosphate deposition within the bone. A study of 24 AN patients between 13 and 18 years of age showed significantly lower trabecular bone mineral content and volumetric bone mineral density of the forearm compared to age and height-matched adolescents [20].

The aforementioned studies demonstrate how low bone mineral density in AN results from multiple pathophysiologic processes. These data provide scientific plausi-bility for the drastically elevated fracture prevalence in patients with AN which is observed in both the short-term as well as in longstanding AN. In a study by Faje et al., 310 patients with active AN aged 12–22 were found to have an increased lifetime fracture prevalence that was 59.8% higher than age-matched comparisons. This frac-ture incidence peaked after the diagnosis of the disorder and occurred even at minimal reductions of BMD [49]. Long-term evaluations of bone health in AN suggest the severe and persistent nature of bone disease in this popu-lation [9, 10, 12, 43]. Decades after complete weight restoration, changes in bone density can still be seen, such as in a study from Switzerland demonstrating significantly reduced bone mass 27 years after complete disease recov-ery [43]. In another study, patients who had recovered from AN for as long as 21 years were still noted to have a decreased BMD [12]. These studies suggest that BMD in patients with AN never completely "catches up" or reaches the peak bone mass that otherwise would have been obtained [12, 43]. In fact, patients whose eating disorder developed in adolescence, which is the time of greatest bone mass accrual, are thought to be more prone to low BMD as near 90% of total bone mass is gained during this time [50].

An increased fracture risk has also been noted in patients with AN. One large study in Denmark evaluated 2149 AN patients and found a 2-fold increase in fracture risk 10 years after the initial diagnosis [10]. Another study found an increased fracture risk of approximately 3 standardized incidence ratio (SIR) for males and females 40 years after disease diagnosis [9]. Due to the retrospective nature of these studies, it is not known if these patients received treatment for low BMD during the course of their disease.

Approach to diagnosis and management

The majority of studies regarding bone mineral density in the eating disorder population are limited by small study populations and narrow demographics, making clear recommendations challenging. For example, male subjects are rarely included in current studies but are an increasingly important presence in the eating disorder population [1]. Clear guidelines are urgently needed given the susceptibility of the typical, young eating disorder patient to lifelong debilitating fractures. Study reviews, expert opinion and rigorous peer-based evaluation would be an optimal approach for creating standards in this heretofore neglected field of eating disorder comorbidities.

Diagnosis of low bone mineral density in eating disorders

A thorough medical and social history, physical exam and basic laboratory evaluation should be performed on all patients with AN suspected of having bone disease. Causes of secondary osteoporosis should be evaluated (Table 1) with special attention to medication use (especially steroids, diuretics, SSRIs, AEDs and depot medroxyprogesterone acetate). Basic lab work including a complete blood count, comprehensive metabolic panel, thyroid function tests, 25-hydroxyvitamin D and total testosterone in men are included in a baseline workup (Table 2). Initial DXA screening for children and adolescents is suggested if amenorrhea has been persistent for at least 6 months and serial screening is recommended yearly [51, 52]. In adults with AN, guidelines do not exist, however our current practice is to check a DXA on any male or female inpatient with an active eating disorder for 6 months or more. The frequency of follow-up screening is recommended every two years for adults according to the National Osteoporosis Foundation (NOF) [53], and in patients with worsening or persistent AN, repeat DXA may be indicated sooner if the results would change management. Patients with malnutrition as a result of ARFID or BN should also receive a screening DXA.

For patients with eating disorders, general management recommendations are discussed below. For eating disorder patients that are peri- or postmenopausal or for males over the age of 50, diagnosis and management follow WHO diagnostic criteria and guidelines, which includes thorough history taking to exclude secondary etiologies, fracture risk assessment using FRAX and pharmacologic therapy if warranted [15, 17].

Treatment of low bone mineral density in eating disorders

Aggressive management of the underlying eating disorder is the mainstay of therapy. The primary goals of this are weight restoration and resumption of spontaneous menses

Table 1 Common causes of secondary osteoporosis

Premenopausal amenorrhea
Hypercortisolism (i.e. Cushing Syndrome)
Celiac disease, inflammatory bowel disease, short gut or other malabsorption syndrome
Osteomalacia
Liver or renal disease
Low testosterone
Low body weight or malnutrition
Hyperthyroidism
Rheumatoid arthritis, Systemic lupus erythematosus, connective tissue diseases or chronic inflammatory conditions
Medications (i.e. steroids, diuretics, AEDs, depot medroxygprogesterone, SSRIs)
Diabetes mellitus
Vitamin D or calcium deficiency
Current cigarette smoking
Alcohol consumption, tobacco use

Table 2 Basic laboratory evaluation of osteoporosis

Serum chemistry panel (includes calcium, phosphorous, albumin, magnesium, liver function tests, alkaline phosphatase, creatinine)
Complete blood count
Thyroid stimulating hormone
25-hydroxyvitamin D
Testosterone panel in men

in females. Adequate intake of vitamin D and calcium should be ensured.

Meta-analyses and systematic reviews analyzing the efficacy of weight restoration in improving BMD show supportive results [28, 54–56]. BMD of the spine can increase up to 3.1% with weight gain although improvements may be slow and may not be detectable for up to 16 months [54]. In females, one study demonstrated that significant BMD improvement was only seen if the weight gain was substantial enough to result in resumption of spontaneous menses [57]. In addition to these supportive data, weight gain is the safest method of improving bone density in patients with AN, helps reverse any other concomitant disease complications and provides the foundation for sustained disease recovery.

Weight-bearing exercise and physical activity are generally recommended in non-eating disorder patients with low bone mineral density [58, 59]. Mechanical loading has osteogenic properties, can positively alter bone geometry and lead to increased bone mineral accrual in youth [60]. Physical activity can also reduce the risk of falls, which in turn decreases fragility fractures [61]. However, in patients with AN, recommendations are limited and study results vary based on the severity of illness and type of mechanical loading [62]. In a study from 2011, even moderate exercise was associated with lower lumbar and total body BMD in ill patients [62]. Exercise in patients with active AN may hasten weight loss and cause further complications associated with low body weight [62]. Adequate nutrition and the presence of spontaneous menses in females is thought to be protective to the bone as historically described by the concept of the Female Athlete Triad. This triad was modified in 2000 and is now described as a spectrum disorder with low energy availability (with or without disordered eating), menstrual dysfunction and low bone mineral density [63, 64]. Data comparing BMD in athletes and non-athletes are lacking but studies on amenorrheic and eumenorrheic athletes have shown that amenorrheic athletes have lower bone mineral density, lower estimated bone strength and abnormal bone microarchitecture compared to eumenorrheic athletes [65, 66]. Providers should make cautious, graded recommendations regarding exercise and weight-bearing activity based on the patient's degree of recovery from their malnutrition.

Vitamin D and calcium stores should be optimized in patients with low BMD and vitamin D deficiency should be treated. Vitamin D is important as it enhances intestinal resorption of calcium and phosphorous, which is essential for establishment of the bone matrix. Conflicting data exist regarding optimal vitamin D and calcium stores, however, no specific studies have been conducted in the eating disorder population. There is no evidence of improved BMD with vitamin D and calcium alone in patients with AN, however, one study did show a strong negative linear relationship between 25OH-D levels in eating disorder patients and hip BMD [67]. Patients with eating disorders are known to have significantly lower serum 25OH-D and 1,25OH-D levels compared to healthy controls despite reportedly similar vitamin D intake [68]. The Endocrine Society defines vitamin D deficiency as a serum level < 20 ng/mL and insufficiency between 21 and 29 ng/mL. Patients with vitamin D deficiency should be treated with 50,000 international units (IU) of vitamin D (frequently ergocalciferol) weekly until serum levels are >30 ng/mL, followed by maintenance dosing for fracture prevention. Patients with vitamin D insufficiency can be started on 600-800 IU daily, however, may require doses between 1500 and 2000 IU daily if their level does not improve to >30 ng/mL. If patients have evidence of low bone mineral density but have normal vitamin D levels, they should still be started on daily maintenance vitamin D supplementation of 600-800 IU [69–71]. Approximately 1200 mg of calcium are suggested as the daily dosage for optimal bone health but recommendations change based on age and sex [71] (Table 3). Alimentary calcium tends to be better tolerated and absorbed than supplementation. If supplements are required, calcium carbonate is typically used unless the patient is on a proton-pump inhibitor (PPI) or H2-blocker, in which case calcium citrate is preferred [71].

For adult, premenopausal patients with eating disorders who have persistent risk for BMD loss (i.e. active disease), a Z-score ≤ −2, a history or fractures or are at a high risk for fractures can be considered for pharmacologic therapy

Table 3 Daily Calcium Intake Reference (adapted from the Institute of Medicine 2010)

Age	Estimated Average Requirement of Calcium (mg/day)	Recommended Dietary Allowance of Calcium (mg/day)	Upper Level Intake of Calcium (mg/day)
14–18 years old	1100	1300	3000
19–30 years old	800	1000	2500
31–50 years old	800	1000	2500
51–70 year old males	800	1000	2000
51–70 year old females	1000	1200	2000

of osteoporosis. The abovementioned recommendations regarding weight restoration, resumption of spontaneous menses and repletion of vitamin D and calcium stores are prerequisites for further pharmacologic management and should be discussed at length with patients.

Specifically for patients with bone age ≥ 15 years, physiologic transdermal estrogen with oral progesterone can be considered for treatment of osteoporosis as it improved BMD in one study of female adolescents with AN [72]. Oral contraceptive pills (OCPs) are insufficient in improving BMD in AN due to a suspected estrogen-induced suppression of insulin-like growth factor I (IGF-I), which is a bone anabolic agent [28, 73, 74]. One study, however, did find a bone density increase of 4% with OCPs in a severely malnourished subset of patients who weighed <70% of their ideal body weight [73], yet these data have not been reproduced elsewhere. OCPs had positive effects on bone density in other populations of healthy, premeno-pausal women, but the evidence is conflicting and OCPs are therefore not recommended for this purpose [75, 76].

Testosterone is a known anabolic bone agent and is deficient in females and adolescent boys with AN [31, 77, 78]. One study in females with AN found that lean mass, but not BMD, increased with use of transdermal testosterone replacement [79]. Hypogonadism is a principal cause of osteoporosis in men without disordered eating and testos-terone administration has been effective in increasing BMD in males with central and primary hypogonadism [80, 81]. For example, one study found an impressive spinal BMD increase of 5% and a 7% lean mass increase after 18 months of intramuscular testosterone therapy (100 mg/week) [80]. Most recently, transdermal testosterone treatment in older men with low testosterone significantly increased BMD after one year [82]. No studies have evaluated the preva-lence of acquired hypogonadism in adult males with AN nor the possible efficacy of testosterone replacement in this population. Similar to estrogen, testosterone should not be used in patients with a bone age ≤ 15 years or in the pres-ence of unfused epiphyses. If testosterone stores are replete and a high fracture risk exists due to ongoing AN, use of other pharmacologic agents could be considered for males.

Bisphosphonates improve bone mineral density by inhibiting osteoclast activity and reducing bone resorption and turnover. They become integrated into the bone matrix and due to their half-life of >10 years, their function is still noted long after treatment cessation. Bisphosphonates have been in use for over 20 years and have been studied in pa-tients with AN. Different formulations of bisphosphonates appear to be equally effective [83] and, in postmenopausal women, they have been shown to reduce the risk of fracture by approximately 50% [84]. Two bisphosphonates (alendro-nate and risedronate) have been approved by the US Food and Drug Administration (FDA) for treatment of premenopausal osteoporosis in patients with steroid-induced osteoporosis and have also been shown to improve BMD in cases of osteoporosis caused by pregnancy, lactation, cystic fibrosis and thalassemia [85–88]. In eating disorder patients, two randomized controlled trials were performed showing efficacy of bisphosphonates (alendronate and risedronate) with an approximate BMD gain of 2–4.4% [79, 89]. The alendronate study, however, found weight restoration to be the most important determinant of BMD improvement at follow-up and BMD was higher at the femoral neck with alendronate only after correcting for change in body weight [89]. Bisphosphonates are classified as pregnancy risk factor C, can cross the blood-placenta barrier and lead to fetal hypocalcemia [90, 91]. In the typical young, female AN patient with the potential to regain fertility, contraception is recommended during and after treatment but their use is generally discouraged. Bisphosphonates have an appealing role in the treatment of male AN patients with osteoporosis given the irrelevance of teratogenic risk, however, this has not yet been studied.

Teriparatide is a recombinant 1–34 parathyroid hormone and an anabolic bone growth agent that stimulates preosteoblasts and calcium reabsorption in the kidneys. It is approved by the FDA for treatment of osteoporosis in adults and has been used in premenopausal women with steroid-induced osteoporosis with a high fracture risk [76] and in patients with idiopathic osteoporosis [92]. Given the black box warning of osteosarcoma, fusion of the epiphyses should be well documented in patients under the age of 25 [92]. One recent randomized controlled trial in eating disorder patients demonstrated a dramatic BMD increase of 6–10% [93]. Given the coupled nature of the bone remodeling properties of teriparatide, prolonged use (>24 months) leads to osteoclast stimulation and therefore bone resorption and loss of BMD. Teriparatide cessation without sequential antiresorptive therapy leads to loss of bone mass in postmenopausal women and declines by 4.8% at the lumbar spine in patients with premenopausal idiopathic osteoporosis [94]. In a randomized controlled trial of post-menopausal women using teriparatide (DATA-Switch study), sequential therapy with denosumab resulted in a continued increase of BMD after cessation of teriparatide [95]. It is likely that patients with AN would require antiresorptive therapy after teriparatide although this has not been evaluated. Long-term effects of teriparatide on the premenopausal population have not been studied and this medication should therefore be used with caution in these patients. Similar to bisphosphonates, teriparatide has a pregnancy risk factor C, however, no long term pregnancy-related risks have been noted.

Denosumab has not yet been studied in patients with eating disorders. However, denosumab is FDA approved for the treatment of post-menopausal osteoporosis [96].

It is a human monoclonal antibody that inhibits osteoclast activation via binding on the RANK ligand. There are lim-ited studies on the use of denosumab in premenopausal women but it is an appealing option in younger populations given its ease of administration (one injection every six months) and shorter half-life compared to bisphosphonates (25.4 days). Denosumab is contraindicated in pregnancy and premenopausal women on this medication should be on contraception during and up to five months after com-pletion of treatment. Patient consent of the risks and bene-fits of this medication should be well documented. Other long-term side effects on the premenopausal population have not been studied.

Romosozumab is a monoclonal antibody that binds and inhibits sclerostin and is a new, emerging option for osteoporosis management in post-menopausal women and may eventually have a role in the treatment of osteoporosis in eating disorder patients. Sclerostin is produced by osteocytes which inhibits bone formation and was found to be elevated in a cohort of females with AN [97, 98].

Conclusion

There are currently no existing guidelines for the treatment of eating disorder patients with low bone mineral density. This review aims to provide a summary of the literature to date and current options for prevention and management, however, a standardized approach for low BMD in this patient population through rigorous peer-review is needed. Future studies are warranted regarding use of osteoporosis agents, such as denosumab, in patients with AN as well as evaluation of the long-term side effects of these medications in younger populations.

Based on the current literature, if a patient with AN has evidence of low BMD, weight restoration with resumption of spontaneous menses is the mainstay of therapy. Patients should be screened for causes of secondary bone mineral loss and a thorough history and physical exam should be performed. Baseline laboratory data should be obtained and vitamin D and calcium stores optimized. Weight-bearing exercise should be avoided initially but can be gradually reintroduced at the discretion of the treatment team if weight gain is achieved. Postmenopausal women or men over the age of 50 with an eating disorder should be managed per WHO recommendations [15]. Premenopausal women or men under the age of 50 who have a Z-score ≤ −2, history of or risk for fracture or ongoing bone mineral loss can be considered for use of pharmacologic osteoporosis agents as a bridge until full weight restoration occurs. Bone age should be greater than 15 years prior to initiating pharmacologic or hormonal treatment in younger patients.

Ultimately, deciding which pharmacologic agent to use is often a question of administration logistics and cost and should be discussed with the patient and tailored to their specific needs. Other factors, such as previous pharmacologic treatment, possibility of future pregnancy, disease severity, medication side effect profile, likelihood of medication compliance and follow up care, can affect selection of the appropriate agent. Evidence of efficacy in eating disorder patients has been demonstrated with the following: physiologic transdermal estrogen plus oral progesterone, bisphosphonates (alendronate or risedronate) and teriperatide. There are no definitive guidelines when treating osteoporosis in males with eating disorders, however, if concomitant hypogonadism is detected, treating with testosterone is reasonable. Denosumab has not been studied in premenopausal eating disorder patients but is an appealing option based on its ease of administration, shorter half-life and lack of skeletal accumulation. Further research is needed in this group of patients who are highly susceptible to rapid loss of bone mineral density to prevent fractures and potentially debilitating and irreversible deformities.

Abbreviations

AEDs: Antiepileptic drugs; AN-BP: Anorexia nervosa-binge purge subtype; AN-R: Anorexia nervosa-restricting subtype; ARFID: Avoidant/restrictive food intake disorder; BAT: Brown adipose tissue; BMD: Bone mineral density; BMI: Body mass index; BN: Bulimia nervosa; D2: Ergocalciferol; D3: Cholecalciferol; DSM – V: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; DXA: Dual-energy X-ray absorptiometry; ED-NOS: Eating disorder not otherwise specified; FDA: US Food and Drug Administration; FRAX: Fracture risk assessment tool; GH: Growth hormone; IGF-1: Insulin-like growth factor-1; IOF: International Osteoporosis Foundation; ISCD: International Society of Clinical Densitometry; NOF: National Osteoporosis Foundation; OCP: Oral contraceptive pills; PPIs: Proton-pump inhibitors; PTH: Parathyroid hormone; RANKL: Receptor activator of nuclear factor kappa-B ligand; SIR: Standardized incidence ratio; SSRIs: Selective serotonin reuptake inhibitors; WHO: World Health Organization

Competing interests

The authors declare that they have no competing interests

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Vegetarian-style dietary pattern during adolescence has long-term positive impact on bone from adolescence to young adulthood: a longitudinal study

Abstract

Background: The amount of bone accrued during adolescence is an important determinant of later osteoporosis risk. Little is known about the influence of dietary patterns (DPs) on the bone during adolescence and their potential long-term implications into adulthood. We examined the role of adolescent DPs on adolescent and young adult bone and change in DPs from adolescence to young adulthood.

Methods: We recruited participants from the Saskatchewan Pediatric Bone Mineral Accrual Study (1991–2011). Data from 125 participants (53 females) for adolescent analysis (age 12.7 ± 2 years) and 115 participants (51 females) for adult analysis (age 28.2 ± 3 years) were included. Bone mineral content (BMC) and areal bone mineral density (aBMD) of total body (TB), femoral neck (FN) and lumbar spine (LS) were measured using dual-energy X-ray absorptiometry. Adolescent dietary intake data from multiple 24-h recalls were summarized into 25 food group intakes and were used in the principal component analysis to derive DPs during adolescence. Associations between adolescent DPs and adolescent or adult BMC/BMD were analyzed using multiple linear regression and multivariate analysis of covariance while adjusting for sex, age, the age of peak height velocity, height, weight, physical activity and total energy intake. Generalized estimating equations were used for tracking DPs.

Results: We derived five DPs including "Vegetarian-style", "Western-like", "High-fat, high-protein", "Mixed" and "Snack" DPs. The "Vegetarian-style" DP was a positive independent predictor of adolescent TBBMC, and adult TBBMC, TBaBMD ($P < 0.05$). Mean adolescent TBaBMD and young adult TBBMC, TBaBMD, FNBMC and FNaBMD were 5%, 8.5%, 6%, 10.6% and 9% higher, respectively, in third quartile of "Vegetarian-style" DP compared to first quartile ($P < 0.05$). We found a moderate tracking (0.47–0.63, $P < 0.001$) in DP scores at individual levels from adolescence to adulthood. There were an upward trend in adherence to "Vegetarian-style" DP and an downward trend in adherence to "High-fat, high-protein" DP from adolescence to young adulthood ($P < 0.01$).

Conclusion: A "Vegetarian-style" DP rich in dark green vegetables, eggs, non-refined grains, 100% fruit juice, legumes/nuts/seeds, added fats, fruits and low-fat milk during adolescence is positively associated with bone health.

Keywords: Dietary patterns, Vegetarian, Adolescence, Bone mineral content, Bone mineral density, Young adulthood

Background

Peak bone mass (PBM) attained by the end of adolescence is an early determinant of osteoporosis risk in older populations [1]. During adolescence, bone linear growth, and subsequent mineral deposition increase substantially [2]. The greatest rate of growth in height during this time is termed as peak height velocity (PHV). The PHV is consid-ered as one of the main indicators of somatic maturation, the stage during which males and females are at a compar-able sexual development milestone [3]. More than 39% of total body PBM is acquired during a 5-year period around PHV, and around 99% is attained by 6 years after attain-ment of PBM [4]. This suggests that modification of the factors that contribute to PBM attainment during adoles-cence might impact the risk of osteoporosis later in life [1].

Nutrition is an important modifiable factor, which could influence bone accrual, maintenance, and loss during one's lifetime [1, 5]. Diet is a complex combination of nutrients and dietary components that correlate or interact with each other. Even though the separate role of key nutrients, or foods, on bone health has been reported previously, these associations might be confounded by any change in the other dietary components. Dietary pattern (DP) approaches describe and quantify the whole diet and consider contributions from various dietary aspects [6]. Findings from DP studies could complement those from studies of single nutrients and foods on bone accrual and may be translated into public health recommendations, which better suit real world dietary habits.

In adults and elderly, several studies have investigated the association between DPs derived by an exploratory method, mainly factor analysis, and bone health [7–20]. However, little is known about the DPs influencing bone health during adolescence [21–24], and their potential long-term implications. Therefore, longitudinal studies that follow participants from adolescence to adulthood are of immense importance because they could bridge the current gap in knowledge.

The objectives of our study are: 1) to examine the association between adolescent DPs and adolescent and young adult bone measurements including total body (TB), femoral neck (FN) and lumbar spine (LS) bone mineral content (BMC) and areal bone mineral density (aBMD), and 2) to evaluate the stability of DPs from adolescence to young adulthood. We hypothesized that a "healthy" DP, with an emphasis on higher intake of fruits, vegetables would be beneficial for adolescence and young adulthood bone health; and DPs remain relatively stable over time from adolescence to young adulthood.

Methods

Participants

We recruited participants from the Saskatchewan Pediatric Bone Mineral Accrual Study (PBMAS) (1991–2011). The mixed longitudinal design of the study has been described in detail elsewhere [3, 4, 25]. In brief, the PBMAS cohort consists of 251 individuals (133 girls and 118 boys; aged 8 to 15 years) recruited from two elementary schools in the city of Saskatoon between 1991 and 1993 who were subsequently followed with annual follow-ups until 2011. There were two four-year breaks in annual measurements: one between 1997 and 2002 and one between 2005 and 2010. The ages of the participants at the final follow-up were between 24 to 32 years. At each measurement occasion, participants underwent dual-energy X-ray absorptiometry (DXA) scans for bone and body composition. Anthropometry, dietary intake, and physical activity were also assessed at each measurement point.

For the present study, the first measurement within the age of PHV ± 2 years was considered as the adolescent measurement. For most participants ($n = 105$), the data collected during 1992 or 1993 were included in the analysis as adolescent data. The data collected during 2010 or 2011 were included in the analysis as young adult data. We included data from 125 participants (age 12.7 ± 2 years, 53 females) for adolescent analysis (cross-sectional) and 115 participants (age 28.2 ± 3 years, 51 females) for adolescence to young adult analysis (longitudinal). All participants or their parents provided informed written consent. Ethics approval was obtained from the University of Saskatchewan and Royal Hospital advisory boards on ethics in human experimentation [25].

Dietary intake

The dietary intakes of participants were assessed using 24-h recalls. To determine accurate estimates of portion sizes, participants had access to pictures of foods. Adolescent dietary intakes were assessed by two to four (mostly three) 24-h recalls collected over a year and were analyzed using the Canadian compatible nutrition assessment software: NUTS Nutritional Assessment System, version 3.7 (Quilchena Consulting Ltd., Victoria, BC, 1988) to estimate the daily total energy and nutrient intakes. The average dietary intakes per day during the study year were stratified with the other annual measurements during the same year. To include in DP analysis, first, we converted quantities of all consumed foods and beverages into grams per day; then, all items were assigned into 25 pre-defined non-overlapping food groups, manually, based on similar nutrient content or culinary usage of them (Table 1). Young adult dietary intakes were assessed using one 24-h recall and estimates of total energy and nutrient intakes were obtained using Food Processor version 8.0 and its revisions (ESHA Research Inc., Salem, Ore, 2003).

Bone mineral content and areal density

Adolescent and young adult BMC and aBMD of TB, FN and LS (L1–L4) were measured using DXA (Hologic

Table 1 Food groupings used for principal component analysis to identify dietary patterns during adolescence

Food groups	Food items
Dark green vegetables	Asparagus, green beans, broccoli, lettuce, green pepper, seaweed, spinach, mixed greens, snow peas
Eggs	Eggs
Non-refined grains	Whole grains and partially whole grains (60%) mostly cereals, mixed granola/grain bar, cracker, oat flakes, wheat germ, whole wheat breads, puffed wheat, brown and wild rice, popcorn, barley
Fruit juice 100%	Apple cider, apple, lemon, lime, orange juice canned or bottled, unsweetened cranberry, etc.
Legumes, nuts and seeds	Beans (black, kidney, lima, navy, small white, soy), chickpeas, hummus, tofu, brazil nuts, coconut, almond, hazelnuts, walnuts, cashew, peanuts, mixed nuts, pecans, peanut butter, sunflower seeds
Added fats	Saturated fats such as butter, margarine, meatless bacon bits and coconut oil, and unsaturated fats such as vegetable oil, cooking oil, mayonnaise, olive oil, pesto
Fruits	All fresh and dried fruits, canned fruits (not sweetened), avocado, olives
Low-fat milk	1%, skim, rice beverage, soy beverage
Fruit drinks	Fruit juice (sweetened), fruit drinks, iced tea
Refined grains	Refined cereals, white bread, white rice, refined pasta, noodles, pop corns, pie crust, pizza pop
Cream	Sour cream, cream (10%, whipped or low fat)
Poultry	Chicken and turkey
Processed meats	Burger patties (beef, ham, chicken, etc.), sausages, bacon, canned meat, dry ribs, fried chicken, nugget
High-fat milk	2%, whole or almond milk
Tomato	Tomato and its products
Red meat	Beef, ham, pork, bison (ground, loin, rib, steak, stew, fried, pot roast, balls, loaf, chop)
Cheese	Cheddar, cream cheese, feta, gouda, mozzarella, parmesan, Swiss, cottage, ricotta, cheese sauce
Yogurt	Yogurt (plain, vanilla or fruit)
Desserts and sweets	Sweet baked products, milk desserts, jelly, chocolate, sugar, jam, syrups, honey and candies
Fish and seafood	Fish, shrimp, lobster, mussels, pickerel, prawns, scallops
Dressings, sauces, gravy	Gravy, dressings, Caesar, French, ranch, Italian, 1000 island, Alfredo, blue cheese, chip dip, Greek, honey garlic, white sauce, sandwich spread, tartar, teen, sundried tomato
Vegetables, others	Carrots, snap beans, cabbage, cauliflower, celery, cucumber, garlic, mushroom, pepper, squash, bean sprouts, beets, onion, eggplant, radish, zucchini, potato, green peas, corn, sweet potato and soups
Chips & fries	Potato chips, fries, corn chips, nacho, hash brown
Soft drinks	Soft drinks (sugar-sweetened or diet)
Others	Salt, spices, seasonings, additives, pickles (dill, beet), low fat sauces (mustard, hot, soy, teriyaki), vinegar

QDR 2000, Hologic, Inc., Waltham, MA, USA) in the array mode; and analysis was conducted using enhanced global software version 7.1 [26]. To minimize operator-related variability in the scan analysis over the years, the same trained person analyzed all scans. The TB scans were analyzed using software version 5.67A and scans of the FN and LS were analyzed using software version 4.66A. The in vivo coefficients of variations, which represent short-term precision, were comparable to the values from other studies employing the QDR 2000 in the array mode (0.60, 0.91 and 0.61 for TB, FN, and LS BMC, respectively).

Physical activity

Physical activity was defined as sports, games, or dance that makes you breathe hard, makes your legs feel tired, or makes you sweat. The physical activity questionnaire (PAQ) was used to assess adolescent physical activity during spare time in the previous 7 days by rating nine items in elementary schools or eight items in high schools (excluding the item regarding activity at recess) scored on a five-point scale [27]. Six of these questions were related to scaling the level of different activities in physical education classes, recess, lunch, right after school, in the evenings and on the weekend. Other three questions were asking about the frequency of physical activity during each day, the number of hours spent for watching TV, and describing the whole week activity from low to very high activity levels [28]. The average score derived from each PAQ ranged from one to five, with higher scores indicating higher levels of physical activity. To assess young adult physical activity, PAQ was modified to a 7-item questionnaire including more age-relevant activities. The school-day structure of questions was replaced with a day section structure (i.e., morning, after lunch, before supper, evening) in the PAQ for adults [28]. The PAQ was administered three times a year during first 3 years of study and two times a year thereafter. The average PA scores derived from PAQs

collected during each year were aligned with the other annual measurements [26].

Anthropometry and age of PHV

Weight and stature were measured following standard protocols for each participant while wearing lightweight clothing and no shoes [25]. To control for somatic maturity, the age of PHV for each participant was estimated. The process for determining PHV has been described elsewhere [26]. In brief, whole-year height increase velocity was computed using serial measurements of height for each participant by age. Using a cubic spline procedure, a growth curve was fitted to each individual's annual height velocities (GraphPad Prism Version 3.00) and the age of PHV was determined from the estimated growth curve [26].

Statistical analysis

The DPs were identified using factor analysis via princi-pal component analysis (PCA). The PCA aggregates the food groups into a smaller number of the distinct factors based on inter-correlation between them [6, 29]. To achieve a simpler structure with higher interpretability, orthogonal rotation (Varimax option) was applied. Over-all, 11 factors were extracted using PCA with an eigen-value > 1 accounting for 66% of the total variance in all food group intakes. Based on the breakpoint in scree plot, we retained 5 major factors (accounting for almost 40% of the total variance) for further evaluation and re-ran the analysis with a five-factor solution. Factor load-ings represent the correlation between food groups and the factors (Table 2). The absolute value represents the strength of the correlation. A positive loading shows a direct association and a negative loading shows an in-verse association between the food group intake and DP score. Food groups with a factor loading ≥0.35 or ≤ − 0.35 were considered informative for interpretation of DPs in our study. Regression scores for each DP were calculated using the regression scores option in SPSS. Calculating regression scores enhances the validity of DP scores and reduces the probability of biased esti-mates of the true scores [30].

Descriptive statistics for all bone variables (TBBMC, TBaBMD, FNBMC, FNaBMD, LSBMC, LSaBMD), and covariate variables (age, the age of PHV, height, weight, physical activity score and total energy intake) were presented as mean ± SD in adolescence and young adulthood. We used independent Student's t-test to compare variables of interest between females and males. Multiple linear regression using stepwise procedure were conducted to evaluate associations between adolescence DP and adolescence bone measurements. To assess the long-term impact of DPs on the bone, we also ran the same modeling with adolescent DP scores as predictor variables, and young adulthood bone measurements as outcome variables. All models were adjusted for sex, the age of PHV, age, height, weight, physical activity score and total energy intake. Covariates measured during adolescence and young

Table 2 Factor loading of food groups in five dietary patterns identified by principal component analysis during adolescence, in participants of Pediatric Bone Mineral Accrual Study (PBMAS), $n = 125$[1]

	Factor Loadings for Dietary Patterns				
	Vegetarian-Style	Western-Like	High-Fat, High-Protein	Mixed	Snack
Dark green vegetables	**0.64**	0.02	− 0.00	0.07	− 0.22
Eggs	**0.63**	− 0.18	0.23	− 0.05	− 0.15
Non-refined grains	**0.54**	− 0.13	− 0.11	0.10	0.20
Added fats	**0.41**	**0.39**	− 0.03	− 0.04	− 0.00
Fruits	**0.40**	0.24	− 0.16	0.13	0.23
Others	− 0.28	0.03	0.08	0.08	0.04
Fruit drinks	0.00	**0.73**	− 0.04	− 0.03	0.04
Refined grains	0.06	**0.66**	0.21	− 0.10	− 0.03
Cream	− 0.06	**0.55**	− 0.01	0.13	− 0.02
Poultry	− 0.27	**0.41**	− 0.04	− 0.10	**0.40**
Processed meats	− 0.05	**0.35**	− 0.12	0.01	− 0.09
High-fat milk	− 0.12	− 0.17	**0.74**	− 0.04	0.18
Tomato	0.22	0.30	**0.59**	− 0.14	− 0.34
Red meat	− 0.07	− 0.05	**0.52**	0.14	− 0.07
Low-fat milk	**0.35**	0.03	**− 0.48**	− 0.01	− 0.16
Legumes, nuts, and seeds	**0.45**	0.11	**0.47**	− 0.09	0.06
Cheese	0.03	0.12	0.06	**0.72**	**− 0.36**
Yogurt	− 0.11	0.04	− 0.12	**0.61**	0.19
Desserts and sweets	− 0.18	− 0.05	0.23	**0.59**	0.08
Fish and seafood	0.24	− 0.10	−0.08	**0.52**	− 0.13
Fruit juice 100%	**0.46**	0.02	− 0.04	**0.49**	0.18
Dressings, sauces, gravy	0.09	− 0.30	0.24	0.08	**0.64**
Vegetables, others	− 0.03	0.22	0.06	− 0.03	**0.58**
Chips & fries	− 0.03	− 0.09	− 0.02	0.00	**0.40**
Soft drinks	0.00	− 0.02	− 0.16	− 0.20	0.20
% Of variance explained	9.2	8.5	7.8	7.7	6.7

[1]Factor loadings ≥0.35 or ≤ − 0.35 have been presented
The bold numbers represent the foods with significant positive or negative loading in each pattern

adulthood were used in the adolescence and young adulthood models, respectively.

Comparisons of the mean adolescence or young adult bone variables across the quartile categories of adolescent DP score were conducted via a multivariate analysis of covariance (MANCOVA) (with a Bonferroni adjustment for multiple comparisons) while adjusting for scores of the other four DPs (as continuous variables), sex, age of PHV, age, height, weight, physical activity score and total energy intake.

To evaluate the stability of DPs from adolescence to young adulthood, we calculated applied DP scores during adolescence and young adulthood, based on the factor loadings for 25 food groups in five DPs derived during adolescence. To control for the overall increase in con-sumption of food groups by age from adolescence to young adulthood, we computed the consumed amount (g) per 1000 kcal of total energy intake for each food group. Then, these energy-adjusted intakes were multiplied by their corresponding factor loading in each DP and were summed up as the DP score. We standardized adolescence and young adulthood DP scores for mean and standard deviation of adolescence DP scores in our sample. Then we calculated tracking coefficients using generalized estimating equations (GEE). Tracking coefficient represents how position of participants in a study population distribution is maintained from baseline to the last follow-up [31]. We regressed adolescence standardized DP scores (independent variable) against young adulthood standardized DP scores (dependent variable) while adjusting for chronological age as the time-dependent variable, and sex and age at adolescence as time-independent variables. The ß coefficient of adolescence standardized DP scores takes values between 0 to 1, representing no tracking and strong tracking, respectively. The ß coefficient for chronological age indicates the change in DP score as z-score or SD for each year increase in age.

The DP analysis and all other statistical analyses were performed using SPSS software, version 24.0 (SPSS, Chicago, lL, USA). $P < 0.05$ was considered significant.

Results

The characteristics of the study population during adolescence and young adulthood are shown in Table 3. Our estimated mean ± SD follow-up period from adolescence to young adulthood was 15.5 ± 3.4 years. The first factor, labeled as "Vegetarian-style" DP, was rich in dark green vegetables, eggs, non-refined grains, 100% fruit juice, legumes, nuts and seeds, added fats, fruits and low-fat milk (including non-dairy milk). The second factor, a "Western-like" DP was associated with higher intakes of fruit drinks, refined grains, cream, poultry and processed meats. The most significant characteristic of the third factor, "high fat, high protein" DP, was high positive loadings for High-fat milk, tomato, red meat and legumes, nuts and seeds and a negative loading for low-fat milk. The fourth factor, a "Mixed" DP, was characterized by a high intake of yogurt, cheese, desserts and sweets, fish and seafood and 100% fruit juice. Dressings and sauces, vegetables (excluding dark green vegetables), chips and fries and poultry had high positive loadings and cheese had a negative loading in the fifth factor, labeled a "Snack" DP (Table 2).

After controlling for covariates (sex, age of PHV and adolescent age, height, weight, physical activity score and total energy intake), multiple linear regression

Table 3 Descriptive characteristics during adolescence and young adulthood by sex[1]

	Females	Males	Total
Adolescence	*n* = 53	*n* = 72	n = 125
Biologic age[2] (year)	0.2 ± 1.7	−0.1 ± 1.8	0.0 ± 1.7
Age (year)	12.0 ± 1.8	13.2 ± 1.8*	12.7 ± 1.9
Age of PHV (year)	11.8 ± 0.8	13.2 ± 0.9	12.6 ± 1.2
Physical activity (score)	2.9 ± 0.7	3.0 ± 0.6	3.0 ± 0.7
Total energy intake (kcal/d)	1714 ± 461	1978 ± 615*	1867 ± 569
Height (cm)	153 ± 11	162 ± 14**	158 ± 13
Weight (kg)	46.0 ± 14	52.4 ± 14*	49.8 ± 14
TBBMC (g)	1402 ± 452	1751 ± 612**	1604 ± 575
TBaBMD (g/cm^2)	0.87 ± 0.10	0.94 ± 0.12*	0.91 ± 0.11
FNBMC (g)	3.3 ± 0.8	4.1 ± 1.0**	3.8 ± 1.0
FNaBMD (g/cm^2)	0.73 ± 0.13	0.81 ± 0.13*	0.77 ± 0.13
LSBMC (g)	35.8 ± 13.4	40.8 ± 16.0	38.7 ± 15.1
LAaBMD (g/cm^2)	0.76 ± 0.15	0.75 ± 0.14	0.76 ± 0.14
Young adulthood	*n* = 51	*n* = 64	n = 115
Biologic age[2] (year)	16.1 ± 3.5	15.0 ± 3.3	15.5 ± 3.4
Age (year)	27.9 ± 3.4	28.3 ± 3.4	28.2 ± 3.4
Physical activity (score)	2.3 ± 0.6	2.3 ± 0.7	2.3 ± 0.6
Total energy intake (kcal/d)	1823 ± 698	2823 ± 1235**	2401 ± 1151
Height (cm)	166 ± 7	179 ± 7**	174 ± 9
Weight (kg)	70.7 ± 16	87.0 ± 14**	80.3 ± 16
TBBMC (g)	2286 ± 321	3020 ± 413**	2706 ± 523
TBaBMD (g/cm^2)	1.12 ± 0.09	1.22 ± 0.10**	1.18 ± 0.11
FNBMC (g)	4.3 ± 0.7	5.6 ± 0.8**	5.0 ± 0.9
FNaBMD (g/cm^2)	0.86 ± 0.10	0.95 ± 0.128**	0.91 ± 0.12
LSBMC (g)	62.0 ± 12.6	76.2 ± 12.8**	70.3 ± 14.5
LSaBMD (g/cm^2)	1.04 ± 0.12	1.06 ± 0.12	1.05 ± 0.12

Abbreviations: *aBMD* areal bone mineral density, *BMC* bone mineral accrual, *FN* femoral neck, *LS* lumber spine, *PBMAS* the pediatric bone mineral accrual study, *PHV* the peak height velocity, *TB* total body

[1]Values are Mean ± SD. *P* values were obtained using independent samples Student's *t* test. *Different from females, $P < 0.01$. **Different from females, $P < 0.001$

[2]Biologic age is calculated as chronologic age minus the age of PHV

showed that the "Vegetarian-style" DP was a positive independent predictor of adolescent TBBMC (β =35.2, P = 0.025; R^2 = 0.84) and young adult TBBMC (β = 55.8, P = 0.021; R^2 = 0.78), TBaBMD (β = 0.016, P = 0.041; R^2 = 0.67). No other adolescent DP was found to be an independent predictor for any of the adolescent or young adult bone variables.

Comparison of adolescent or young adult bone variables across adolescent DP score quartiles showed that, those in the third quartile of "Vegetarian-style" DP had 5.7%, 8.5%, 6%, 10.6% and 9% higher adolescent TBaBMD (Table 4), and young adult TBBMC, TBaBMD, FNBMC and FNaBMD (Table 5), respectively, compared to their peers in the lowest quartile, after adjusting for covariates and other four DP scores as continuous variables.

Tracking coefficients for standardized scores of five DPs and change in the score by age from adolescence to young adulthood are presented in Table 6. The greater tracking coefficients show the higher stability of DPs at the individual level. Since DP scores have been standardized for the baseline DP scores, ß coefficient for age variable represents the amount of change in z-score. Overall, energy-adjusted scores increased for "Vegetarian-style" and decreased for "High-fat, high-protein" DP, from adolescence to young adulthood (Table 6).

Discussion

In our prospective study, we found that a "Vegetarian-style" DP rich in dark green vegetables, eggs, non-refined grains, 100% fruit juice, legumes, nuts and seeds, added fats, fruits and low-fat milk during adolescence was associated positively with adolescent TBBMC and TBaBMD. We also found that participants who had higher adherence to the "Vegetarian-style" DP during adolescence had higher TBBMC, TBaBMD, FNBMC and FNaBMD during young adulthood, average 15 years later. Tracking DP scores showed that participants moderately maintained their position in the study population distribution from adolescence to young adulthood, which means DPs were relatively stable over time. However, the overall adherence to "Vegetarian-style" DP increased from adolescence to young adulthood.

In the present study, the favorable effects of the "Vegetarian-style" DP were only observed in TB and FN bone measurements, but not in LS bone. This might be due to the different proportions of cortical and trabecular bone compartments in different skeletal sites. The trabecular bone is the predominant bone compartment in LS, while TB and FN mainly contain cortical bone [32, 33]. Trabecular bone is metabolically more active than cortical bone and might be influenced by everyday changes in hormone or environmental factors. Hence adaptations in bone might last longer in cortical compared to trabecular bone [34].

Our study is unique as it evaluated the long-term impact of adolescent DPs on young adult bone. To our knowledge, there are only four studies that evaluated the DPs during adolescence in association with bone health [21–24]. Even though three of these studies were similar to our study in their prospective design (follow-up period ranged from 22 months to 6 years) [21, 23, 24], identified DPs are not directly comparable, because of the differences in DP approaches, food groupings and dietary habits and other characteristics of the study population [3, 6]. However, our findings of a positive association between "Vegetarian-style" DP and bone measurements are in accordance with the results from two studies which used reduced-rank regression (RRR) to derive DPs. The RRR has the advantage of deriving DPs associated with bone variables such as BMD and BMC [21] or intermediate factors such as protein, calcium, and potassium [2], as response variables. In Korean girls (aged 9–11 years, n = 198), the RRR-derived "fruits, nuts, milk beverages, eggs, and grains" DP was associated positively and "egg and rice" DP was associated negatively with BMC gain after 22 months [21]. Also, a higher intake of low-fat dairy, whole grains, and vegetables, as components of a DP rich in protein, calcium and potassium in Australian adolescents (aged 14 years, n = 1024) was associated with higher BMD and BMC at age 20 years [24]. Overall, higher intakes of fruit and vegetables, milk and alternatives, nuts and grains were the common components in all DPs which determined to be beneficial for bone [2, 21]).

Our results are also in line with the findings from previous DP studies in adults and elderly populations suggesting that a high intake of fruit and vegetables, whole grains, poultry and fish, nuts and legumes and low-fat dairy products labeled as "healthy" DP is beneficial for bone health [7–10, 12–14, 16, 17]. Vegetables, fruits, and 100% fruit juices are rich in potassium, magnesium, vitamins C, K and folate and carotenoids [35]. Potassium and magnesium may contribute to acid-base balance [35] and calcium metabolism [36, 37] to prevent bone loss. Vitamin C, carotenoids, and other antioxidants may affect bone health through their antioxidant properties, which suppress osteoclast activity [38, 39]. Vitamin C also acts as a cofactor for osteoblast differentiation and collagen formation [38, 40]. Vitamin K also plays a role in bone matrix formation where mineralization happens [41]. Low-fat milk and its alternatives are the main contributors of calcium and magnesium in diet [42], which have a structural role in bone health [43]. Calcium from vegetable sources also has been shown to be positively effective in bone maintenance in older ages [44]. They are also a source of protein, vitamin D, vitamin B12, zinc and riboflavin [42]. An adequate protein intake is essential for bone matrix formation and maintenance. Eggs, legumes, nuts and seeds, as meat alternatives, are good

Table 4 Adolescence bone variables across the quartile groups of each dietary patterns derived during adolescence[1]

	Dietary pattern score quartiles[2]				*P* value
	Quartile1 (n = 31)	Quartile2 (*n* = 31)	Quartile3 (n = 31)	Quartile4 (*n* = 32)	
Vegetarian-style					
TBBMC	1555.34 ± 33.15	1579.43 ± 31.58	1649.63 ± 32.15	1634.61 ± 32.15	0.18
TBaBMD	0.88 ± 0.01[a]	0.90 ± 0.01[a,b]	0.93 ± 0.01[b]	0.91 ± 0.01[a,b]	0.025
FNBMC	3.64 ± 0.01	3.69 ± 0.01	3.86 ± 0.01	3.79 ± 0.01	0.31
FNaBMD	0.75 ± 0.01	0.78 ± 0.01	0.80 ± 0.01	0.76 ± 0.01	0.22
LSBMC	37.08 ± 1.18	39.68 ± 1.18	39.03 ± 1.19	38.9 ± 1.19	0.52
LSaBMD	0.73 ± 0.01	0.77 ± 0.01	0.77 ± 0.01	0.75 ± 0.01	0.20
Western-like					
TBBMC	1612.62 ± 33.61	1623.64 ± 31.58	1594.61 ± 32.25	1588 ± 33.32	0.86
TBaBMD	0.91 ± 0.01	0.91 ± 0.01	0.91 ± 0.01	0.90 ± 0.01	0.74
FNBMC	3.78 ± 0.11	3.68 ± 0.11	3.8 ± 0.12	3.8 ± 0.12	0.92
FNaBMD	0.79 ± 0.01	0.76 ± 0.01	0.77 ± 0.01	0.78 ± 0.01	0.82
LSBMC	39.21 ± 1.22	39.52 ± 1.14	38.18 ± 1.21	37.79 ± 1.21	0.73
LSaBMD	0.76 ± 0.01	0.76 ± 0.01	0.76 ± 0.01	0.75 ± 0.01	0.93
High-fat, high-protein					
TBBMC	1630.53 ± 32.15	1597.64 ± 32.14	1586.65 ± 32.15	1603.71 ± 34.28	0.82
TBaBMD	0.91 ± 0.01	0.90 ± 0.01	0.90 ± 0.01	0.91 ± 0.01	0.92
FNBMC	3.79 ± 0.01	3.88 ± 0.01	3.67 ± 0.01	3.68 ± 0.01	0.40
FNaBMD	0.77 ± 0.01	0.79 ± 0.01	0.77 ± 0.01	0.77 ± 0.01	0.74
LSBMC	38.91 ± 1.19	38.91 ± 1.19	39.08 ± 1.28	37.79 ± 1.28	0.91
LSaBMD	0.74 ± 0.01	0.76 ± 0.01	0.77 ± 0.01	0.76 ± 0.01	0.77
Mixed					
TBBMC	1580.01 ± 32.38	1608 ± 30.28	1657 ± 30.73	1572 ± 32.75	0.22
TBaBMD	0.89 ± 0.01	0.91 ± 0.01	0.92 ± 0.01	0.90 ± 0.01	0.24
FNBMC	3.79 ± 0.01	3.68 ± 0.01	3.88 ± 0.01	3.69 ± 0.01	0.41
FNaBMD	0.77 ± 0.01	0.77 ± 0.01	0.80 ± 0.01	0.76 ± 0.01	0.50
LSBMC	38.68 ± 1.21	37.77 ± 1.10	40.86 ± 1.10	37.31 ± 1.21	0.16
LSaBMD	0.75 ± 0.01	0.75 ± 0.01	0.78 ± 0.01	0.75 ± 0.01	0.37
Snack					
TBBMC	1587.11 ± 30.77	1639.27 ± 30.22	1590.44 ± 31.17	1601.11 ± 32.54	0.59
TBaBMD	0.90 ± 0.01	0.92 ± 0.01	0.90 ± 0.01	0.91 ± 0.01	0.37
FNBMC	3.81 ± 0.09	3.85 ± 0.09	3.68 ± 0.09	3.88 ± 0.09	0.43
FNaBMD	0.79 ± 0.01	0.78 ± 0.01	0.75 ± 0.01	0.78 ± 0.01	0.31
LSBMC	38.09 ± 1.12	41.05 ± 1.12	37.31 ± 1.12	38.44 ± 1.22	0.12
LSaBMD	0.76 ± 0.01	0.79 ± 0.01	0.73 ± 0.01	0.75 ± 0.01	0.055

Abbreviations: *aBMD* areal bone mineral density, *BMC* bone mineral accrual, *FN* femoral neck, *LS* lumber spine, *TB* total body

[1]Values are Mean ± SE. Mean adolescence bone variables were adjusted for sex and adolescent age of peak height velocity, age, height, weight, physical activity score, total energy intake and other four dietary pattern scores as continuous variables and were compared across quartiles of adolescence dietary pattern scores using MANCOVA with Bonferroni adjustment for multiple comparisons. Labeled means in a row without a common superscript letter differ, $P < 0.05$

[2]Participants in Quartile four have the highest adherence to the DPs in adolescence

sources of protein [45]. Dietary fiber from non-refined grains and other plant sources might also have a beneficial impact on bone through decreasing glycemic load and inhibiting hyperinsulinemia which in turn prevents urinary calcium loss induced by insulin [46]. Added fats including, mainly, butter, margarine, and mayonnaise as one of components of the "Vegetarian-style" DP might play a role in providing adequate dietary energy for adolescents during their growth spurt, when they are consumed along with other components of "Vegetarian-style" DP. Lower intake

Table 5 Young adulthood bone variables across the quartile groups of each dietary patterns derived during adolescence[1]

	Dietary pattern score quartiles[2]				*P* value
	Quartile1 (n = 29)	Quartile2 (*n* = 29)	Quartile3 (n = 29)	Quartile4 (*n* = 28)	
Vegetarian-style					
TBBMC	2592.38 ± 46.12[a]	2693.36 ± 46.12[a,b]	2813.68 ± 47.22[b]	2709.64 ± 49.25[a,b]	0.016
TBaBMD	1.14 ± 0.01[a]	1.18 ± 0.01[a,b]	1.21 ± 0.01[b]	1.18 ± 0.01[a,b]	0.017
FNBMC	4.69 ± 0.12[a]	5.02 ± 0.12[a,b]	5.19 ± 0.12[b]	5.08 ± 0.12[a,b]	0.042
FNaBMD	0.87 ± 0.02[a]	0.92 ± 0.02[a,b]	0.95 ± 0.02[b]	0.89 ± 0.02[a,b]	0.020
LSBMC	66.27 ± 1.91	71.75 ± 1.91	72.17 ± 2.04	68.91 ± 2.04	0.14
LSaBMD	1.00 ± 0.02	1.06 ± 0.02	1.08 ± 0.02	1.04 ± 0.02	0.09
Western-like					
TBBMC	2742.45 ± 47.45	2688.48 ± 47.62	2745.88 ± 48.22	2629.84 ± 48.24	0.28
TBaBMD	1.18 ± 0.01	1.17 ± 0.01	1.20 ± 0.01	1.15 ± 0.01	0.24
FNBMC	5.04 ± 0.11	4.91 ± 0.11	5.15 ± 0.12	4.90 ± 0.12	0.39
FNaBMD	0.91 ± 0.02	0.90 ± 0.02	0.93 ± 0.02	0.90 ± 0.02	0.71
LSBMC	70.10 ± 2.01	71.31 ± 1.90	71.34 ± 2.02	66.12 ± 2.02	0.25
LSaBMD	1.05 ± 0.02	1.05 ± 0.02	1.07 ± 0.02	1.01 ± 0.02	0.35
High-fat, high-protein					
TBBMC	2715.42 ± 48.68	2692.14 ± 50.25	2684.42 ± 48.58	2712.85 ± 47.85	0.96
TBaBMD	1.18 ± 0.01	1.18 ± 0.01	1.17 ± 0.01	1.18 ± 0.01	0.98
FNBMC	5.02 ± 0.11	5.20 ± 0.11	4.74 ± 0.11	5.08 ± 0.12	0.07
FNaBMD	0.90 ± 0.02	0.93 ± 0.02	0.88 ± 0.02	0.92 ± 0.02	0.27
LSBMC	67.90 ± 2.01	72.40 ± 2.11	68.60 ± 2.04	70.0 ± 2.04	0.45
LSaBMD	1.02 ± 0.02	1.07 ± 0.02	1.04 ± 0.02	1.06 ± 0.02	0.41
Mixed					
TBBMC	2713.25 ± 48.32	2700.38 ± 48.32	2721.25 ± 48.32	2668 ± 49.12	0.88
TBaBMD	1.17 ± 0.01	1.19 ± 0.01	1.18 ± 0.01	1.17 ± 0.01	0.78
FNBMC	5.11 ± 0.12	5.11 ± 0.12	4.90 ± 0.12	4.88 ± 0.12	0.28
FNaBMD	0.91 ± 0.02	0.93 ± 0.02	0.89 ± 0.02	0.89 ± 0.02	0.46
LSBMC	68.67 ± 2.02	70.18 ± 2.02	72.52 ± 2.02	67.52 ± 2.02	0.36
LSaBMD	1.04 ± 0.02	1.06 ± 0.02	1.07 ± 0.02	1.02 ± 0.02	0.50
Snack					
TBBMC	2673.32 ± 45.45	2780.77 ± 47.32	2652 ± 46.87	2699 ± 47.35	0.24
TBaBMD	1.17 ± 0.01	1.20 ± 0.01	1.17 ± 0.01	1.17 ± 0.01	0.58
FNBMC	5.01 ± 0.12	5.01 ± 0.12	4.80 ± 0.13	5.07 ± 0.13	0.64
FNaBMD	0.92 ± 0.02	0.90 ± 0.02	0.88 ± 0.02	0.92 ± 0.02	0.41
LSBMC	68.22 ± 1.9	72.04 ± 2.02	68.11 ± 2.02	70.51 ± 2.02	0.45
LSaBMD	1.05 ± 0.02	1.07 ± 0.02	1.03 ± 0.02	1.04 ± 0.02	0.58

Abbreviations: *aBMD* areal bone mineral density, *BMC* bone mineral accrual, *FN* femoral neck, *LS* lumber spine, *TB* total body

[1]Values are Mean ± SE. Mean young adulthood bone variables were adjusted for sex and age of peak height velocity and young adult age, height, weight, physical activity score, total energy intake and other four adolescence dietary pattern scores as continuous variables and were compared across quartiles of adolescence dietary pattern scores using MANCOVA with Bonferroni adjustment for multiple comparisons. Labeled means in a row without a common superscript letter differ, $P < 0.05$

[2]Participants in Quartile 4 have the highest adherence to the DPs in adolescence

of meat seems to be beneficial, as this seems to be one of the key differences between "Vegetarian-style" DP and other four DPs. Taken together, the "Vegetarian-style" DP represents a combination of beneficial nutrients and dietary components with potential synergic or interacting effects. Therefore no single nutrient or dietary components could be pointed out as the one responsible for the beneficial impact of the DP on bone.

Table 6 Tracking coefficients and change in score by age for dietary patterns derived during adolescence[1]

	Tracking dietary patterns			Change in dietary pattern score		
	ß (adolescence score)	95% CI	*P* value	ß (age)	95% CI	*P* value
Vegetarian-style	0.59	0.48, 0.71	< 0.001	0.026	0.00, 0.04	0.008
Western-like	0.47	0.40, 0.53	< 0.001	− 0.008	− 0.029, 0.012	0.42
High-fat, high-protein	0.51	0.41, 0.60	< 0.001	− 0.019	−0.034, − 0.005	0.009
Mixed	0.54	0.39, 0.69	< 0.001	−0.003	−0.033, 0.028	0.85
Snack	0.63	0.55, 0.70	< 0.001	−0.003	−0.023, 0.018	0.80

Abbreviations: *CI* confidence intervals

[1]Generalized estimating equations was used for modeling association between adolescence and adulthood standardized and energy-adjusted dietary pattern scores while controlling for sex, age, and age at adolescence; $n = 115$. Tracking coefficient (ß coefficient for adolescent dietary pattern) shows how participants maintained their position in the study population distribution, between adolescence and young adulthood. Tracking coefficient for age represents z score change in dietary pattern score from adolescence to young adulthood

Our study has several strengths. This is the first study that evaluated DPs during adolescence in association with young adult bone health. In our sample, all participants during young adulthood had their PBM confirmed by a plateau in bone mineral accrual curve, representing a steady status of bone [4]. We also controlled for somatic maturity by including the age of PHV as a covariate in our models. Adolescent dietary intake data were collected using multiple, mostly three, 24-h recalls over a year for each participant, which is preferred to food frequency questionnaires [47], the method used by most previous studies. In addition, we analyzed the impact of the whole diet, instead of a single food or nutrient, on bone.

The main limitation of our study was the small sample size ($n = 125$ for adolescent analysis, and $n = 115$ for young adult analysis), which did not allow us to run the separate analysis for females and males or run other DP approaches such reduced-rank regression method. Small sample size also limited us from adding more covariates in the model such as young adult DPs, smoking status, oral contraceptive use or reproductive history (in females). Even though we did not control the models for young adult DPs, we assessed change in DPs from adolescence to young adulthood to overcome this limitation. Two further limitations of our study are reliance on only one 24-h recall in young adulthood and using two different nutrient assessment systems from adolescence to young adulthood. However, our focus was food group intake and these two systems were only used to measure total energy intake.

Conclusions

Our results suggest that a diverse and well-balanced DP, rich in dark green vegetables, eggs, non-refined grains, 100% fruit juice, legumes, nuts and seeds, added fats, fruits and low-fat milk during adolescence has a beneficial impact on bone health during adolescence and this positive impact on bone accrual can be carried into young adulthood. Further population-based studies are needed to confirm our findings and generalize these results to other populations.

Abbreviations

aBMD: areal bone mineral density; BMC: Bone mineral content; DP: dietary pattern; DXA: dual-energy X-ray absorptiometry; FN: femoral neck; LS: lumbar spine; MANCOVA: multivariate analysis of covariance; PAQ: physical activity questionnaire; PBM: peak bone mass; PBMAS: Pediatric Bone Mineral Accrual Study; PCA: principal component analysis; PHV: peak height velocity; TB: total body

Competing interests

The authors declare that they have no competing interests.

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The role of a dairy fraction rich in milk fat globule membrane in the suppression of postprandial inflammatory markers and bone turnover in obese and overweight adults: an exploratory study

Abstract

Background: Inflammation is associated with increased bone resorption; the role of inflammation in postprandial bone turnover has not been explored. Consumption of milk fat globule membrane (MFGM) reduces inflammation in animal models. This study aimed to measure postprandial changes in bone turnover after intake of high saturated fat test meals, with- and without the anti-inflammatory ingredient MFGM.

Methods: Subjects ($n = 36$ adults) were obese (BMI 30–39.9 kg/m^2) or overweight (BMI 25–29.9 kg/m^2) with two traits of Metabolic Syndrome. Subjects consumed a different test meal on four occasions at random; blood draws were taken at baseline and 1, 3, and 6 h postprandial. Test meals included whipping cream (WC), WC + MFGM, palm oil (PO) and PO + MFGM. Biomarkers of bone turnover and inflammation were analyzed from all four time points.

Results: Test meal (treatment) by time interactions were significant for bone resorption marker C-telopeptide of type 1 collagen (CTX) ($p < 0.0001$) and inflammatory marker interleukin 10 (IL-10) ($p = 0.012$). Significant differences in overall postprandial response among test meals were found for CTX and soluble intercellular adhesion molecule (sICAM), with the greatest overall postprandial suppression of CTX occurring in meals containing MFGM. However, test meal by MFGM interactions were non- significant for bone and inflammatory markers. Correlations between CTX and inflammatory markers were non-significant.

Conclusion: This exploratory analysis advances the study of postprandial suppression of bone turnover by demonstrating differing effects of high SFA meals that contained MFGM; however MFGM alone did not directly moderate the difference in postprandial CTX response among test meals in this analysis. These observations may be useful for identifying foods and ingredients which maximize the suppression of bone resorption, and for generating hypotheses to test in future studies examining the role of inflammation in postprandial bone turnover.

Trial registration: Clinicaltrials.gov NCT01811329. Registered 11 March 2013.

Keywords: Postprandial, Bone Turnover, Inflammation, Milk Fat Globule Membrane, C-telopeptide of type 1 collagen (CTX)

Background

Biomarkers of bone resorption have been observed to fluctuate over the course of a day, with a peak occurring at night and a nadir seen in the day, particularly in the late afternoon. This pattern appears exaggerated with food intake [1–3]. Consumption of glucose suppresses bone resorption marker C- telopeptide of type I collagen (CTX) by 45–50% approximately 120 min after intake in healthy subjects [4, 5]. Mixed meals induce a similar but delayed reduction in CTX approximately 180 min after eating [5, 6]. In contrast to bone resorption, bone formation markers such as N-terminal serum type 1 procollagen (P1NP) change less drastically in the postprandial state [1, 4]. Explanations for the postprandial suppression of bone turnover have focused primarily on insulin and incretin hormones, but additional factors such as inflam-matory mediators may contribute to the observed reduc-tion in circulating CTX in the postprandial period [5].

In the Post-Prandial Inflammation (PPI) study, our group showed that the addition of a dairy fraction rich in milk fat globule membrane (MFGM) reduced postprandial concentrations of cholesterol, inflammatory markers and insulin in overweight and obese subjects who consumed test meals high in saturated fatty acids (SFA) [7]. MFGM was selected as a key ingredient due to its reported anti-inflammatory properties [8]. Composed of sphingolipids and glycerophospholipids, as well as proteins, MFGM covers the apical surface of lipid droplets produced by mammary glands [8, 9]. Whole buttermilk that was created during butter churning in decades past was a naturally rich source of MFGM, but today MFGM can be isolated, purified and added to other foods [9]. MFGM has been shown to reduce inflammation [10], improve endurance capacity and lipid metabolism [11] in animals, as well as reduce frailty in elderly women [12]. To date, effects of MFGM on bone outcomes have not been investigated in clinical studies. The PPI study provided the opportunity to conduct exploratory analyses of potential associations between postprandial bone turnover and postprandial inflammation using MFGM.

The aims of the present project were to examine postprandial changes in bone turnover after intake of high SFA challenge meals (with and without MFGM), and to investigate the relationships between the responses of inflammatory markers and bone turnover markers to the test meals. Since inflammation has been associated with increased bone resorption [13], and we previously showed that the addition of MFGM to a high SFA challenge meal reduces inflammatory markers [7], we hypothesized that due to anti-inflammatory effects of MFGM, high SFA challenge meals containing MFGM would attenuate postprandial bone turnover markers to a greater extent than high SFA challenge meals without MFGM.

Methods

Participants

Details of the PPI study have been previously published [7]. Briefly, subjects were recruited from the Davis and greater Sacramento areas of California and included 36 adults (19 women and 17 men). Inclusion criteria were 18–65 years of age and a body mass index (BMI) classified as obese (BMI 30–39.9 kg/m^2) or overweight (BMI 25–29.9 kg/m^2) plus two traits of Metabolic Syndrome (MetS). Per the American Heart Association definition, MetS traits include blood pressure ≥ 130/85 mmHg, fasting plasma triglyceride ≥ 150 mg/dl, fasting plasma high density lipoprotein (HDL) cholesterol < 40 mg/dl for men and < 50 mg/dl for women, waist circumference > 40 inches for men and 35 inches for women, and fasting glucose ≥ 100 mg/dl [14]. Exclusion criteria included gastrointestinal disorders, type 2 diabetes, immune-related disorders, cancer, self-reported eating disorder, use of anti-inflammatory pain medication, use of over the counter anti-obesity agents or corticosteroids in the last 12 weeks, initiation of statin therapy in the last 12 weeks, initiation of fish, krill, flax, borage or primrose seed oils within the last 12 weeks, use of dietary supplements with concentrated soy isoflavones, resveratrol or other polyphenols, initiation, change or cessation of hormonal birth control in the last 6 months, known allergy or intolerance to study food, adherence to a vegetarian diet, consumption of >1% of energy from trans-fats, > 1 serving of fish per week, > 14 grams of fiber per 1000 kcal/day, <16:1 total omega-6:omega 3 fatty acid ratio, >10% weight loss or gain in the past 6 months, poor vein assessment determined by phlebotomist, use of tobacco products, initiation of a new exercise program in the last month, and pregnancy, lactation, or plans to become pregnant in the next 6 months. Fulfillment of enrollment criteria was determined through questionnaires, analysis of a fasting blood sample for blood lipids and glucose, and anthropometric measurements (height, weight, waist circumference) that were taken during the subjects' screening visits. The study protocol was approved by the Institutional Review Board of the University of California at Davis, and all procedures performed in the study were in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The study was registered at clinicaltrials.gov under NCT01811329.

Study design

Phone screenings were used to determine subject eligibility, after which the individual reported to the Western Human Nutrition Research Center (WHNRC) to complete consent forms. Subjects were randomized to one of four

treatment sequences in a repeated measures Latin Square design (Fig. 1). The advantage of this design for a repeated measures experiment is that it ensures a balanced fraction of all treatment combinations when subjects are limited and the sequence effect of treatment can be considered to be negligible. Investigators were blind to treatment order. A washout period of 1–2 weeks was observed between treatments to prevent carry-over effects across treatments. A random allocation sequence generator (randomization.-com; seed#4234) was used to assign treatment order.

Subjects were instructed to abstain from alcohol, NSAIDs, and other anti-inflammatory supplements 72 h before each test day and from vigorous exercise and consuming seafood 24 h before each test day. Additionally, subjects recorded their diets 24 h before each test day. Nutrition Data System for Research (NDSR; University of Minnesota) was used to assess the 1-day diet record for compliance with the pre-study instructions.

The study took place at the WHNRC in Davis, CA. Subjects fasted for 10–12 h prior to each study day. Subjects completed a modified gastrointestinal questionnaire [15] and provided a fasting blood draw at the beginning of each study day. Blood pressure, heart rate, weight and waist circumference measurements were also recorded. Subjects then consumed the "breakfast" test meal within 20 min, and postprandial blood draws were taken at 1, 3, and 6 h (Fig. 1). Subjects were not allowed to consume any additional food throughout the study day but could drink bottled water ad libitum. Subjects were also instructed to minimize their physical activity during the remaining time of the test day by either staying at the test center for the entire 7-hour period or traveling by car if they chose to leave between blood draws. If subjects left and returned to the center, they were instructed to arrive 15 min prior to their scheduled blood draw to allow for a 10 min rest period before the venipuncture.

Test meals

Meals consisted of a smoothie, as well as a bagel with strawberry preserves. Smoothies were made from whipping cream (WC) or palm oil (PO); milk fat globule membrane (MFGM) was added to one WC smoothie (WC + MFGM) and one PO smoothie (PO + MFGM). In this study, MFGM was sourced from the complex milk lipid fraction powder BPC50 (Fonterra Co-operative Group Ltd., Auckland, New Zealand) [16]. The composition of BPC50 includes the following (%wt/wt): 52% protein (13.2% membrane-derived protein), 6.6% lactose, and 36.2% total fat (22.5% triglycerides and 13.7% phospholipids, 0.63% gangliosides (GD3), and 5.2% ash) [17–19]. BPC50 contains the following MFGM-derived proteins in greatest abundance: fatty acid binding protein, butyrophilin, lactadherin, adipophilin, xanthine oxidase, and mucin [18]. Because the WC and PO smoothies did not contain BPC50, whey protein isolate was added to match the protein content. The nutrient composition of the test meals is shown in Additional file 1. Test meal ingredients are listed in the Additional file 2.

Each test meal was customized to provide 40% of each subject's total energy intake (EI), as determined by the National Academy of Sciences equation from the Institute of Medicine Dietary Reference Intake. This equation accounted for gender, age, weight, height and physical activity [20]. The Baecke Physical Activity questionnaire was used to determine habitual physical activity [21]. The composition of each test meal was approximately 55% fat (49–87 grams per individual EI), 30% carbohydrate (61–107 grams per individual EI, and 15% protein (31–55 grams per individual EI). The NDSR was used to estimate the nutrient composition of each test meal. MFGM replaced 31% of the fat in each meal (34% of total kcal, 53.2–93.1) grams depending on individual EI). Per study protocol, subjects consumed each meal in its entirety, rinsed the beverage cup with bottled water and drank the rinse water.

Blood analyses

A trained phlebotomist at the WHNRC collected blood by venipuncture at each time point. Whole blood was centrifuged in a tabletop ultracentrifuge for 15 min at

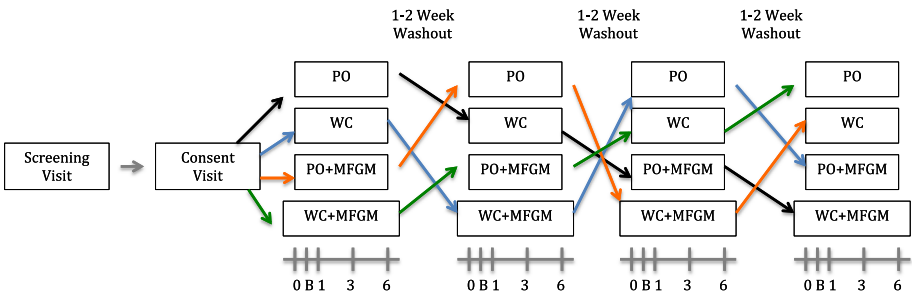

Fig. 1 Study design. Test meals: palm oil (PO), palm oil plus milk fat globule membrane (PO + MFGM), whipping cream (WC), whipping cream plus milk fat globule membrane (WC + MFGM). Venipuncture timeline: 0 = baseline, B = breakfast test meal, 1 = 1 hour postprandial, 3 = 3 hours postprandial, 6 = 6 hours postprandial. *N* = 36

4 °C at 1300 × g within 30 min of collection. Plasma was then separated into 1.5 mL aliquots and immediately frozen at −70 °C until analysis. Serum was allowed to clot on ice for 30 min, centrifuged for 15 min at 4 °C at 1300 × g and transferred into 1.5 mL aliquots and frozen at −70 °C until analysis.

Bone biomarkers

C-telopeptide of type 1 collagen (CTX) was measured by enzyme linked immune-sorbent assay (ELISA) (Immunodiagnostic Systems, Inc., Gaithersburg, MD, USA). Type 1 C-terminal collagen propeptide (C1CP) was also measured by ELISA (Quidel Corporation, San Diego, CA, USA).

Inflammatory markers

Inflammatory biomarker analyses were conducted at all four time points. An electro-chemiluminescence detection system using multi-array technology (SECTOR Imager 2400, Meso Scale Discovery) was used to analyze interleukin-6 (IL-6), interleukin-18 (IL-18), interleukin-1 (IL-1), tumor necrosis factor alpha (TNFα), C-reactive protein (CRP) and soluble intercellular adhesion molecule (sICAM) per manufacturer's instructions. Plasma was used to measure IL-18; serum was used to measure all other inflammatory markers. In brief, 25–50 μL of serum or plasma was added to pre-coated plates con-taining capture antibodies. After incubation, plates were washed, and a labeled detection antibody was added. The bound detection antibodies emit light upon electro-chemical stimulation, and a plate reader was used to quantify each protein of interest.

Metabolic parameters

Assessment of plasma glucose, triglycerides, HDL-cholesterol and insulin was completed at the clinical laboratory at the University of California Medical Center (Sacramento, CA) using standard clinical techniques.

Clinical characteristics

Anthropometric data were collected at the time of screening. Measurements included height (Ayrton Stadiometer Model S100; Ayrton Corporation), body weight (6002 Wheelchair Scale; Scale-tronix), waist circumference measured in the standing position midway between the lower rib margin and ileac crest (QM2000 Measure Mate; QuickMedical), blood pressure and resting heart rate (Carescape V100 with Critikon Dura-cuff for either adults or large adults; GE Medical Instruments). BMI was calculated as kg/m^2. Bone density was also measured by dual energy X-ray absorptiometry (DXA, Lunar Prodigy instrument; GE Medical Instruments).

Statistical analyses

Sample size to detect a minimum significant difference between treatment groups was determined for the original PPI study, and power calculations were based on previously published plasma inflammatory marker data [22] and preliminary oxylipin studies in our lab. We defined the minimal detectable difference as the difference between the maximum and minimum responses; for example, the magnitude of this difference was 34.1 for prostaglandin E2 (PGE2). With a significance level set at a two-sided alpha of 0.05 and power held at 80%, we calculated that a sample size of 36 subjects was needed for this four-way crossover trial.

Descriptive statistics (mean ± standard deviation) were calculated for subjects' baseline characteristics (Tables 1 and 2). Data were tested for normality with the Shapiro-Wilk test and transformed as appropriate. A repeated measures mixed model analysis (including the random effects of subject and subject by treatment) was used to test the effects of time, treatment (test meal) and time by treatment interaction for the bone marker variables (C1CP, CTX) and inflammatory variables (IL-6, IL-18, TNF-α, CRP, IL-10, sICAM) (Table 3).

Additionally, a summary measure (incremental area under the curve, iAUC) using the trapezoid method, was used for comparison of overall postprandial responses among test meals [23]. For bone markers and inflammatory variables, the mean incremental area under the

Table 1 Subject baseline characteristics[a]

	Mean ± SD	MetS criteria[b]
Age (years)	42.9 ± 14.0	—
Weight (kg)	92.9 ± 12.2	—
Height (m)	1.7 ± 0.1	—
BMI, (kg/m^2)	31.7 ± 2.6	—
Total Body Fat (%)	36.7 ± 7.8	—
Total Body Fat, Male (%)	30.9 ± 6.2	—
Total Body Fat, Female (%)	41.9 ± 4.9	—
Waist circumference (inches)	39.3 ± 3.2	—
Waist circumference, Male (inches)[c]	41.1 ± 3.1	>40
Waist circumference, Female (inches)[c]	37.9 ± 2.2	>35
Systolic blood pressure (mmHg)	123.9 ± 13.6	≥130
Diastolic blood pressure (mmHg)	75.0 ± 10.3	≥85
HDL cholesterol (mg/dl)	48.3 ± 14.1	—
HDL cholesterol, Male (mg/dl)[c]	43.2 ± 11.7	<40
HDL cholesterol, Female (mg/dl)[c]	52.9 ± 14.7	<50
Fasting glucose (mg/dl)	91.0 ± 7.4	≥100
Fasting triglycerides (mg/dl)	122.5 ± 57.8	≥150

[a]Measurements taken at screening visit ($n = 36$). *BMI* body mass index, *HDL* high-density lipoprotein
[b]MetS as defined by the American Heart Association [14]. *MetS* metabolic syndrome
[c]Male $n = 17$, Female $n = 19$

Table 2 Bone mineral measurements[a] of subjects at baseline (Mean ± SD)

	BMC (g)	BMD (g/cm^2)	T-score
Males ($n = 17$)			
Lumbar Spine L1-L4	81.00 ± 13.3	1.13 ± 0.1	0.39 ± 1.1
Total Right Hip	48.83 ± 8.7	1.12 ± 0.1	0.58 ± 0.9
Total Left Hip	46.81 ± 14.0	1.12 ± 0.1	0.61 ± 0.9
Females ($n = 19$)			
Lumbar Spine L1-L4	62.17 ± 8.6	1.05 ± 0.1	0.05 ± 1.0
Total Right Hip	31.49 ± 8.2	0.98 ± 0.1	0.33 ± 1.0
Total Left Hip	34.29 ± 5.0	1.00 ± 0.1	0.43 ± 1.0

[a]Measurements taken by dual energy X-ray absorptiometry, *BMC* bone mineral content, *BMD* bone mineral density, T score = comparison to young adult average

transformed curve (iAUtC) of each test meal was compared by repeated measures analysis of covariance, including fixed effects of group and subject, and transformed hour 0 as a covariate (Table 4).

To examine whether postprandial changes in inflammatory variables may have mediated postprandial changes in the bone markers, within-subject correlations between iAUtC of inflammatory variables and iAUtC of bone markers were examined, controlling for hour 0 values (Table 5). Correlation coefficients were calculated as partial Pearson correlations; mixed model regression (with a random effect of subject) was used to calculate p-values for each correlation in order to account for the fact that the four measurements were not independent of each other. Additionally, we calculated correlations between CTX and selected metabolic variables (glucose, HDL cholesterol, triglycerides and insulin) at each time point (Additional file 3).

GraphPad Prism 6.0c (GraphPad Software, Inc., La Jolla, CA) and SAS for Windows release 9.4 (SAS Institute, Cary, NC) were used for statistical analyses.

Results

Out of 207 potential subjects who were screened, 38 subjects were enrolled. Two subjects were disqualified due to initiation of medication that could confound the results and to scheduling difficulties. The final subject population included 17 males and 19 females. Details of subject enrollment, CONSORT Diagram and follow up have been previously published [7]. All subjects ($n = 36$) consumed all four test meals. One subject did not complete the postprandial blood draws after the PO + MFGM test meal due to difficulties with the venipuncture. The missing data for the 1, 3 and 6 h time points for this subject were accounted for in the statistical analyses per SAS protocol. For the correlations, the missing points were omitted from the analysis. For the various mixed model analyses, the non-missing points were included, and the missing points were excluded, but the nature of the model is that it implicitly imputes the missing values when estimating differences between the means for the treatments or the time points.

Baseline characteristics of the subjects were assessed at the consent visit and are shown in Table 1. Subjects were predominately Caucasian (67%) or Hispanic (28%). On average, subjects were obese and met MetS criteria for waist circumference and HDL cholesterol. Mean bone mineral content (BMC), bone mineral density (BMD), and T scores of male and female subjects are shown in Table 2. Bone measurements were taken once during the study to establish the baseline bone health of each subject. Mean T-scores for lumbar spine, total right hip and total left hip were within the normal range for male and female subjects.

Effects of treatment and time were significant for CTX ($p \leq 0.0001$), and significant effects of time ($p < 0.0001$) were also observed for IL-6, IL-18, TNF-α, CRP and sICAM (Table 3). There were significant treatment by time interactions for CTX ($p < 0.0001$) and IL-10 ($p = 0.012$) (Table 3).

Postprandial CTX concentrations (mean ± SD) after each test meal are presented in Fig. 2. Differences in CTX response to the test meals were most apparent at 6 h postprandial; CTX concentrations remained lower at this time point after consumption of WC + MFGM and PO + MFGM test meals compared to WC and PO test meals.

Significant differences in overall postprandial response (iAUtC) among the test meals were found for CTX and sICAM. Test meal by MFGM interactions were not significant for any of the bone or inflammatory markers (Table 4).

The correlation between the iAUtCs of C1CP and IL-6 was statistically significant ($r = -0.20$; $p = 0.045$), but other correlations between bone markers and inflammatory variables were non-significant (Table 5).

Discussion

This exploratory study is the first to examine the potential role of inflammation in postprandial bone turnover. In line with previous reports of postprandial bone turnover [5, 6] and as we observed with some of the inflammatory markers [7], bone resorption marker CTX significantly changed over time in the postprandial state. Interestingly, we observed that meals containing MFGM induced the greatest overall suppression of CTX, particularly at 6 h postprandial; however, test meal by MFGM interactions were non-significant. Furthermore, although a weak correlation was found between bone formation marker C1CP and IL-6, correlations between CTX and the inflammatory variables were not statistically significant. Taken together, these data suggest that suppression of postprandial inflammation may have a

Table 3 Concentrations of postprandial bone biomarkers and inflammatory markers (Mean ± SD)

	Postprandial time point						
	0 hour	1 hour	3 hour	6 hour	Treatment effect *p*-value	Time effect *p*-value	Time x treatment interaction *p*-value
C1CP (ng/ml)					0.093	0.084	0.195
PO + MFGM	134.4 ± 140.6	131.1 ± 134.1	128.1 ± 119.9	131.3 ± 127.7			
PO	122.0 ± 133.7	127.1 ± 138.7	117.5 ± 124.2	127.9 ± 152.0			
WC + MFGM	130.0 ± 135.2	126.2 ± 137.0	130.1 ± 146.5	129.3 ± 125.9			
WC	153.1 ± 269.9	137.8 ± 201.7	123.6 ± 133.8	129.2 ± 146.3			
CTX (ng/ml)					0.0001	<0.0001	<.0001
PO + MFGM	0.57 ± 0.27	0.33 ± 0.14	0.24 ± 0.12	0.32 ± 0.16			
PO	0.53 ± 0.22	0.31 ± 0.13	0.24 ± 0.12	0.44 ± 0.22			
WC + MFGM	0.56 ± 0.27	0.33 ± 0.16	0.22 ± 0.11	0.26 ± 0.14			
WC	0.55 ± 0.26	0.31 ± 0.11	0.20 ± 0.09	0.38 ± 0.20			
IL-6 (pg/ml)					0.449	<0.0001	0.975
PO + MFGM	0.72 ± 1.37	0.59 ± 1.26	0.54 ± 0.98	0.76 ± 1.40			
PO	0.74 ± 1.08	0.59 ± 0.87	0.61 ± 1.09	0.76 ± 1.16			
WC + MFGM	0.78 ± 1.43	0.63 ± 1.27	0.60 ± 1.20	0.86 ± 1.48			
WC	0.70 ± 1.10	0.63 ± 1.18	0.60 ± 1.13	0.87 ± 1.51			
IL-18 (pg/ml)					0.671	<0.0001	0.245
PO + MFGM	11.11 ± 3.33	11.32 ± 3.32	10.72 ± 2.74	10.80 ± 3.39			
PO	11.24 ± 3.10	10.51 ± 3.34	9.629 ± 3.15	10.96 ± 2.95			
WC + MFGM	11.79 ± 3.64	10.63 ± 4.11	10.40 ± 3.67	10.81 ± 3.17			
WC	11.05 ± 3.44	10.98 ± 3.03	10.09 ± 2.69	10.18 ± 3.06			
TNFα (pg/ml)					0.679	<0.0001	0.303
PO + MFGM	2.34 ± 0.67	2.36 ± 0.66	2.27 ± 0.62	2.22 ± 0.68			
PO	2.49 ± 0.71	2.36 ± 0.56	2.29 ± 0.63	2.36 ± 0.64			
WC + MFGM	2.54 ± 0.83	2.36 ± 0.81	2.32 ± 0.81	2.32 ± 0.73			
WC	2.44 ± 0.70	2.38 ± 0.67	2.27 ± 0.57	2.32 ± 0.63			
CRP (mg/l)					0.207	<0.0001	0.608
PO + MFGM	1.49 ± 0.85	1.51 ± 0.86	1.46 ± 0.85	1.52 ± 0.87			
PO	1.55 ± 0.86	1.65 ± 0.87	1.58 ± 0.88	1.62 ± 0.87			
WC + MFGM	1.49 ± 0.85	1.56 ± 0.90	1.51 ± 0.87	1.56 ± 0.88			
WC	1.49 ± 0.83	1.58 ± 0.90	1.50 ± 0.84	1.53 ± 0.86			
IL-10 (pg/ml)					0.278	0.206	0.012
PO + MFGM	0.49 ± 1.17	0.52 ± 1.19	0.52 ± 1.12	0.57 ± 1.14			
PO	0.56 ± 1.37	0.54 ± 1.35	0.54 ± 1.22	0.51 ± 1.32			
WC + MFGM	0.52 ± 1.08	0.57 ± 1.09	0.57 ± 1.04	0.58 ± 1.17			
WC	0.52 ± 1.13	0.58 ± 1.15	0.58 ± 1.21	0.55 ± 1.28			
sICAM (mg/l)					0.168	<0.0001	0.114
PO + MFGM	0.96 ± 0.51	0.97 ± 0.51	0.95 ± 0.51	0.97 ± 0.53			
PO	0.97 ± 0.51	1.058 ± 0.54	1.00 ± 0.51	1.044 ± 0.54			
WC + MFGM	0.93 ± 0.50	0.99 ± 0.53	0.97 ± 0.54	0.97 ± 0.51			
WC	0.92 ± 0.48	1.00 ± 0.52	0.96 ± 0.49	0.96 ± 0.49			

Actual (untransformed) values are presented here; analysis was done on the transformed data. $N = 36$
Test meals: palm oil plus milk fat globule membrane (PO + MFGM), palm oil (PO), whipping cream plus milk fat globule membrane (WC + MFGM), whipping cream (WC)
Bone variables: type 1 C-terminal collagen propeptide (C1CP), C-telopeptide of type 1 collagen (CTX)
Inflammatory variables: interleukin-6 (IL-6), interleukin-18 (IL-18), tumor necrosis factor alpha (TNFα), C-reactive protein (CRP), interleukin-10 (IL10), soluble intercellular adhesion molecule (sICAM)

Table 4 Postprandial response (iAUtC[1] ± SD) of bone biomarkers and inflammatory markers by test meal

	Test meals				Test Meal x MFGM interaction *p*-value
	PO + MFGM	PO	WC + MFGM	WC	
C1CP	−0.18 ± 0.9	0.07 ± 1.2	0.01 ± 0.9	−0.28 ± 0.8	0.859
CTX	−1.52 ± 1.0[b]	−1.16 ± 0.9[a]	−1.67 ± 0.9[c]	−1.49 ± 0.9[bc]	0.332
IL-6	−0.70 ± 1.5	−0.78 ± 1.4	−0.84 ± 1.5	−0.47 ± 1.0	0.846
IL-18	−1.93 ± 10.6	−5.58 ± 11.4	−6.68 ± 11.0	−3.81 ± 11.5	0.106
TNF-α	−0.12 ± 0.7	−0.36 ± 0.6	−0.49 ± 0.7	−0.26 ± 0.7	0.102
CRP	0.04 ± 0.3	0.12 ± 0.2	0.10 ± 0.2	0.08 ± 0.2	0.119
IL-10	0.71 ± 1.8	−0.25 ± 1.8	0.37 ± 1.7	0.19 ± 1.7	0.567
sICAM	0.13 ± 0.5[b]	0.37 ± 0.4[a]	0.28 ± 0.4[ab]	0.32 ± 0.5[ab]	0.080

[1]Mean incremental area under transformed curve
Significant differences in iAUtC of bone biomarkers and inflammatory markers among test meals are indicated by superscript letters
N = 36
Test meals: palm oil plus milk fat globule membrane (PO + MFGM), palm oil (PO), whipping cream plus milk fat globule membrane (WC + MFGM), whipping cream (WC)
Bone variables: type 1 C-terminal collagen propeptide (C1CP), C-telopeptide of type 1 collagen (CTX)
Inflammatory variables: interleukin-6 (IL-6), interleukin-18 (IL-18), tumor necrosis factor alpha (TNFα), C-reactive protein (CRP), interleukin-10 (IL-10), soluble intercellular adhesion molecule (sICAM)

minimal role in the suppression of postprandial bone turnover and that MFGM did not directly moderate the difference in postprandial CTX response among test meals. However, further studies are warranted to confirm these observations.

The mechanisms of postprandial suppression of bone turnover are incompletely understood. During fasting (and especially during overnight fasts) the body must mobilize nutrients from the bone for calcium homeostasis and cell growth processes, and therefore bone resorption increases. It has been hypothesized that since organic compounds and nutrients are readily available to the skeleton after eating, bone resorption is unnecessary in the postprandial state [1]. Differing effects of nutrients and foods on postprandial bone resorption have been reported. A small study of healthy adults compared carbohydrate, fat and protein and found that all macronutrients suppressed postprandial CTX compared to baseline levels; however the postprandial CTX response to fat ingestion was more blunted [24]. In contrast, other researchers have observed a trend for greater CTX suppression with lower protein, higher fat foods [2]. The present study demonstrates differences in postprandial CTX suppression among high SFA meals containing an ingredient with anti-inflammatory properties (MFGM).

Previous work has revealed that bile acids [25], insulin [5] and incretin hormones such as glucose dependent insulinotropic peptide (GIP) may contribute to postprandial suppression of bone resorption [24, 26]; however, the PPI protocol did not include any measurements of bile acids or incretin hormones, or insulin at 6 h postprandial. Per the PPI protocol, insulin was measured at 0, 1 and 3 h postprandial, and we examined correlations between CTX and other selected metabolic variables

Table 5 Within-subject correlations of bone biomarkers and inflammatory markers[a]

Inflammatory variable	C1CP correlation coefficient	*p*-value	CTX correlation coefficient	*p*-value
IL-6	−0.20	0.045	−0.08	0.421
IL-18	−0.09	0.338	−0.12	0.202
TNF-α	−0.15	0.118	0.05	0.613
CRP	0.14	0.159	0.08	0.445
IL-10	0.02	0.877	−0.10	0.307
sICAM	0.03	0.748	0.01	0.935

[a]Correlations calculated for incremental area under the transformed curve, controlling for hour 0 values of the two variables. Correlation coefficients were calculated as partial Pearson correlations; mixed model regression (with a random effect of subject) was used to calculate p-values for each correlation in order to account for the fact that the four measurements were not independent of each other. *N* = 36
Bone variables: type 1 C-terminal collagen propeptide (C1CP), C-telopeptide of type 1 collagen (CTX)
Inflammatory variables: interleukin-6 (IL-6), interleukin-18 (IL-18), tumor necrosis factor alpha (TNFα), C-reactive protein (CRP), interleukin-10 (IL-10), soluble intercellular adhesion molecule (sICAM)

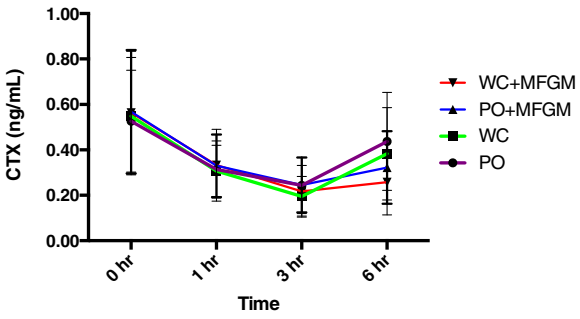

Fig. 2 Postprandial CTX concentrations over time after consumption of four high SFA test meals. Test meals: palm oil (PO), palm oil plus milk fat globule membrane (PO + MFGM), whipping cream (WC), whipping cream plus milk fat globule membrane (WC + MFGM). *N* = 36

(glucose, HDL cholesterol, triglycerides and insulin) at each time point separately. Other studies have suggested associations between bone turnover and cholesterol [27, 28], so the positive correlations that we observed between CTX and HDL cholesterol and negative correlations between CTX and triglycerides warrant further study (Additional file 3). We found no significant correlations between CTX and insulin at any of the three time points (Additional file 3).

We may have been unable to detect significant test meal by MFGM interactions due to the fact that the test meals were not matched for individual SFA's (Additional file 1). Additionally, the PPI nutrient analysis did not include micronutrients such as calcium, which may influence bone turnover in the postprandial state [29]. Moreover, the study was designed to compare effects of the test meals against each other, so there was no control group with which to make comparisons. Furthermore, the sample size was calculated for the original PPI study based on plasma inflammatory marker and oxylipin data rather than bone turnover markers. Our sample size ($n = 36$) is comparable to that of other crossover studies using bone turnover markers [5, 30]; it is unlikely that the sample size was a major limitation in these exploratory analyses but this remains a possibility. Lastly, our protocol allowed subjects to leave the WHNRC if they traveled by car and returned 15 min early and rested for 10 min prior to their scheduled blood draw. In using this particular protocol, we intended to balance the need to minimize exercise for the sake of the experiment with the need to accommodate the free-living subjects who volunteered for our study. A total of 27 subjects chose to leave between blood draws, and it is possible that their activities while away from the WHNRC could have influenced our results. Future studies should follow a more stringent protocol that does not allow subjects to leave between blood draws.

Despite the aforementioned limitations, our observations may be useful for identifying foods and ingredients which maximize the suppression of bone resorption for therapeutic purposes, and for generating hypotheses to test in future studies. Identifying foods and ingredients (e.g. MFGM), as well as meal patterns (e.g. six small meals vs. three larger meals), which can suppress bone resorption for a longer period in the postprandial state, may lead to osteoporosis prevention strategies in the long term. Additionally, it is possible that MFGM may have interacted with other ingredients or nutrients in the meal to give the observed response. The possible interactive effects of MFGM with other food ingredients, as well as the effects of MFGM on longer-term bone outcomes such as BMD, will require further investigation.

Conclusions

In this exploratory study, we have shown that bone resorption (as measured by the biomarker CTX) is significantly suppressed in the postprandial state, particularly after consumption of high SFA meals that contain MFGM. However, MFGM was not found to be a direct moderator of the CTX response. To further elucidate the potential role of inflammation in postprandial bone turnover, future studies based on our observations should 1) match test meals for individual fatty acids and micronutrients as well as macronutrients, 2) include measurements of postprandial bile acids, incretin hormones and insulin, and 3) assess interactions of MFGM with other food ingredients.

Additional files

Additional file 1: Nutrient composition of test meals. (DOCX 21 kb)

Additional file 2: Test meal ingredient list. (DOCX 14 kb)

Additional file 3: Correlations of CTX and selected metabolic variables after intake of high saturated fat test meals. (DOCX 17 kb)

Abbreviations

MFGM: Milk fat globule membrane; PO: Palm oil; PPI: Postprandial inflammation; WC: Whipping cream; WHNRC: Western Human Nutrition Research Center

Competing interests

AMZ received a stipend from the National Dairy Council to present a talk at a symposium in 2013.

At the time of data collected and analysis ED was a doctoral student at the University of California Davis and had no COI to disclose; currently ED is employed by National Dairy Council. TSR, NR, ERG, JBG, JTS and MDVL declare that they have no competing interests.

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The impact of type 2 diabetes on bone metabolism

Abstract

Diabetes complications and osteoporotic fractures are two of the most important causes of morbidity and mortality in older patients and share many features including genetic susceptibility, molecular mechanisms, and environmental factors. Type 2 diabetes mellitus (T2DM) compromises bone microarchitecture by inducing abnormal bone cell function and matrix structure, with increased osteoblast apoptosis, diminished osteoblast differentiation, and enhanced osteoclast-mediated bone resorption. The linkage between these two chronic diseases creates a possibility that certain antidiabetic therapies may affect bone quality. Both glycemic and bone homeostasis are under control of common regulatory factors. These factors include insulin, accumulation of advanced glycation end products, peroxisome proliferator-activated receptor gamma, gastrointestinal hormones (such as the glucose-dependent insulinotropic peptide and the glucagon-like peptides 1 and 2), and bone-derived hormone osteocalcin. This background allows individual pharmacological targets for antidiabetic therapies to affect the bone quality due to their indirect effects on bone cell differentiation and bone remodeling process. Moreover, it's important to consider the fragility fractures as another diabetes complication and discuss more deeply about the requirement for adequate screening and preventive measures. This review aims to briefly explore the impact of T2DM on bone metabolic and mechanical proprieties and fracture risk.

Keywords: Type 2 diabetes, Bone metabolism, Bone mineral density, Fracture

Background

Type 2 diabetes mellitus (T2DM) is associated with an increased risk of fracture, although bone mineral density (BMD) is unaffected or even higher in diabetic patients [1]. The reasons involve likely a combination of features, including the duration of disease, inadequate glycemic control, greater risk of falling as a consequence of hypoglycemia, osteopenia, impairment of bone quality, and side effects of medication, which could lead to a higher risk of bone fragility and fractures [1].

Unfortunately, there is little scientific knowledge approaching the impact of diabetes and of most anti-diabetic treatments on bone quality and fracture risk. Thus, this review aims to briefly explore the impact of T2DM on bone metabolic and mechanical proprieties and fracture risk. Moreover, an accompanying review about the pros and cons of the available pharmacologic treatments for T2DM on bone mineral density and risk of fractures in humans is provided in this issue of *Diabetology & Metabolic Syndrome* by Vianna et al. (doi:10.1186/s13098-017-0274-5).

T2DM and higher risk of bone fracture

The prevalence of T2DM has augmented with the growth in obesity epidemics, mainly because of the lifestyle changes imposed by the modern life. Patients with poorly controlled T2DM are at increased risk for diabetic complications, including macrovascular disease, retinopathy, nephropathy, and neuropathy. Recently, an increased risk of fragility fractures has been recognized as another significant diabetes complication [2]. According to Rotterdam study, individuals with T2DM have a 69% increased risk of having fractures when compared with healthy controls. Paradoxically, T2DM subjects had greater BMD

of the femoral neck and lumbar vertebrae [3]. The discrepancy between BMD and fracture incidence observed in T2DM patients could be attributed to a frailer bone material causing failure at lower stress or to the impaired biomechanical skeletal properties [4]. Osteoporosis is one of the most important causes of reduced bone mineral density, and it is estimated to affect 200 million women worldwide. It accounts for more than 8.9 million fractures annually in women over age 50 [5]. T2DM and osteoporosis are both chronic diseases that may coexist and progressively increase in prevalence and are boosted by aging [6, 7].

It has been observed that T2DM negatively affect bone strength regardless of BMD [1, 8]. The greater risk of fracture is demonstrated by the health, aging and body composition study, where the relative risk (RR) of fracture was 1.64 (95% CI 1.07–2.51) in those with diabetes compared to those without, even after adjustments for hip BMD and additional risk factors for fracture [9]. Typically, T2DM patients have a normal BMD, so this increased risk is probably due to abnormalities in bone material strength and bone biomechanical quality [10]. Some cross-sectional studies in T2DM patients using high-resolution peripheral quantitative computed tomography (HR-pQCT) and magnetic resonance imaging (MRI) revealed quality defects in both cortical and trabecular bone [10]. Farr et al. [10] by assessing bone quality with HR-pQCT in 30 postmenopausal T2DM patients at the distal radius and distal tibia, found that the cortical thickness in T2DM subjects was lower than in controls. Moreover, bone microindentation testing dis-played lower bone material strength (BMS) in post men-opausal women with T2DM compared to those without diabetes [11]. Patsch et al. [12], investigated bone micro-architecture changes in postmenopausal T2DM patients with or without fractures at radius and tibia by using dual-energy X-ray absorptiometry (DXA) and HR-pQCT. They concluded that T2DM patients with fractures had higher pore-related deficits and a greater cortical pore volume than diabetic patients without fractures. Corti-cal defects often accompanied the impaired mechanical properties, such as increased failure load and low bone bending strength, that led to a reduction in overall bone strength and increase in fracture risk [13]. It seems like that bone trabecular and cortical microarchitecture are both deranged in T2DM and may contribute to bone fragility [11, 14]. Bone remodeling decreases, as demon-strated by histomorphometric analysis of bone, which is an additional contributor to the increased the risk of fragility fractures in T2DM patients [15, 16].

Patients with T2DM have an elevated risk of all clinical fractures, particularly African-American and Latino populations [16]. Ageing, prior fracture, corticosteroid use, longer duration of diabetes and poor glycemic control are all contributory factors. Complications comorbidities and diabetic complications such as sensory neuropathy and visual impairment imply in greater risk of falling [4]. Moreover, falling risk may also be associated, at least partially, to increased rates of hypoglycemia, postural hypotension, and vascular disease, contributing to increased risk of fragility fracture [17–19].

Cross-talk between glucose homeostasis and bone metabolism

Recent evidence of common regulatory control of both glycemic and bone homeostasis enables to recognize the intimate relationship between these two entities and similarly the likelihood of antidiabetic agents to impact the bone quality. The shared regulatory control includes accumulation of advanced glycation end products (AGEs), insulin, insulin-like growth factor-1 (IGF-1), peroxisome proliferator-activated receptor gamma (PPARγ), the incretin hormones like glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide 1 and 2 (GLP-1 and GLP-2), the bone-derived hormone osteocalcin and sclerostin.

The impact of vitamin D levels on glycemic control and bone mineral density in postmenopausal women with T2DM have also been studied [20]. Vitamin D [25 (OH) D3] plays a fundamental role in bone metabolism and might impact the development and control of diabetes [21, 22]. Some studies have reported an inverse relationship between HbA1c levels and serum levels of 25 (OH) D3 [22], while others have found that 25 (OH) D3 supplements improve glucose control in T2DM [22, 23]. Physiologically, vitamin D seems to stimulate the expression of the insulin receptor. Therefore vitamin D deficiency might be associated with insulin resistance [24]. Recently, Perez-Diaz et al. [20] have attempted to evaluate the impact of vitamin D levels on glycemic control and bone metabolism. They failed to demonstrate a clear relationship between 25 (OH) D3 levels and glucose control or osteoporotic fractures, even though reported that patients with poor glycemic control had lower 25 (OH) D3 levels than controls.

Advanced glycation end products (AGEs)

The hyperglycemia affects both cellular and extracellular bone matrix. The presence of glucose induces the formation of intermediate products containing highly reactive dicarbonyls, which ultimately leads to the production of irreversible accumulation of advanced glycation end products compounds [25], from a non-enzymatic glycation process [26]. The congeries of AGEs determines the formation of defective collagens and reactive oxygen species (ROS), inducing structural changes in bone through

posttranslational modifications [27]. At the organic bone matrix, these reactions may lead to impaired bone strength [28, 29]. Higher levels of circulating AGEs are reported to increase fracture risk [30].

AGEs bind to the receptor for AGE (RAGE), which is a member of the immunoglobulin superfamily, and it is the AGE-RAGE interaction that mediates generation of ROS, vascular inflammation, macrophage and platelet activation, and stimulates the migration of inflammatory cells [31]. All these reactions contribute to the development and progression of diabetic macro- and microangiopathy and result in a more brittle bone with reduced strength and less ability to deform before fracturing [32].

Immune cells also express RAGE and incite activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a central transcription factor of the immune and inflammatory response [31]. The AGE-RAGE linkage in immune cells results in upregulation of inflammatory cell adhesion molecules and chemokines, releasing, even more, RAGE ligands, and sustaining the inflammatory tissue response, modulating the response of activated macrophages to increase the damaging signals in the tissues and suppressing the repair and remodeling reactions [31]. In a microenvironment with incremental inflammatory cytokines, AGEs may induce osteoclastogenesis and osteoblast dysfunction, which may ultimately result in the development of osteoporosis (Fig. 1) [33]. Pentosidine, the most studied AGE in T2DM patients, accumulates in the cortical and trabecular bone and negatively impact the bone strength and probably leads to functional changes in osteoblasts and the bone mineralization process [34, 35].

The outcome of these reactions is reduced strength and impaired biomechanical properties of both trabecular and cortical bone, including disturbance in osteoblast function and attachment to collagen matrix, damaging the healthy development [30, 35–37].

Insulin and IGF-1

Insulin is an anabolic hormone which acts on bone tissue through its receptors (IRS-1 and IRS-2) expressed by osteoblasts, stimulating bone formation. Insulin increases osteoblast proliferation and promotes collagen synthesis. In the same way, insulin growth factor-1 (IGF-1) acts increasing osteoblast recruitment and bone matrix deposition and diminishing collagen degradation. Studies have exhibited a positive correlation between IGF-1 and BMD, and a negative correlation with hip and vertebral fracture [38, 39].

The peroxisome proliferator-activated receptor gamma (PPARγ)

The PPARγ protein is an essential regulator of lipid, glucose, and insulin metabolism. There are two isoforms in humans, PPARγ1 and PPARγ2. PPARγ1 is expressed in a variety of cell types, including osteoclasts, promoting their differentiation and bone resorption [40]. The PPARγ2 expression restricts to cells of adipocytic lineage [41]. In bone, PPARγ2 plays a significant role in the regulation of mesenchymal cell (MSC) differentiation toward osteoblasts and adipocytes. When this isoform is activated, cells of osteoblast lineage are converted to terminally differentiated adipocytes, disturbing the delicate balance between bone marrow adipocytes and osteoblasts (Fig. 2) [42].

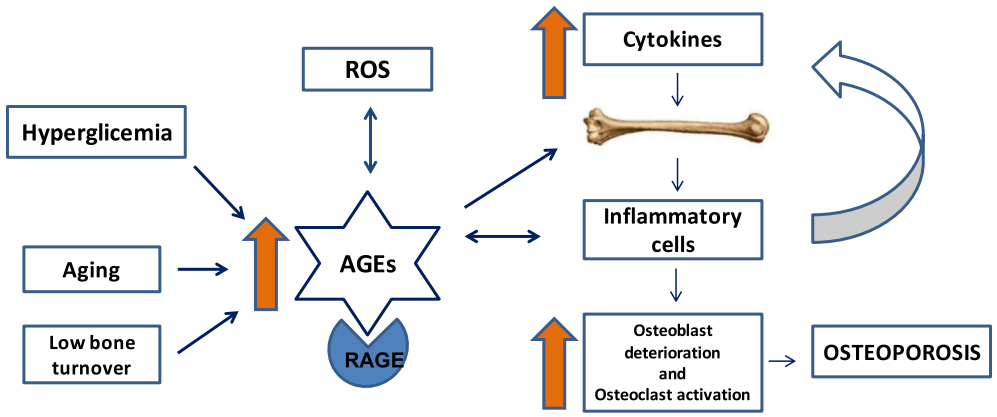

Fig. 1 The relationship between the accumulation of AGEs within the bone. Increased oxidative stress, high glycemic levels, ageing and reduced bone turnover are the main contributors to increased formation and accumulation of AGEs in bone. They induce an inflammatory process that results in activation of osteoclastogenesis, osteoblast dysfunction and accelerated development of the osteoporosis process (Adapted from Sanguineti et al. [33])

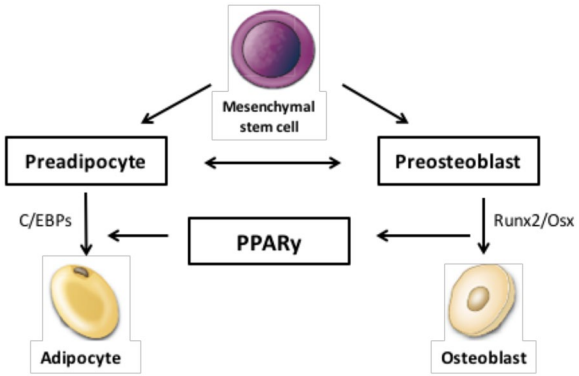

Fig. 2 PPAR regulates mesenchymal cell differentiation. PPAR induces adipogenesis and suppresses osteoblastogenesis, by inhibiting Runx2 function, resulting in a reduction of osteoblasts in the bone marrow. *C/EBPs* CCAAT/enhancer binding proteins, *Osx* osterix, *Runx2* runt-related transcription factor 2 (Adapted from Kawai et al. [43])

The role of enteric hormones

The glucose-dependent insulinotropic peptide (GIP) and the glucagon-like peptides 1 and 2 (GLP-1 and GLP-2) are hormones released by gut enteroendocrine K-cells in the duodenum and proximal jejunum and from L-cells located in the distal ileum and colon, respectively [44]. GIP and GLP-1 are secreted just after nutrient ingestion. They are already released into circulation in their active hormonal form and bind to a specific G protein-coupled receptors present in several cells and target tissues. Both hormones have their bioactivity limited by rapid degradation and inactivation by the enzyme dipeptidyl peptidase-4 (DPP-4), which is present in plasma and expressed in most tissues [45]. The incretin hormones (GIP and GLP-1) stimulate insulin release from β-cells to inhibit glucagon production by the α-cells [46]. Incretin receptors are also expressed in both osteoblasts and osteoclasts. These nutritional hormones are recognized to be significant in bone turnover since as soon as a meal is ingested, bone resorption is suppressed [47, 48]. In times of energy and nutrient excess, the balance is tipped for bone formation, whereas while energy and nutrient are lacking, bone resorption increases [47]. GIP and possibly GLP-1 and GLP-2 may link nutrient ingestion to suppression of bone resorption and stimulation of bone formation [49]. Studies indicate that GLP-2 may affect bone remodeling by disassociating bone resorption and bone formation [50], acting mainly as an antiresorptive hormone [50], while GIP can act both as an antiresorptive and anabolic hormone [49, 51].

Bone turnover markers: focus on osteocalcin

An additional approach to evaluate the impact of diabetes on bone metabolism is to assess the serum markers of bone turnover (BTM), particularly the formation markers osteocalcin (OC) and amino-terminal propeptide of procollagen type 1 (PINP), which are decreased in these patients [52, 53]. Shu et al. [54] investigated structural and biochemical skeletal parameters in T2DM patients and shown that postmenopausal women with T2DM had lower levels of bone formation markers when compared to controls, while their bone structure was not modified. They found lower OC and PINP levels in diabetic subjects, and these levels correlated inversely with glucose levels and fat mass. This concept supports the idea that biochemical indices of bone formation are lower in T2DM patients than in controls. Moreover, the resorption marker CTX (serum C-terminal telopeptide from type 1 collagen) is shown by some authors to be reduced in T2DM individuals [52, 55], while other revealed no difference [56].

Interestingly, OC seems also to have a role in energy metabolism. In its undercarboxylated form, OC stimulates insulin secretion and enhances insulin sensitivity in both adipose and muscle tissue. An inverse association between OC and metabolic syndrome has been demonstrated, suggesting that reduced levels of osteocalcin may impact in the pathophysiology of T2DM [57, 58]. Consequently, the skeleton has been considered a new endocrine organ that participates and influences glucose homeostasis.

The Wnt/ß-catenin pathway

Sclerostin is another regulator of bone metabolism and is expressed by osteocytes. It inhibits the Wnt/ß-catenin pathway by binding to low-density lipoprotein receptor-related protein (LPR) 5 or 6 and negatively regulates bone formation [59]. The Wnt/ß-catenin pathway induces osteoblastogenesis and thereby enhances bone formation. Canonical Wnt signaling suppresses osteoclastogenesis by inducing osteoprotegerin, and, also, suppresses bone resorption by an osteoprotegerin-independent mechanism acting directly on osteoclast precursors. The dual effect of Wnt on cells of the osteoblast and osteoclast lineage results in an increase in bone mass. So, when sclerostin bind to Wnt co-receptors, inhibition of osteoblastogenesis and bone formation occurs (Fig. 3) [59]. Patients with T2DM have higher serum levels of sclerostin, which are associated with increased risk of vertebral fractures. Studies also show that sclerostin levels is directly related to both duration of T2DM and glycated hemoglobin, and inversely related to levels of bone turnover markers [52, 60].

The impact of vitamin D

The hyperglycemia seems to play a major role on the vitamin D-calcium axis through impairment renal calcium

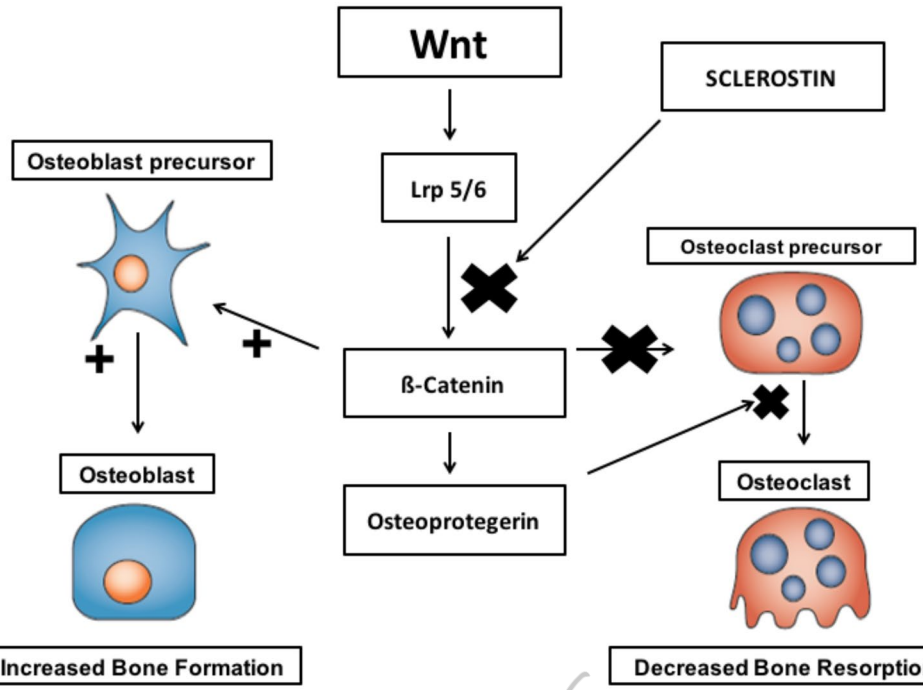

Fig. 3 Canonical Wnt signaling and bone remodeling. T2DM patients present a greater amount of sclerostin, which blocks the Wnt pathway and inhibits osteoblastogenesis. *Lrp* lipoprotein receptor-related protein (Adapted from Canalis et al. [59])

absorption [61]. High glycemic levels contribute to the reduced number of 1,25(OH)$_2$D3 (1,25-dihydroxy vitamin D) receptors on osteoblasts and limit the ability of the osteoblast to synthesize osteocalcin in response to 1,25(OH)$_2$D3 [61]. However, the vitamin D performance in affecting T2DM and fracture risk is currently uncertain [31].

Summary

As reviewed in the topics above, several direct and indirect mechanisms in T2DM may affect the bone metabolism and quality, as well the risk of fractures. Table 1 review and summarize the effects of type 2 diabetes on bone.

Table 1 Summary of the mechanisms by which T2DM negatively affects the bone

	Mechanisms	Effects on bone
AGEs	Osteoclastogenesis and osteoblast dysfunction [28]	Low bone quality [29, 30] Increased risk of fragility fracture [28]
Insulin and IGF-1	Increases osteoblast proliferation and promotes collagen synthesis [38]	Negative correlation with hip and vertebral fracture [39]
PPARγ	Differentiate MSC into adipocytes [42]	Suppression of osteoblastogenesis [43]
Enteric hormones (incretins)	Energy intake releases GIP and GLP-2 [47–50]	Low incretin levels decrease bone formation and augment resorption [47–50]
Osteocalcin	Low levels in T2DM [54]	Low levels decrease bone formation [57, 58]
Wnt/B-catenin pathway: sclerostin	High levels in T2DM [59]	High sclerostin levels increase bone resorption [59]
Vitamin D3	Low levels in T2DM [31] Reduction of 1,25(OH)$_2$D3 receptors [61]	Reduction of osteocalcin synthesis [61]

The indirect and direct effects of compromised glucose/insulin metabolism on bone induces a decreased bone turnover, a reduced bone quality and an augmented risk of fractures

AGEs advanced glycation end-products, *IGF-1* insulin-like growth factor-1, *PPARγ* peroxisome proliferator-activated receptor gamma, *MSC* mesenchymal stem-cells, *GIP* glucose-dependent insulinotropic polypeptide, *GLP-2* glucagon-like peptide-2, *T2DM* type 2 diabetes mellitus, *1,25(OH)$_2$D3* 1,25 dihydroxy vitamin D

Conclusion

Patients with T2DM have an augmented risk for fragility fractures, not predictable by BMD measurements. This higher risk is probably multifactorial.
Despite
these features, there are no current recommendations regarding routine screening or initiation of preventative medications for osteoporosis in patients with diabetes. Adequate glycemic control prevents this risk and reduces the micro-and macrovascular complications, which consequently, can contribute to diminish the production of AGE's, reduce the vascular damage in the bone tissue and lessen the risk of falls. As reported, bone and energy metabolism are closely related, and this connection occurs since the differentiation of adipocytes and osteoblasts from the same mesenchymal stem cells. In hyperglycemic patients, bone formation decreases and all mechanisms described so far contribute to the poorer bone formation and quality, increasing fracture risk. Currently, it is essential to consider the fragility fractures as an additional diabetes complication, recognize the diabetes bone disease as a specific pathology, and discuss more deeply about the requirement for adequate screening and preventive measures.

Abbreviations

AGEs: advanced glycation end products; BMD: bone mineral density; BMS: bone material strength; BTM: bone turnover markers; CTX: C-terminal telopeptide of type 1 collagen; DPP-4: dipeptidyl peptidase-4; DXA: dual-energy X-ray absorptiometry; GIP: glucose-dependent insulinotropic peptide; GLP-1: glucagon-like peptide 1; GLP-2: glucagon-like peptide 2; HR- pQCT: high-resolution peripheral quantitative computed tomography; IGF-1: insulin-like growth factor-1; IRS-1: insulin receptor substrate-1; IRS-2: insulin receptor substrate-2; LPR: low-density lipoprotein receptor-related protein; MRI: magnetic resonance imaging; MSC: mesenchymal cell; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB); OC: osteocalcin; P1NP: amino-terminal propeptide of procollagen type 1; PPARγ: peroxisome proliferator-activated receptor gamma; RAGE: AGEs' receptors; ROS: reactive oxygen species; RR: relative risk; T2DM: type 2 diabetes mellitus; $1,25(OH)_2D3$:

Competing interests

The authors declare that they have no competing interests.

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