REVIEW

Nutrition and Bone Marrow Adiposity in Relation to Bone Health

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Summary

Bone remodeling is energetically demanding process. Energy coming from nutrients present in the diet contributes to function of different cell type including osteoblasts, osteocytes and osteoclasts in bone marrow participating in bone homeostasis. With aging, obesity and osteoporosis the function of key building blocks, bone marrow stromal cells (BMSCs), changes towards higher accumulation of bone marrow adipose tissue (BMAT) and decreased bone mass, which is affected by diet and sex dimorphism. Men and women have unique nutritional needs based on physiological and hormonal changes across the life span. However, the exact molecular mechanisms behind these pathophysiological conditions in bone are not well-known. In this review, we focus on bone and BMAT physiology in men and women and how this approach has been taken by animal studies. Furthermore, we discuss the different diet interventions and impact on bone and BMAT in respect to sex differences. We also discuss the future perspective on precision nutrition with a consideration of sex-based differences which could bring better understanding of the diet intervention in bone health and weight management.

Key words

Nutrition • Diet composition • Bone • Bone marrow adiposity • Sex differences

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Introduction

Bone is a complex, continually changing tissue that provides mechanical support for ligaments, tendons and joints, protects vital organs from damage, and serves as a reservoir for phosphate and calcium in maintaining regular mineral homeostasis [1,2]. Bone matrix contains several types of collagens including type I collagen [1] as well as non-collagenous proteins and growth factors, which are unique to bone tissue and important for mineralization.

The bone tissue consists of two main parts: dense cortical bone, forming a solid outer layer, and porous trabecular (spongy) bone, mainly found at bone ends and inner parts. Cortical bone makes up 80 % of the skeleton, providing structure, while trabecular bone has a larger surface area and is less dense and more susceptible to rapid loss during increased bone turnover [2]. The composition and structure of these bones support the skeleton's mechanical functions [1].

Under hard core of bone, there is bone marrow (BM) which consists of various cell types, including: i) hematopoietic stem cells (HSCs) and progenitor HSCs, which can differentiate into different types of blood cells, such as red blood cells (erythrocytes), white blood cells (leukocytes), myeloid precursors for osteoclast formation (bone resorptive cells) and platelets (thrombocytes); ii) bone marrow stromal cells (BMSCs) which can differentiate towards adipocytes or bone forming cells – osteoblasts and chondrocytes; iii)) fibroblasts that produce connective tissue and support the structure of the BM; iv) endothelial cells, and v) nerve cells [3,4].

Another compartment of BM is bone marrow adipose tissue (BMAT) arising from BMSCs. BMAT makes up to 10 % of whole-body fat mass in lean and healthy adults [5]. This overlooked fat depot has been considered as an inert filler of the bone cavity for a long time. However, increased scientific interest in last decades brought new findings on BMAT to be characterized as a secretory and metabolically active organ that responds to nutritional challenges and secretes cytokines that indirectly influence bone and energy metabolism [3,5-7].

In this review we present the overview of the literature in animal and human studies investigating bone and BMAT physiology in respect to sex differences. Furthermore, we discuss the impact of diet interventions and the contribution of different nutrients to bone health and BMAT accumulation. As most of the studies has been performed in males, it raises a question for the precise nutrition how different dietary demands can be applied in both sexes with consideration on healthy aging and longer life expectancy.

Bone structure and bone marrow composition in males and females

It has been well-documented that genetic and non-genetic (diet, exercise, age, and sex) factors influence bone strength and quality [8]. Notably, sex differences in bone morphology, mechanical properties and response to mechanical loading have been reported in various mouse models [9,10]. Yao *et al*. [10a] observed significant sex differences in trabecular and cortical bone geometry and morphology of 4-month-old C57BL/6J mice, where male mice had inherently more bone compared to female mice, with significantly higher cortical and trabecular bone volume and thickness [11]. Considering sex difference in humans, the study comparing 18-year-old male and female participants indicated that despite comparable body size, males have greater bone mineral content (BMC) and bone mineral density (BMD) at the hip and distal tibia and greater tibial cortical thickness which may confer greater skeletal integrity in males [11]. In humans, the sexual dimorphism is expressed in bone length, BMD and geometry, providing men with a potential advantage in bone mechanical resistance compared to women [12]. Importantly, the study using high-resolution peripheral quantitative computer tomography [13] showed larger total bone area in distal radius and tibia in young men compared to women. Moreover, this study showed, that in young men trabecular number and thickness were 7-20 % higher than in women in both sites and cortical porosity was 31-44 % decreased in young women than in young men. The distal radius cortex of young women carried 14 % more load compared to young men. However, bone strength was 34-47 % greater in young men compared to women [13]. More studies have analyzed the difference of cortical and trabecular parameters of lumbar vertebra [14]. Single-energy quantitative computed tomography (CT) of lumbar vertebrae in subjects of both sexes younger than 40 years versus group older than 65 years showed no difference in cortical bone of younger subject, but there was significant decrease of cortical volume in women compared to males caused by aging [15]. However, trabecular volume was significantly decreased in adult females compared to males with no significant age difference. On the other hand, trabecular BMD was significantly lower in old female group compared to old males. Another study showed age- and sex-related changes of lumbar bone microstructure, e.g. decreased cortical and trabecular BMD in females leading to increased fracture load of analyzed L1-L3 vertebrae compared to men in 6-year follow-up study (patients were over 50 years old with no previous lumbar fractures) [14].

Bone matrix composition and the total collagen content might not significantly differ between males and females, however, there could be differences in the composition or structure of collagen between sexes [16]. On the other hand, in mature individuals, the male skeleton contains roughly 1400 g of calcium, while the female skeleton contains about 1200 g [17]. However, there are no specific data on sex-specific content of phosphorus in bones. Therefore, further investigation might be necessary to better understand physiological differences between males and females on the BMC and mineralization process.

In terms of BM cellular composition, several studies have identified differences between male and female hematopoietic systems [18]. Nakada *et al.* [19] found that males and females have similar basal numbers of HSCs and their multipotent progenitor cells. However, female HSCs undergo more frequent self-renewing divisions without depletion of the stem cell pool driven by estrogen receptor alpha (ERα) signaling. Another animal study showed that increased estrogen levels decrease B lymphopoiesis [20]. Singer *et al.* [21] reported that obese males have increased myelopoiesis and

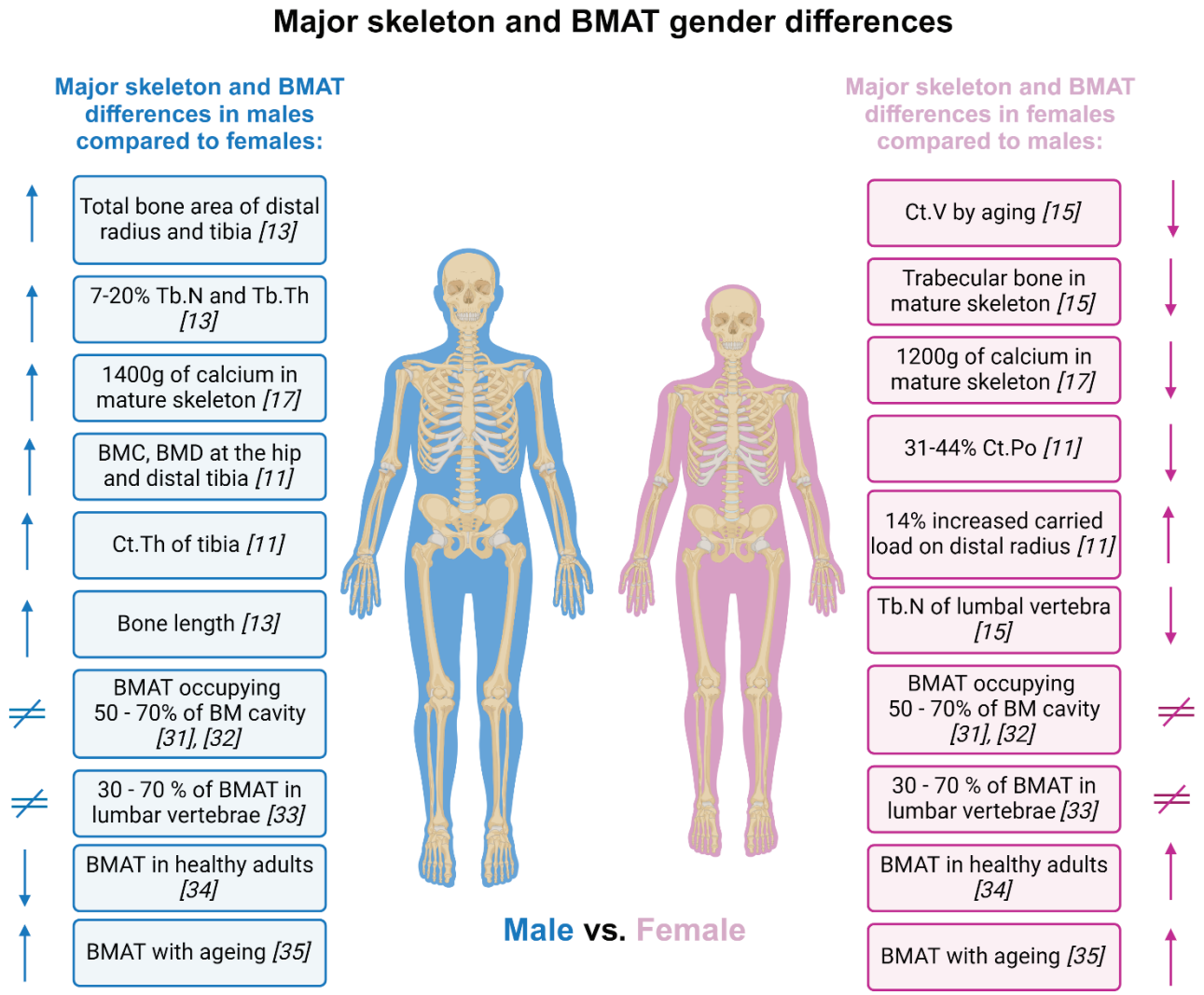

Fig. 1. Major skeleton and BMAT sex differences in adult and elder humans (Created with Biorender.com).

increased pro-inflammatory response of macrophages compared to age-matched females. However, more studies using single cell RNA sequencing are needed to dissect sex dimorphism in BM composition, especially on BMSC heterogeneity under physiological conditions and how it is affected with aging, osteoporotic or metabolic challenges in relation to bone homeostasis. Main differences in BM composition between sexes have been reported in context of BM adiposity, which is discussed later in this review. The major differences in skeleton between males and females are depicted in Figure 1.

During the lifetime, bone undergoes remodeling (coupling of bone formation and bone resorption) which is the process of changing size or shape of bones in response to physiological or mechanical stimuli [1]. An imbalance of these two processes leads to bone impairment and bone loss [22]. Several studies documented the role of growth hormone and sex steroids as key regulators of bone development and growth [23,24]. It suggests that sex differences in long bone structure may arise from the different hormonal environments in female and male BM as BMSCs express ERα affecting BMSC differentiation potential and expansion in BM [25]. Additionally, the impact of sex steroids on the response of bone cells to mechanical loading is emphasized, as demonstrated by studies on sex steroid receptor knock-out mice [26].

Another key factor contributing to the impact on bone remodeling in respect to sex differences is aging [27]. In perimenopausal and early postmenopausal women the remodeling is increased, slowing down with further aging but remaining faster than in premenopausal women [28]. Remodeling is also slightly increased in aging men [27] but there is still a lack of studies directly comparing bone remodeling processes in men and women.

Clinical study including adults with a mean age of 50 years, found that female participants have lower trabecular parameters compared to men [29]. Khosla *et al.* [30] demonstrated the same results in the cohort of 90-year-old participants. This discrepancy in bone parameter characteristics suggests potential differences in skeletal integrity between males and females, serving as a critical point for this review.

BMAT development and physiology in males and females

BMAT, derived from BMSCs, represents important part of BM cavity and its presence changes with age, sex and skeletal site. At the birth red BM is mostly filled with HSCs, which is substituted during adulthood (around the age of 25 years) with yellow BM filled with BMAT occupying around 70 % of the BM volume, mostly in distal bones [31]. In the literature, two types of BMAT have been described: constitutive BMAT (cBMAT), which is located within the yellow BM of the distal skeleton, where hematopoiesis is nearly absent, and regulated BMAT (rBMAT), which is localized in the proximal skeleton, where bone remodeling and hematopoiesis are active. rBMAT can be modulated by different factors such as nutrition, aging and endocrine status, while cBMAT is much more inert [32]. rBMAT changes in response to ovariectomy (OVX), obesity, caloric restriction (CR) and irradiation, are typically accompanied by hematopoietic abnormalities and/or bone loss [33]. Therefore, the changes in BMAT volume are important to be measured in relation to bone health and also in respect to sex differences.

BMAT volume changes through lifetime and its distribution and composition are also affected by sex [34]. In humans, the expansion of BMAT in long bones occurs from distal to proximal sites and is more prevalent at distal sites after birth. BMAT is easily detected in distal epiphyses of long bones by age of 6-7 years and in the midshaft around 12-14 years of age in both sexes [35]. Then, in adults aged about 25 years, BMAT is occupying approximately 50-70 % of the total BM cavity, with at least some bone marrow adipocytes (BMAds) also present in sternum, ribs, pelvis and vertebrae [35,36]. In lumbar vertebrae, BMAT expands varying from 30 % to 70 % in both sexes between 8 and 57 years of age, respectively [37]. Healthy adult women tend to have higher levels of BMAT compared to healthy adult men, this difference is particularly evident in the hips and femur. In women, BMAT is often distributed in the lower extremities, including the hips and thighs, whereas in men is more concentrated in the trunk and abdomen [38,39]. Notably, the accumulation of BMAT with age is also influenced by sex. Females younger than 55 years have approximately 5-10 % lower level of BMAT than age-matched males [40]. Moreover, the significant increase of BMAT in postmenopausal women causes about 10 % higher BMAT content than in males at the age over 60 years [41]. With aging, both men and women experience an increase in BMAT [38,41]. However, the rate and extent of this increase may vary between sexes influenced by age and pathophysiological and metabolic status. In postmenopausal women, there is a significant increase in BMAT due to hormonal changes [42]. Estrogens play a crucial role in BMAT regulation. Reduction in estrogen levels, particularly during menopause in women, is associated with an increase in BMAT [43,44]. Androgen levels in men may also influence BMAT, low endogenous testosterone is associated with high BMAT in older men [42]. These changes are summarized in Figure 1.

In contrast, mice have lower BM adiposity than humans, but the sequence and timing in bone marrow adipocyte development are very similar. In mice, BMAT is present in caudal vertebrae as early as one week after birth. BMAT may also be found in sacrum and lower lumbar vertebral bodies in adults, but it is rare to see BMAT in cervical and thoracic vertebrae. BMAT in distal tibias are readily detectable at four weeks and continue to accumulate until the cavity is filled at around eight weeks of age (adults) [45]. BMAT in proximal tibia and femur appears later than in distal tibia and caudal vertebrae. Male mice usually develop BMAT later and less extensively in proximal tibia than age-matched females [5,45], which is a little bit different compared to humans. Importantly, the different expansion of BMAT in males and females during lifetime may differently contribute to the bone homeostasis and by secretion of bioactive molecules to the regulation of bone remodeling and energy metabolism [5,46]. Moreover, since BMAT is derived from BMSCs, sex differences in BMAT expansion may be caused by different differentiation capacity of BMSCs in males and females [4]. However, more studies are needed to understand the molecular mechanism behind these changes.

Moreover, the understanding of potential ethnic and sex differences in the relationship between BMD and BMAT is important for future studies focused on

developing of the prevention and treatment strategies for bone loss and fracture risk. Shen *et al.* [47] showed that healthy men and premenopausal women had higher total body BMD levels than postmenopausal women for the same amount of BMAT. The increased BMAT in postmenopausal women is linked to osteoporosis and impaired bone quality [48]. Understanding these sex differences in BMAT is essential for investigating its role in bone health, metabolic disorders, and for designing targeted interventions in various conditions affecting bone and metabolism. However, up to now, prospective studies examining BMAT variations with age, sex and skeletal sites are still lacking.

Dietary factors affecting bone and BMAT

Besides micronutrients (minerals, vitamins), there are also macronutrients like carbohydrates, proteins, fat and fiber that are important parts of diet composition affecting bone homeostasis. All of these components play a critical role in maintenance of bone health and it is necessary to keep them in a healthy balance. They can have positive or negative impact on bone and fat metabolism. Therefore, we will provide an overview of animal and clinical studies using different dietary interventions in the context of bone health and fracture risk in relation to sex differences (summarized in Tables 1 and 2).

Diets enriched in carbohydrates and their effect on bone and BMAT

Carbohydrates, especially glucose, are usually considered as main sources of energy for cellular metabolism in many cell types [49,50]. However, chronic exposure to high glucose levels in the body represents a pathological condition impairing glucose metabolism and leading to insulin resistance, diabetes and bone loss [51,52]. Several *in vitro* studies using osteoblastic cell lines (MC3T3-E1) documented a negative impact of high glucose (22-30.5 mM) on osteoblast differentiation [51-53] inducing higher reactive oxygen species production, decreased proliferation and cellular mineralization. Negative effect of high-sucrose diet on bones in rodents of both sexes has been known for a long time [54,55]. More recent studies are adding further information about the effect of different saccharides on bones. Yarrow *et al.* [56] reported a negative effect of diet enriched with 40 % fructose in 8-week-old Sprague-Dawley male rats on bone homeostasis compared to control diet. After 12 weeks of treatment bone marrow adipocyte density measured in histology slides was increased, while bone volume and trabecular number of proximal tibia measured by micro-CT (μCT) were decreased by high-fructose diet compared to control diet (more details in Table 1). On the other hand, drinking of 10 % fructose during 28 days in adult male Sprague-Dawley rats had a deleterious effect on osteocyte density, while no difference on BMAT was observed in femur compared to control diet [57]. Moreover, differentiation analysis of primary BMSCs showed decreased osteoblast and increased adipocyte differentiation supporting *in vivo* bone phenotype.

Testing the effect of different sweetener in drinking water (glucose, fructose, sucrose etc.) on bone properties of 35-day-old female Sprague-Dawley rats during 8 weeks [58] showed no significant changes in terms of bone mass and bone strength between the groups. However, the increased consumption of glucose altered mineral homeostasis which led to decreased phosphorus and calcium intake and increased calcium excretion compared to fructose beverage suggesting that glucose exerts more detrimental effect on bones than fructose in female rats, but this study did not investigate BMAT in treated rats.

Negative effects on bone quality were observed in 9-week-old C57BL/6 female mice which were treated for 10 weeks with high-fat/high-sucrose diet [59]. μCT analysis of tibias showed decreased bone mass along with lower mechanical properties in high-fat/high sucrose diet compared to control diet. Osteoclastogenesis expressed as Receptor activator of NFκB ligand/osteoprotegerin ratio was not affected, but the expression of cyclooxygenase-2 was increased suggesting increased inflammation. On the other hand, Minematsu *et al.* [60] reported a positive effect of 24-week feeding of high-fat/high-sucrose diet on bone quality in aging model of over one-year-old Wistar male rats measured by μCT analysis of trabecular and cortical bone volume and biochemical analysis of Tartrate-resistant acid phosphatase (TRAP) and calcium levels, while BMAT was not measured.

In humans, the most pronounced effect of higher intake of sugar on bones has been observed in teenagers who consume excess refined carbohydrates and sugars, by increased fracture risk associated with drinking of sweetened beverages [61,62]. Moreover, hyperglycemia commonly driven by high-saccharide diet is strongly associated with increased osteoporotic fracture risk in

older patients [63]. However, there is still a lack of studies directly comparing the effect of high-saccharide diet on bone homeostasis in adult men and women. Previous studies were mostly focused on the effect of different dietary conditions (including diet enriched with saccharides) on fracture risk or BMD in elderly population showing a negative association of higher glycemic index with increased prevalence of fractures in both sexes [64,65]. The different results in animal and clinical studies just point out the lack of comprehensive studies comparing effect of increased saccharide intake in both males and females with a more focus on measurement of BMAT in the context of bone health and fracture risk.

Calorie-restricted diet vs diet enriched in fatty acids and their impact on bone and BMAT

BMAT is unique in its origin and its response to dietary changes. It is known that both caloric restriction (CR) and high-fat diet (HFD) may increase BMAT in rodents and humans [66-70] (see listed in Tables 1 and 2). Many studies have considered BMAT composition and quantity in the context of bone health and metabolic risk. In mice models, both nutrient challenges (CR and HFD) cause enhanced BM adiposity, whereas the extramedullary responses are quite distinct [6,71].

Moreover, expansion of BMAT with CR (10 kcal% fat) has been consistently observed across sex, age and durations from 6 to 19 weeks, but the reduction of bone mass is not uniformly observed in all mouse models. CR of male mice causes bone loss during their active growth (three weeks to about three months of age) [70,72]. On the other hand, female mice have much slighter bone changes despite the significant changes in BMAT after CR. Although CR causes bone loss in very young actively growing female mice, the effects on bone gradually diminish with age [69,73]. It is possible that estrogen contributes to differences between sexes. Moreover, estrogen deficiency drives BM adiposity in mice and can act as an interactive component associated with dietary changes [74,75]. Devlin *et al.* [70] studied the effect of 9-week CR in 3-week-old male mice and they reported significant elevation in BMAT volume which was associated with decreased cortical bone mass in the femur. Interestingly, another study of 12-week CR in 6-month-old female Sprague Dawley rats reported decreased body weight but an increase in bone marrow adipocytes in proximal femur and tibia, and decreased BMD of the proximal tibia [76].

The effects of CR on BMAT in human studies are not clear, with some reports showing an increase in BMAT [68,77,78], whereas others demonstrating no change or less BMAT [79,80]. However, discrepancies between these studies may be affected by different age, sex, treatment protocols or the method of BMAT evaluation (Table 2). Interestingly, CR is considered to have health benefits including reduced adiposity, improved metabolism and increased lifespan; however, a downside includes effects on bone and BMAT. Fazeli *et al.* [66] revealed in an acute 10-day high-calorie feeding protocol followed by a 10-day fasting protocol in healthy men that BMAT elevated in response to both interventions, but the pathophysiology and cues for these changes may differ. Furthermore, 18 months of randomized dietary interventional study with 138 participants (including men and women, mean age 47.8±9.1 years) showed that physiological weight loss can transiently reduce BMAT (MRI quantification) in adults with a more prominent effect in younger adults of both sexes [81]. Another study determined by proton magnetic resonance spectroscopy that women with anorexia nervosa have elevated BMAT content in the lumbar spine as well as in the femoral diaphysis and metaphysis compared with normal weight control group [67]. Taken together, preclinical models of CR and various nutritional status can differently affect BMAT volume and composition in animal models and humans with different response in respect to sex, age, strain, site-specificity and treatment protocol. Thus, it seems that the mechanisms underlying these changes are different between men and women. Giving the evidence of BMAT accumulation under different metabolic conditions, future strategies are needed to define causal mechanism and better reveal the age and sex contribution.

The diets with different content of saturated and unsaturated fatty acids (FA) can have detrimental or beneficial effect on bone, fat metabolism and homeostasis. The role of saturated and unsaturated FA in bone and fat homeostasis can exhibit sex-specific differences. In general, females tend to have higher levels of essential body fat mass for reproductive and hormonal reasons [11,82]. However, dietary patterns play a key role in how saturated and unsaturated fats affect bone metabolism [83]. Consuming an excessive number of calories or maintaining an unbalanced diet, typically characterized by the presence of saturated and trans-unsaturated FA, is associated with an elevated body mass index, obesity and complications that impact both bone

and fat metabolism [84]. HFD enriched in saturated FA induces increased inflammation and oxidative stress, and thus affecting bone density and increasing fracture risk [6,85,86]. Studies using HFD in rodents aimed to induce metabolic changes leading to increased BMAT and bone impairment. The impact appears to depend on factors like the duration of the HFD and the percentage of fat content. Prolonged exposure to a HFD (4 kcal%) may initially increase bone mass, but over time, it seems to lead to decreased bone formation and turnover, potentially associated with metabolic impairment in male mice [87] and ovariectomized 6-month-old female rats [88]. Diets with higher fat content (60 kcal%) are generally associated with more detrimental effects by increasing BMAT and bone loss [6], decreasing cortical and trabecular thickness and increasing bone porosity more in males than females [89,90]. On the contrary, lower fat content diets (42 kcal%) may have an anabolic effect on bone, at least over a more extended period, in mice of both sexes [91]. Our previous study and others [6,89] using 8-week-old male C57BL/6J mice showed that 12-week treatment with 60 kcal% HFD decreased bone volume and increased trabecular separation and cortical porosity of proximal tibia. Moreover, bone formation rate was decreased in tibia as well as in vertebrae compared to control diet. Furthermore, BMAT analysis using hematoxylin-eosin staining and osmium-tetroxide staining showed increased adiposity in proximal tibia after HFD diet, which was accompanied by increased adipocyte differentiation of primary mouse BMSCs. According to these studies, the higher is saturated fat content, the more damage is delivered to a bone. Findings from other studies focused on the effects of obesity on BMAT changes are well summarized in our previous review [7].

Conversely, it is considered that the incorporation of unsaturated FA, particularly omega-3 polyunsaturated FA (omega-3 PUFAs), have a more favorable impact on BMAT and overall bone and fat homeostasis in both males and females. Furthermore, unsaturated FA play a role in fat homeostasis by positive influence on overall body fat composition. Docosahexaenoic acid (DHA; 22:6n-3) and eicosatetraenoic acid (EPA; 20:5n-3) as main representatives of omega-3 PUFAs help to reduce the accumulation of excess fat and improve glucose metabolism in metabolic complications [92,93].

Animal studies employing HFD supplemented with omega-3 PUFAs in osteoporotic models demonstrated a reduced negative impact on bone loss and BMAT [94,95]. Our recent study using omega-3 PUFAs supplementation in C57BL/6N male mice for two months [96] or other study using 6-month dietary intervention [97] decreased BMAT and prevented bone impairment. Human study focused on different types of omega-3 PUFAs and hip fracture risk, involving men and postmenopausal women, found that higher alpha-linolenic acid consumption was linked to a decreased risk of hip fractures in women but not in men. Interestingly, there was no correlation between intake of EPA+DHA and the risk of hip fractures [98], which was confirmed by other study focused on male and female participants aged 65 years or older [99]. Senile osteoporotic women treated with combination of PUFAs and calcium maintained lumbar and increased femoral neck BMD compared to control group [100]. While current evidence in humans does not strongly support a positive relationship between omega-3 PUFAs and human osteoporosis prevention or treatment, it suggests potential benefits when incorporating omega-3 PUFAs into the diet rich in calcium, vitamins, and minerals or concentrated oil mixtures with other PUFAs. To comprehensively explore the effect of omega-3 PUFAs on fracture risk, further large-scale investigations are needed, particularly focusing on the treatment with different types of omega-3 PUFAs on bone quality. The intake of EPA, DHA and EPA+DHA has been found to be significantly higher in males than females in several age categories [101]. While there may be some sex-specific differences in bone health and susceptibility to conditions like osteoporosis, the effects of saturated and unsaturated FA on bone and BMAT are generally similar for both sexes. More details are summarized in Table 2. A diet rich in unsaturated fats and low in saturated fats is recommended to promote healthier bone and fat homeostasis in both males and females.

Protein-enriched diet and its effect on bone and BMAT

The optimal dietary protein intake has been studied for decades, as non-pharmacological approach how to maintain skeletal health in adults. It is also becoming clear that protein and their individual amino acids (AA) can have different effects on cell function and impact on bone formation. However, it is still unclear whether dietary protein exerts a positive or negative effect on bone health. Protein undernutrition is a known factor in the pathogenesis of osteoporotic fracture in the elderly, but the mechanisms of bone loss

resulting from this deficiency are still poorly understood. The details of these studies are summarized in Tables 1 and 2. On the cellular level, it has been shown that protein malnutrition induced increased adipocyte differentiation of BMSCs isolated from 2-month-old male Balb/c mice fed for 3 weeks with 2 % low-protein diet compared to 12 % control protein diet. This protein malnutrition led to impaired hematopoietic microenvironment and inducing the BM failure [102]. Similar results were observed in another study using C57BL/6N male mice with the same protein supplementation on impaired HSC differentiation towards lymphoid, granulocytic and megakaryocytic-erythroid lineage [103]. In animal models, both low and high dietary protein intakes have shown to suppress the acquisition of bone mass and the increase in bone strength during growth in comparison to moderate protein intake [104-108]. Takeda *et al.* [104] showed the effect of different levels of protein diet in growing 5-week-old male rats after 2 months of dietary intervention. Measurement of BMD and bone strength by dual-energy X-ray absorptiometry (DXA) and three-point bending test revealed suppressed acquisition of bone mass and increased bone strength with low protein diet compared to medium and high protein diets. Furthermore, Dubois-Ferrière *et al.* [105] reported that mechanical properties measured by three-point bending test and bone microstructure were decreased in 6-month-old Sprague-Dawley female rats fed with low-protein diet for 10 weeks. Another study [106] using a selective isocaloric protein-restricted diet in adult female rats showed similar detrimental effect on both cortical and trabecular parameters, through the impact on the insulin-like growth factor 1 (IGF-1) and sex hormone regulation. Prevention design of the study using casein or soy protein supplementation in CR-induced bone loss in 8-month-old Sprague-Dawley male rats did show a beneficial effect on bone mass [109]. Moreover, Wright *et al.* [108] investigated the effect of 12-week feeding of four CR diets varying in predominate protein source (beef, milk, soy, casein) and protein quantity (control diet 15 % vs. high-protein diet 35 %) on bone and body composition outcomes in 32-week-old female rat model of postmenopausal obesity. Overall, CR had a negative impact on bone parameters with different extent depending on the protein source. Thus, these results suggest that specific protein source recommendations may be needed to attenuate the adverse alterations in bone quality following a high-protein CR diet in a model of postmenopausal obesity. Other animal studies supporting these results are summarized in Table 1. However, none of these studies measured BMAT in the context of bone health.

The effects of protein diet in humans have been studied in various conditions. However, it is not well-known whether this dietary intervention can modulate the BMAT volume. Trudel *et al.* [110] investigated the effect of high-protein diet and bed rest interventions (two bed rest campaigns consisted of 7 days of baseline data collection, 21 days of head-down tilt and 6 days of recovery) in healthy men on the lumbar BMAT volume showing no change of lumbar bone marrow fat fraction measured by MRI. In a different study combining bed rest and a high-protein, leucine-supplemented diet, 8 healthy women aged 25-40 years, showed no change in lumbar fat fraction [111]. Even 18 months of protein supplementation in diet did not improve lumbar spine BMD measured by DXA in 208 older women (70.5±6.4 years) [112]. Cao *et al.* [113] demonstrated that short-term consumption of high-protein diets (31 days) during CR did not disrupt calcium homeostasis and it was not accompanied by any changes of BMC and BMD in young adult men and women aged 20-21 years. Arjmandi *et al.* [114] found that one-year supplementation with soy protein positively modulated markers of bone formation in postmenopausal women (≤ 65 years), but on the other hand this amount of protein was unable to prevent lumbar and whole-body bone loss. Contrary, Holm *et al.* [115] showed that whey protein supplementation resulted in superior improvements in femoral neck BMD and bone formation during 24 weeks of strength training in postmenopausal women (55±1 years). The observed differences following such a short intervention emphasize the significance of post-exercise nutrient supply on musculoskeletal maintenance. All published studies of protein enriched diet and its effect on bone and BMAT are summarized in Table 2.

Taken together, animal and clinical studies regarding the impact of protein intake on bone health and BMAT are not very conclusive as the studies differ in the design, targeted group of subjects and methods of bone and BMAT evaluation. Further studies are needed to study the impact of low or high protein intake on bone and BMAT parameters in relation to prevention strategies to decrease fracture risk.

The diet with essential branched chain amino acids (BCAAs) and its effect on bone and BMAT

Several studies suggest that further attention is warranted to the impact of specific AA on skeletal health, rather than just considering protein content as a whole. BCAAs, which include valine, leucine, and isoleucine, account for upwards of 40 % of the preformed AA. These are essential AA that must be acquired by dietary intake [116]. However, there is a lack of studies on the effects of BCAAs on skeletal health and BMAT. In animal studies, *in vitro* BCAAs supplementation increased metabolic activity and proliferation of BMSCs and enhanced the immunomodulatory capacity of BMSCs by decreasing the p-NFκB/NFκB ratio and increasing synthesis of the anti-inflammatory mediators TGF-β and PGE_2 [117]. Furthermore, Mu *et al.* [118] reported that BCAA supplementation in 4-month-old male C57BL/6J mice increased body weight, lean mass, and fat mass with increased adipose tissue inflammation and worsen insulin sensitivity compared to mice fed with low-protein diet. These data suggest that dietary protein levels and BCAAs play a role in modulating whole-body metabolism. However, in this study they did not measure impact on bone and BMAT. More animal and human studies investigating the effect of BCAAs on BMAT and bone formation are needed in respect to sex differences.

Amino acids enriched diet and its effect on bone and BMAT

Another AA supplementation in diet showed that N-acetylcysteine in HFD diet in male C57BL/6 mice fed for 17 weeks showed protection from HFD-induced bone impairment measured in distal femur, which was accompanied by decreased bone resorption but the measurement of BMAT parameters was missing [119]. Cysteine is linked to the methionine metabolism. Animal study using a 12-week feeding of methionine-enriched diet induced increased bone fragility and reduced bone quality in Wistar rats, especially in the cancellous bone [120] without BMAT measurement. However, the feeding of methionine-restricted diet for 5-12 weeks, which was aimed to improve glucose metabolism in young male and female mice as well as in aged male mice (C57BL/6J), caused similar negative impact on bone lengths and trabecular parameters accompanied by decreased osteoblast differentiation, while preserving the bone strength compared to control group [121-123].

Selenium in the form of selenocysteine is critical for bone remodeling. Recent study by Kim *et al.* [124] defined a negative effect of selenoprotein W on bone mass by stimulating osteoclastogenesis in bone as selenoprotein W-deficient mice exhibit high bone mass phenotype. Selenoprotein is usually made from selenocysteine. In rats, femoral BMD was increased by 77 % together with improved bone growth andn development with supplementation of L-Se-methylselenocysteine in selenium-deficient rats [125].

Even, a combination of different AAs could have a positive effect on bone health as showed in the study of Ding *et al.* [126] using 8-week-old male C57BL/6J mice treated for 2 months with low-protein diet supplemented with either a triad of serine, valine and threonine or a triad of phenylalanine, tyrosine and tryptophan. This AA-supplemented diet had a positive effect on BMSC proliferation and osteoblast differentiation.

In humans, the presence of specific AA in the diet has been linked to various aspects of bone health. Studies have indicated that a diet enriched with vitamin D, calcium, and leucine can potentially increase BMD in sarcopenic older adults [127]. Furthermore, post-menopausal women with high BMD levels were found to have higher concentrations of certain AA, including leucine, valine, and tyrosine, suggesting potential associations between these AA and BMD [128]. Conversely, lower intake of phenylalanine in patients of both sexes (8-16 years) has been linked to reduced BMD values [129]. Additionally, studies have demonstrated that tryptophan supplementation can stimulate BMSC proliferation and differentiation, potentially through the upregulation of the RUNX2 expression factor [130,131]. Despite a positive association between high tryptophan intake and hip BMD in individuals aged over 45 years, it was concluded that excessive tryptophan consumption may not play a critical role in bone health [132].

Glutamine-enriched diet

Glutamine represents a non-essential AA, which plays important role in regulation of oxidoreductase activity and inflammation [133]. Glutamine enrichment of the diet has been shown to have a positive effect on bone metabolism [134]. Glutamine metabolism plays a pivotal role in regulating BMSC proliferation, lineage allocation and osteoblast differentiation [134,135]. Recent study using knock-out of key enzyme of glutamine metabolism, glutaminase, reported negative effect on bone formation manifested by reduced osteoblast numbers and increased adipocyte

differentiation, highlighting the critical involvement of glutamine metabolism in BMSC function and bone health in mice [134].

Furthermore, Blais *et al.* [135] showed that monosodium glutamate supplementation of low-protein diet in 8-week-old Balb/C female mice increased glutamine plasma levels, increased BMD, trabecular and cortical bone microarchitecture, osteoblast differentiation and improved bone quality compared to mice under protein restriction. However, supplementation did not restore these parameters to the levels obtained in animals fed with control diet [135].

In addition, glutamine contributes to proline production, an important AA for collagen synthesis and connective tissue formation. This cascade of effects underscores the positive impact of glutamine on bone tissue, reinforcing its significance in bone health [136]. Hanaa *et al.* [137] reported the potential beneficial effect of oral administration of glutamine in ovariectomized female Sprague Dawley rats (starting 3 months after OVX and lasting for further 3 months) documented by increased $1,25(OH)_2D_3$, IGF-1 and TGF-β levels, along with improved BMC and BMD. Notably, glutamine supplementation fosters the production of glutathione, a potent calcium enhancer through calcium sensing receptor activation. However, more studies are needed to investigate the effect of glutamine supplementation on bone health and BMAT formation in different animal models and human studies.

Conclusions and future perspectives

The importance of healthy and well-balanced diet is crucial, as shown by several animal and clinical studies of different ages and metabolic conditions. It helps to counteract the negative effects of obesity, osteoporosis and aging on bone health, reducing the risk of fractures. Presence of BMAT in different stages of life span may differ between sexes. However, its impact on bone mass in males and females are still not well-known (Fig. 2). Even though there are some mild differences in nutrient levels among sexes, the impact of dietary intervention and nutrient supplementation on bone health is similar and the major determinant of bone health. There are differences between men and women in steroid hormone levels, which could primarily drive the heterogeneity of BMSC and HSC populations in BM affected by ERα signaling in response to hormonal or nutritional stimuli participating in bone formation.

Fig. 2. The effect of different diet interventions on bone and BMAT in humans and mice with respect to sex differences. ↑ increased, ↓ decreased, N/A – not available (Created with Biorender.com).

However, more detailed and well-controlled clinical studies are needed to determine the best nutrient-enriched diet designed for each subject individually as their metabolism can differ and much of what is known about bone health and BMAT analysis is based on the research conducted in male mice. We move into the era of precision nutrition, understanding these sex-based differences may help to optimize recommendations and interventions chosen to support bone health and weight management.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

AA, Amino acids; AAD, Average American diet; AD, Adipocyte; ALA, Alpha-linolenic acid; ALP, Alkaline phosphatase; AN, Anorexia nervosa; ArA, Arachidonic acid; BCAA, Branched chain amino acids; BM, Bone marrow; BMAds, Bone marrow adipocytes; BMAT, Bone marrow adipose tissue; BMC, Bone mineral content; BMD, Bone mineral density; BMSCs, Bone marrow stromal cells; BMI, Body mass index; BO, Borage oil; BV, Bone volume; BV/TV, Bone volume fraction; cBMAT, Constitutive BMAT; CO, Corn oil; CR, Caloric restriction, CT, Computed tomography; µCT, Micro-computed tomography; Ct.Po, Cortical porosity; Ct.Th, Cortical thickness; DHA, Docosahexaenoic acid; DXA, Dual-energy X-ray absorptiometry; ED, energy deficit; ELISA, Enzyme-linked immunosorbent assay; EPA, Eicosatetraenoic acid; ERα, Estrogen receptor alpha; F, Female; FA, Fatty acids; FO, Fish oil; GLA, Gamma-linolenic acid; HFCS-5, High-fructose corn syrup; HFD, High-fat diet; HFDD, HFD deficient in D3 and calcium; HFD+FO, High-fat diet supplemented with fish oil; HFD/F, High-fat diet supplemented with fructose; HFD/HSD, High-fat/high-sucrose diet; HP, High-protein; HSD, High-sucrose diet; ^{1}H-MRS, Proton magnetic resonance spectroscopy; HSCs, Hematopoietic stem cells; IGF-1, Insulin-like growth factor 1; LA, Linoleic acid; LP, Low-protein; M, Male; MR, Methionine restriction; MRI, Magnetic resonance imaging; MSG, Monosodium glutamate; MUFAs, Monounsaturated fatty acids; N/A, Not available; NP, Normal protein; OA, Oleic acid; OB, Osteoblast; OVX, Ovariectomy; PGE_2, Prostaglandin E_2; p-NFκB/NFκB, ratio of phosphorylated to total Nuclear Factor κB; pQCT, peripheral quantitative computed tomography; PTT, triad of phenylalanine, tyrosine and tryptophan; PUFAs, Polyunsaturated fatty acids; RANKL/OPG, Receptor activator of NFκB ligand/osteoprotegerin ratio; rBMAT, Regulated BMAT; RSG, Rosiglitazone; RUNX2, Runt-related transcription factor 2; SFAs, Saturated fatty acids; SFO, Sunflower oil; SO, Safflower oil; SVT, triad of serine, valine and threonine; Tb.N, Trabecular number; Tb.Sp, Trabecular separation; Tb.Th, Trabecular thickness; Tb.V, Trabecular volume; TGF-β, transforming growth factor β; TRAP, Tartrate-resistant acid phosphatase

Table 1. Dietary factors affecting bone and BMAT in animal models.

Metabolic condition	Animal model	Age	Sex	Duration of the diet	Composition of the diet	Methods	Effect on bone	Effect on BMAT	Reference
Diet enriched in carbohydrates									
High-saccharide diet	Sprague-Dawley rats	60 days	M	12 weeks	high-fructose (40% fructose, 10% glucose) or high-glucose diet (50% glucose)	histology histomorphometry 3-point bending test µCT	High fructose diet vs. high glucose diet: ↑ bone volume of distal femur; ↑ Tb.V of tibia; ↑ bone mechanical properties	N/A	Bass *et al.* 2013 [138]
HFD enriched with fructose (HFD/F)	Sprague-Dawley rats	8 weeks	M	12 weeks	30% (sugar-free) control HFD; 30% HFD+ 40% fructose	µCT; bone mechanical testing; histology; plasma analysis	HFD and HFD/F vs. control diet: ↑ BV/TV and Tb.N of proximal tibia; ↓ cancellous BMD, BV/TV, Tb.N, osteoblast surface and circulating osteocalcin levels; ≠ between HFD and HFD/F	↑ BMAT	Yarrow *et al.* 2016 [56]
High fructose intake	Sprague-Dawley rats	Adult	M	28 days	Control (H_2O); 10% fructose solution	Bone histomorphometry measurement of reossification area; primary BMSC analysis of OB and AD differentiation	↓ osteocyte and osteoclast density; ≠BMAT; ↓ bone regeneration; ↓ OB differentiation, ↓ *Runx2* expression; ↑ AD differentiation, ↑ Peroxisome proliferator-activated receptor gamma expression	≠ BMAT	Felice *et al.* 2014 [57]
High sugar beverages	Sprague-Dawley rats	35 days	F	8 weeks	Control (H_2O); 13% fructose/glucose/sucrose/ HFCS-5	Bone morphometry; bone turnover markers; DXA; three-point bending test	With high-glucose: ≠ bone mass; ≠ bone strength; ↓ calcium and phosphate intake; ↑ calcium excretion	N/A	Tsanzi *et al.* 2008 [58]
HFD/HSD	C57BL/6 mice	9 weeks	F	10 weeks	Low-fat diet (68% complex carbohydrates; 0% sucrose; 6% fat, 26% protein), HFD/HSD (0% complex carbohydrates, 39.5% sucrose, 39.5% fat, 21% protein)	µCT; three-point bending test; gene expression analysis	With HFD/HSD: ↓ tibia mass, length and Ct.Th; ↓ maximal load; ≠ RANKL/OPG ratio; ↑ cyclooxygenase 2 expression	N/A	Lorincz *et al.* 2010 [59]

HFD/HSD	Wistar rats	12 months	M	24 weeks	Control diet (4.7% crude fat); HFD/HSD (13.8% crude fat, 25% sucrose)	μCT; plasma analysis	With HFD/HSD: ↑ BV/TV, Tb.N, Tb.Th, Ct.Th, cortical volume fraction, medullary volume of tibia and femur; ↑ TRACP and calcium levels	N/A	Minematsu *et al.* 2018 [60]
HFD	C57BL/6J mice	6 weeks	M	12-20 weeks	Control diet (13.5% calories from fat); 60% HFD; weight loss group (HFD for 12 weeks followed by control diet for 8 weeks)	Body weight; μCT; mechanical testing of femurs; osmium staining of BMAT	With HFD: ↑ body weight; ↓ Tb.V, BMC and Tb.N, ↓ fracture resistance	↑ BMAT in HFD vs. control diet; ↓ BMAT in weight loss vs. HFD	Scheller *et al.* 2016 [89]
Caloric restriction (CR)									
CR	C57BL/6J mice	5 weeks	F	5 weeks	Control diet (10% kcal/fat) or a 30% CR diet + leptin (1-2 mg/kg/day)	DXA; μCT; histology, histomorphometry analysis; osmium tetroxide staining	With leptin treatment: ≠ trabecular or cortical microarchitecture	With leptin treatment: ↓ BMAT expansion	Devlin *et al.* 2016 [69]
CR	C57BL/6J mice	3 weeks	M	3-9 weeks on the diet	Control phytoestrogen-free diet (10% kcal fat); 30% CR	DXA; μCT; histology; histomorphometry analysis; three-point bending test	CR vs. control group: ↓ trabecular bone volume, number and thickness and ↓ bone strength with inhibited bone formation and bone resorption	↑ BMAT	Devlin *et al.* 2010 [70]
CR	mice	14 weeks	M	10 weeks	10% restriction at 14 weeks of age, increased to 25% restriction at 15 weeks, increased to 40% restriction at 16 weeks and maintained until 24 weeks of age	DXA; pQCT; histomorphometry analysis; radiography	With CR: ↓ femur BMC, BMD, cortical thickness, and fracture strength and ↑ spine BMC, BMD, and trabecular bone volume fraction	↑ BMAT	Hamrick *et al.* 2008 [72]
CR	Sprague-Dawley rats	4 months	F	12 weeks	Control diet Control diet with β-blocker 40% CR diet 40% CR diet with β-blocker	DXA; pQCT; histomorphometry analysis; immunohistochemistry	With β-adrenergic blockade: ↓ metaphyseal bone loss	↑ BMAT	Baek *et al.* 2012 [76]
CR	C57BL/6J mice C3H/HeJ mice Ocn-Wnt10b mice	9 weeks	M	6 weeks	control group; 30% CR group	μCT; osmium staining	N/A	↑ BMAT	Cawthorn *et al.* 2014 [5]

CR	C57BL/6 mice	11 weeks	F	6 weeks	Control group; 30% CR group	MRI; histomorphometry; qRT-PCR	With CR: ↓ Femoral BV; ↓ Tb.Th and Ct.Th	↑ BMAT	McGrath *et al.* 2020 [73]
Diet enriched in fatty acids									
HFD	C57BL/6J mice	8 weeks	M	12 or 20 weeks	60 kcal% HFD (35% fat, 26% protein, 26% carbohydrate, 8.8% sucrose) and control diet (6% fat, 30% protein, 63% carbohydrate, 7.7% sucrose)	μCT; histomorphometry analysis	HFD compared to control diet: ↓ trabecular bone mass and ↓ Ct. Th	↑ BMAT	Tencerova *et al.* 2018 [6]
HFD	C57BL/6J mice	3 weeks	M	12 weeks	HFD diet (60% fat); control diet (10% fat);	μCT; DXA; histomorphometry	HFD vs. control diet: ≠ bone mass	HFD vs. control diet: ↑ BMAT	Doucette *et al.* 2015 [71]
HFD	C57BL/6 mice	12 weeks	M	11 weeks	HFD diet (45 kcal%/fat); Control diet (12 kcal%/fat)	μCT; bone histomorphometry	HFD vs. control diet: ↑ bone mass but over time ↓ bone mass	HFD vs. control diet: ↑ BMAT	Leczka-Cernik *et al.* 2015 [87]
HFD and HFD deficient in D3 and calcium (HFDD)	Sprague-Dawley rats OVX	6 months	F	N/A	control diet; HFD; HFDD; OVX with HFD (OVX-HFD); OVX with HFDD (OVX-HFDD)	Glucose tolerance test; serum analysis; Masson-Goldner trichrome staining; histomorphometry; immunohistochemistry	With HFD: ↑ bone calcium content and bone strength of OVX rats; With HFDD: ↓ BMC, ↓ BMD; ↓ bone calcium content; ↓ bone strength	N/A	Wang *et al.* 2017 [88]
HFD	C57BL6/J mice	6 weeks	M	12, 16 or 20 weeks	Control diet or 60% HFD diet	μCT; osmium staining	HFD vs. control diet: ↓ Tb.Th and Ct.Th; ↑ bone loss in tibia	HFD vs. control diet: ↑ BMAT	Scheller *et al.* 2016 [89]
HFD	C57BL/6 mice	4 weeks	M F	10 weeks	control diet or 60 % HFD diet	μCT; AD and OB differentiation of BMSC	HFD vs. control diet: ↑ bone loss; ≠ cortical bone parameters and strength	N/A	Gautam *et al.* 2014 [90]
HFD	LG/J and SM/J mice	5 months	M F	N/A	control diet relatively high in fat (42% calories from fat); low fat diet (15% calories from fat)	μCT; three-point bending test	Low fat diet vs. control HFD diet: ↑ mechanical properties of the bones	N/A	Silva *et al.* 2019 [91]

HFD	C57BL/6J mice OVX	8 weeks	F	12 weeks	HFD (60%fat) or control diet (10% fat);	Glucose tolerance test; µCT; qRT-PCR; Western blot; RNA sequencing	HFD vs. control diet: ↑ cellular senescence; ↓ bone mass	↑ BMAT at estrogen deficiency	Ali *et al.* 2022 [75]
HFD and HFD + FO	C57BL/6N mice	12 weeks	M	8 weeks	Control diet (3.4% w/w lipid content); HFD (35% w/w lipid content, primary corn oil); HFD+FO (46% w/w DHA, 14% EPA)	µCT; histology; three-point bending test; *in vitro* analysis; plasma analysis	HFD + FO vs. HFD: ↑ Tb.BV/TV and Tb.N of proximal tibia; ↓ Ct.Po., ↑ Ct.Th., ↑ bone strength; ↑ N-terminal propeptide of type I procollagen/TRAP ratio; ↑ osteoblastogenesis and ↓ adipogenic and osteoclastic differentiation	HFD+ FO vs. HFD: ↓ BMAds number, volume and diameter	Benova *et al.* 2023 [96]
n-3 and n-6 PUFA enriched diet	F344 x BNF1 rats	12 months	M	20 weeks	N6+N3 diet (n-6/n-3 PUFA ratio 10); N6 diet (n-6/n-3 PUFA ratio 242); N3 diet (n-6/n-3 PUFA ratio 0.16)	serum analysis, DXA	N6 diet vs. N6+N3 group: ≠ ALP activity, ↓ BMC and ≠ BMD; N3 vs. N6+N3 diet: ↑ ALP activity, ↑ BMC and BMD	N/A	Shen *et al.* 2006 [94]
n-3 and n-6 PUFA enriched diet	SAMP8 mice	4 weeks	F	10 months	Control diet n-6/n-3 PUFA ratio (9.13); SFO (enriched diet in favor of ω6; n-6/n-3 PUFA ratio 18.35); BO (enriched in GLA; n-6/n-3 PUFA ratio 20.67); FO (enriched by EPA and DHA; n-6/n-3 PUFA ratio 3.52)	µCT	FO vs. other groups: ↑ bone volume; SFO and BO vs. control diet: ↑ bone volume	FO vs. other groups ↓ BMAT % SFO, BO vs. control diet: ↓ BMAT %	Bani Hassan *et al.* 2019 [95]
HFD and HFD + FO	C57BL/6 mice	6 weeks	M	6 months	Control diet (10% energy as fat) HFD (45% energy as fat) containing either 0%, 3%, or 9% energy as FO (0FO, 3FO, and 9FO, respectively)	µCT ; serum analysis	3FO vs. 9FO: ↑ BV, BV/TV, Tb.N, and ↓ Tb.Sp in femur; 3FO vs. 0FO: ↑ BV/TV 3FO vs. 9FO: ↑ BV/TV and ≠ cortical parameters; FO ↓ concentrations of serum TRAP	N/A	Cao *et al.* 2020 [97]
HFD and HFD + FO	C57BL/6 mice	13 months	F	5 months	Control diet (10% energy as fat); HFD (45% energy as fat) containing either 0%, 3%, or 9% energy as FO (0FO, 3FO, and 9FO, respectively)	DXA	FO+RSG vs. FO: ↓ BMD and induced bone loss	N/A	Cugno *et al.* 2021 [139]

HFD + FO	Balb/c mice OVX	8 weeks	F	24 weeks	CO (5%; low in n-3 fatty acids) FO (5% FO + 0.5% CO; high in n-3 fatty acids)	DXA, histology, *in vitro* analysis	FO-OVX and CO-OVX vs. sham: ↓ BMD in distal femur; CO-OVX vs. FO-OVX: ↑ bone loss; n-3 FA vs. n-6 FA: ↓ osteoclast maturation	N/A	Sun *et al.* 2003 [140]
n-3 PUFA enriched diet	B6.C3H-6T (6T) mice and C57BL/6J (B6J) mice	12 weeks	F	12 months	SO (22%) FO (22%)	DXA, *in vivo* μCT; histology; three-point bending test	B6J-FO vs. B6J-SO:↑ BMD, ↓ osteoclast number, ≠ mechanical properties; 6T-FO vs. 6T-SO: ≠ osteoclast number and surfaces; ↓ ultimate force and plastic energy, associated with ↓ Ct.Th	6T-FO mice had ↑BMAT vs.B6J mice on FO diet	Bonnet *et al.* 2014 [141]
HFD and HFD + FO	C57BL/6J mice	8 months	F	8 weeks	Control diet (15% protein, 75% carbohydrate, 10% fat, 0.6% Ca) HFD (45%, either enriched with MUFAs (15% protein, 39% carbohydrate, 46% fat, 0.7% Ca) SFAs (19% protein, 37% carbohydrate, 44% fat, 0.7% Ca)	DXA; μCT analysis	≠ effect on final calcium balance, secretion or excretion in all groups; SFAs vs. control diet: ↓ total body and femur BMD and BMC; SFAs vs. HFD diet: ≠ effects on BMC or BMD	N/A	Wang *et al.* 2016 [142]
SO enriched diet	C57BL/6 × C3H fat-1 mice	3 weeks	M F	9 weeks	modified AIN-93G diet (containing 10% SO, high in LA)	DXA, three-point bending test, femur neck fracture test	M vs. F: ↑ femur weight, length, toughness and stiffness at femur midpoint; ↑ BMC and BMD with ↑ percentage composition of total n-3 PUFA (EPA, DHA) and n-6 PUFAs (arachidonic acid)	N/A	Lau *et al.* 2009 [143]
Protein-enriched diet									
Low-protein diet containing 2% protein;	Balb/c mice	2 months	M	3 weeks	Protein source - casein (>85% protein) Control diet contained 12 % casein; the low-protein diet contained 2% casein	Histology; Western blot; ELISA BMSC AD differentiation	low-protein diet vs. control diet: ↑ adipogenesis of BMSC and BM failure	N/A	Cunha *et al.* 2013 [102]

Normoproteic diet 12%, Hypoproteic diet 2%	C57BL/6 NTaq mice	2 months	M	35-40 days	protein source casein (>85% protein) control normoproteic diet contained 12% casein hypoproteic diet contained 2% casein	Flow cytometry; qRT-PCR; BMSC differentiation and proliferation measurement	hypoproteic diet vs. normoproteic diet: ↓ differentiation potential of BMSC; altered the regulatory function of BMSCs and promotes proliferation	N/A	Hastreiter *et al.* 2021 [103]
Isocaloric low-protein diet	Sprague Dawley rats	6 months	F	10 weeks	control (15% casein) and isocaloric low-protein (2.5% casein) diet	µCT; three-point bending test	low protein diet vs. control diet: ↓ bone material level properties ; ↓ bone area, total area, and maximum second moment of inertia	N/A	Dubois-Ferrière *et al.* 2014 [105]
Isocaloric synthetic diets containing varying amounts of casein	Sprague-Dawley rats OVX	Adult	F	16 weeks	Isocaloric synthetic diets with 15, 7.5, 5, and 2.5% casein, + daily dose of vitamin D dissolved in peanut oil	DXA; histomorphometry analysis; three-point bending test	2.5% casein diet vs. other diets: ↓ bone mineral mass and strength	N/A	Ammann *et al.* 2000 [106]
Casein or soy enriched diet	Sprague-Dawley rats	8 months	M	24 weeks on protein diet after 2 weeks of CR	control diet containing 20% casein or soy	Serum biochemistry; Immunohistochemistry; Immunofluorescence	with protein diet after CR-induced bone loss: ≠ bone parameters	N/A	Duque *et al.* 2020 [109]
Casein-containing diet	Sprague-Dawley rats	1 month	F	10 weeks	10% casein, 7.5% casein, or 5% casein with normal or low level of Ca/P	DXA; µCT; qRT-PCR; biochemistry analysis	low-Ca-P diet with reduced protein intakes: ↓ of bone mass and ↓ bone strength	N/A	Fournier *et al.* 2014 [107]
Casein/soy/CR diet	Sprague-Dawley rats	2 months	F	10 weeks	casein diet; soy diet; 40% CR+ casein diet; 40% CR + soy diet	three-point bending test; DXA; µCT; serum biochemistry	Soy diet without CR:↑ BMD and BMC; ≠ effect on bone strength soy diet with CR ↓ BMD and BMC	N/A	Kioka *et al.* 2022 [144]
HFD with caloric restriction based on protein supplementation	Sprague-Dawley OVX rats	7 months	F	12 weeks	12 weeks of obesity-inducing diet (HFD/HSD + 15% protein) followed by 12 weeks of CR diet with different levels and source of proteins (normal protein 15%; high protein (HP)-milk 35%; high protein (HP)-beef 35%; high protein (HP)-soy 35%)	DXA; µCT; histology; three-point bending test	With CR: ↓ bone quantity and microarchitecture, ↓ body composition parameters. With HP-beef diet: ↑ trabecular separation and ↑ endocortical bone formation rates, ↓ bone retention and trabecular BMC compared to HP-soy With HP-milk diet: ≠ weight loss induced bone loss	N/A	Wright *et al.* 2022 [108]

Protein enriched diet combined with exercise	Wistar rats	5 weeks	F	2 months	10%, 20% and 40% protein diet groups + Exercise group rats were trained 6 days per week on a treadmill (25-30 m/min, 60 min) or no-exercise group	DXA; three-point bending test	With 10% protein diet: ↓ bone mass and bone strength	N/A	Takeda *et al.* 2012 [104]
Amino acids enriched diet									
Cysteine supplementation	C57BL/6 mice	6 weeks	M	17 weeks	HFD (45% energy from fat) enriched with N-acetylcysteine (1 g/kg)	Osteoclast culture; qRT-PCR in osteoclast; µCT	With HFD + cysteine: ↑ BV, BV/TV, Tb.Th and BMD in distal femur. ↓ osteoclast number; ↓ osteoclast differentiation from BM cells; ↓ bone resorption	N/A	Cao and Picklo 2014 [119]
Glutamine supplementation of low-protein diet	Balb/C mice	10 weeks	F	12 weeks	Monosodium glutamate (MSG) supplementation in low-protein (LP) diet (6% energy from soy protein)	µCT; determination of the femur protein fraction; determination of free amino acids in plasma	With LP diet + glutamine supplementation: ↑ glutamine plasma concentration LP diet: ↓ BMD gain; With higher concentrations of MSG (0.5/1/2%): ↑ BMD gain, ↑ the trabecular and cortical bone microarchitecture; ↑osteoblast activity; ↑ bone quality	N/A	Blais *et al.* 2019 [135]
Glutamine supplemen-tation	Sprague Dawley OVX rats	N/A	F	3 months	Control diet combined with orally administered L-glutamine dissolved in 10% lactose in a dose of 3.2 g/kg/day	DXA; histology	With control diet + glutamine: ↑ BMD and BMC in femur	N/A	Hanna *et al.* 2009 [137]
Diet restricted in methionine	C57BL/6J mice	3 weeks	M	5 weeks	Control diet with 0.86% methionine or methionine-restricted diet (0.12%, MR)	µCT analysis, four-point bending test, qRT-PCR, osmium tetroxide staining; BMAT evaluation	With HFD restricted in MR: ↓ bone length, ↓ mechanical properties, ↓ Runx 2 mRNA, ↓ OB differentiation	With HFD restricted in methionine: ↑ BMAT in femur	Plummer *et al.* 2016 [123]

HFD restricted in methionine	C57BL/6J mice	8 weeks and 9 months	M F	12 weeks	Control HFD with methionine (0.84% w/w) or HFD restricted in methionine (0.12% w/w)	Whole body and femur length; µCT	With HFD restricted in methionine: ↓ body length; ↓ femur length in young M+F mice and aged M mice; ↓ Cortical BMD in M mice and aged F mice; ↓ trabecular BMD in young mice and aged F; ↓ cortical BMC in all MR mice while trabecular BMC ↓ in young mice No sex differences	N/A	Ouattara *et al.* 2016 [121]
Diet enriched in methionine	Wistar rats	10-12 weeks	F	12 weeks	2.4% methionine-enriched diet	Histomorphometry, Bone turnover markers t	With HFD enriched in MR: ↑ bone fragility, ↓ bone quality; ↑ bone loss in the cancellous bone	N/A	Herrmann *et al.* 2007 [120]
Amino acid supplemented low-protein diet	C57BL/6J mice	18 months	N/A	2 months	Low-protein diet and low-protein diet with AA supplementation: triad of serine, valine and threonine (SVT) and triad of phenylalanine, tyrosine and tryptophan (PTT)	three-point bending test, DXA, µCT, primary BMSC differentiation	With low-protein diet with AA: ↑ OB proliferation and differentiation. SVT ↓ bone mass but PTT revert effects of low-protein diet in terms of femoral and spinal BMD and BV/TV	N/A	Ding *et al.* 2018 [126]

↑- increased; ↓- decreased; ≠ not changed.

Table 2. Dietary factors affecting bone and BMAT in humans.

Clinical study	Age	Sex	Duration of the diet	Composition of the diet	Methods	Effect on bone	Effect on BMAT	Reference
Diet enriched in carbohydrates								
Carbonated beverages	14.3±1.8 years girls 14.6±1.6 years boys	M F	N/A	Various carbonated beverages consumption	Food intake; bone fracture risk analysis	↑ strong association between drinking cola beverage and bone fracture in girls; total caloric intake inversely associated with fracture risk in boys	N/A	Wyshak *et al.* 1994 [61]
Carbonated beverages	9-16 years	M F	N/A	Soft drinks and milk consumption	DXA; metacarpal morphometry	Positive association between cola drinking and increased fracture rate of wrist and forearm; ≠ between different beverages in BMD	N/A	Ma and Jones 2004 [62]
Mediterranean diet in patients with high glycemic index	F 60-80 years M 55-80 years	M F	7 years	Mediterranean diet (MedDiet; supplemented with extra-virgin olive oil (50 ml/day)); MedDiet supplemented with mixed nuts (30 g/day)	Bone fracture assessment; dietary assessment	Patients with ↑ glycemic index and glycemic load had significantly ↑ risk of osteoporotic fractures	N/A	Garcia-Gavilan *et al.* 2018 [64]
Carbohydrate quality index in postmenopausal women	45-65 years	F	N/A	Low carbohydrate diet and diet with higher glycemic index	DXA; dietary assessment; carbohydrate quality index analysis	Diets with ↑ glycemic index had ↑ fracture risk; low-carbohydrate diet score and carbohydrate quality index ↓ fracture risk of osteopenia in osteoporotic subjects	N/A	Nouri *et al.* 2023 [65]
Calorie-restricted diet								
CR (anorexia nervosa (AN))	29-42 years	F	N/A	AN group and control group	^{1}H-MRS; MRI; DXA	BMAT correlates inversely with BMD	AN patients ↑ BMAT compared to control group	Bredella *et al.* 2009 [67]
CR	47.8 ± 9.1 years	M F	6-18 months	low-fat diet or low-carbohydrate diet	MRI	N/A	Transiently ↓ BMAT	Ofir *et al.* 2023 [81]
CR + HFD	22-44 years	M F	10 days on HFD, then 10 days on CR	HFD diet (30-40% fat, 45-55% carbohydrates, ≥25% protein); CR diet (drink water *ad libitum*)	DXA; MRI	N/A	HFD: ↑ BMAT CR: ↑ BMAT	Fazeli *et al.* 2021 [66]

Obesity								
Cross-sectional study in lean and obese subjects	≥ 18 years	F	N/A	Obese and lean group (according to composition of visceral adipose tissue)	CT; ^{1}H-MRS	BMAT correlates inversely with BMD	↑ VAT is accompanied with ↑ BMAT	Bredella *et al.* 2011 [77]
Cross-sectional study in obese subjects in respect to sex	13.6 ± 1.4 years	M F	N/A	Boys and girls with obesity (BMI percentile 98.5 ± 1.2%)	MRI	N/A	↑ thoracic and lumbar BMAT in men compared to women	Vander Wyst *et al.* 2021 [78]
Cross-sectional study in obese subjects in respect to sex	33.7 ± 6.8 years	M F	N/A	Men and women obese groups (BMI 33.1 ± 7.1 kg/m^2; range 18.1-48.8 kg/m^2)	^{1}H-MRS	N/A	↑ ectopic and serum lipid levels associated with ↑ BMAT	Bredella et al. 2013 [68]
Longitudinal study	± 16.7 years	M	Study after 6 years; follow up after 8 years	No dietary intervention	DXA	n-3 FA (especially DHA) positively associated with peak BMD and negatively correlated with oleic acid and MUFAs in young men	N/A	Högström *et al.* 2007 [145]
Diet enriched in fatty acids								
Longitudinal study	25-40 years premenopausal 50-65 years postmenopausal	F	12 months	Control groups (1.0 g calcium) treatment group (1.0 g calcium, 4.0 g primrose oil and 440 mg marine FO)	DXA	BMD ↑ in both groups; ≠ between premenopausal and postmenopausal females; Postmenopausal vs. premenopausal F: ↓ total body BMD, ↑ bone turnover markers	N/A	Bassey *et al.* 2000 [146]
Flaxseed dietary supplementation	45-65 years	F	12 months	40 g flaxseed/day or placebo (wheat germ)	Serum analysis, DXA	Flaxseed vs. placebo: ≠ BMD	N/A	Dodin *et al.* 2005 [147]
Senile osteoporosis	±79.5 years	F	18 months	Treatment group: 6 g of a mixture of evening primrose oil and FO (60% LA, 8% GLA, 4% EPA, 3% DHA) + 600 mg/day calcium Control group: 6 g of coconut oil as placebo (97% saturated fat; 0.2% LA) + 600 mg/day calcium	Serum and urine analysis; FA analysis; bone densitometry – Lunar DPX-L	Treatment vs. control group: FA + Calcium maintain lumbar and ↑ femoral neck BMD	N/A	Kruger *et al.* 1998 [100]

Short-term n-3 PUFA supplementation	18-67 years	F M	12 weeks	1.48 g/day n-3 PUFAs (0.63 g EPA, 0.85 g DHA) or placebo – olive oil	Serum analysis	n-3 PUFAs vs. placebo: ≠ on bone resorption	N/A	Appleton *et al.* 2011 [148]
Long-term n-3 PUFA supplementation (Rheumatoid arthritis patients)	47-69 years	F M	12 weeks of diet and 8 weeks of washout	Treated group: n-3 fortified dairy (1.1 g ALA, 0.7 g EPA, 0.4 g DHA) Control group: standard dairy	Lipid extraction and FA analysis; blood and urinary analysis	Treated group vs. control group: ↓ urinary marker of bone resorption	N/A	Dawczynski *et al.* 2009 [149]
Hyperlipidemic adults	48.6 ± 1.6 years	F M	6 weeks of diet and 3 weeks of washout	Treated groups: LA diet (n-6/n-3 ratio 3.5); ALA diet (n-6/n-3 ratio 1.6) Control group: Average American diet (AAD, n-6/n-3 ratio 9)	Serum analysis	ALA diet: ↓ N-terminal telopeptide- marker of bone resorption compared to AAD	N/A	Griel *et al.* 2007 [150]
n-6 PUFA supplementation	65 years and older	F M	10 years	Dietary EPA and DHA intakes calculated from questionnaire responses	Statistical analysis; DXA	≠ between intake of EPA/DHA combined and the risk of hip fractures	N/A	Virtanen *et al.* 2010 [99]
Protein-enriched diet								
Head-down-tilt-bed-rest + protein supplementation	20-45 years	M	3 weeks	high protein intake (1.2 g/kg body weight/d) + whey protein with alkaline salts; control group – 1.2 g/kg body weight/day of protein	MRI	N/A	≠ in lumbar vertebral fat fraction	Trudel *et al.* 2019 [110]
Soy protein diet	≤ 65 years	F	12 months	Soy containing diet (25 g protein and 60 mg isoflavones)	DXA; Immunoassay analysis of serum and urine	≠ hip BMD and BMC; ↑ bone formation markers in both group	N/A	Arjmandi *et al.* 2005 [114]
Protein diet	20 ± 1 years	F M	1 month	Protein at 0.8; 1.6; 2.4 g/kg/day. Ten days of weight maintenance preceded 21 days of energy deficit (ED), during which total daily ED was 40%, achieved by reduced dietary energy intake (w30%) and increased physical activity (w10%)	DXA; ELISA	≠ BMC, BMD ↑ bone turnover markers in serum	N/A	Cao *et al.* 2014 [113]

Strength training with nutrient supplementation	55 ± 1 years	F	24 weeks	Nutrient group: - 730 kJ, composed of 10 g of protein (whey protein), 31 g of carbohydrate, 1 g of fat, 5.0 µg of vitamin D, and 250 mg of calcium; the placebo (control) group received 102 kJ as 6 g of carbohydrate and 12 mg of calcium	DXA; MRI	↑ femoral neck BMD; ↑ bone formation	N/A	Holm *et al.* 2008 [115]
Bed rest and a high protein diet	25-40 years	F	2 years	Bedrest + exercise group; bed rest + nutrition group or bed rest only (control group)	MRI	N/A	≠ in lumbar vertebral fat fraction	Trudel *et al.* 2009 [111]
Protein supplementation	70.5 ± 6.4 years	F M	18 months	Protein supplementation (160 kcal/45 g powder)	DXA	≠ lumbar spine BMD	N/A	Kerstetter *et al.* 2015 [112]

↑- increased; ↓- decreased; ≠ not changed.

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