Journal of Osteoporosis

Secondary Osteoporosis: Endocrine and Metabolic Causes of Bone Mass Deterioration

Guest Editors: Tomasz Miazgowski, Michael Kleerekoper, Dieter Felsenberg, Jan J. Štěpán, and Paweł Szulc

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Editorial

Secondary Osteoporosis: Endocrine and Metabolic Causes of Bone Mass Deterioration

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Secondary osteoporosis results from medical conditions or treatments that interfere with the attainment of peak bone mass and/or may predispose to accelerated bone loss. Although secondary osteoporosis is less common, it is becoming more frequently diagnosed. Apart from the well-defined risk of secondary osteoporosis in patients requiring long-term corticosteroids therapy, an increasing list of dietary, lifestyle, endocrine, metabolic, and other causes of bone mass deterioration has been identified (Table 1). Recently it has been demonstrated that, in contrast to primary osteoporosis which is associated with age, gender, and family history, secondary osteoporosis shows a prevalence in men similar to that in women (men 21% versus women 17.5%) [1]. However, at presentation with a recent clinical vertebral or nonvertebral fracture, at least 27% of patients have previously unknown contributors to secondary osteoporosis, which are treatable or need follow-up [2]. Therefore, a careful medical examination always should be carried out in each patient with a recent fracture to exclude potentially reversible causes of bone loss. In comparison with primary osteoporosis, treatment of secondary osteoporosis is usually more complex and requires treating the underlying disease.

The main focus of this special issue is on current research that advances our understanding of the mechanisms underlying the endocrine and metabolic causes of bone mass deterioration. In the paper "*Bone mineral density accrual determines energy expenditure with refeeding in anorexia nervosa and supersedes return of menses*," the authors in the clinical study examined the disproportionate increase in resting energy expenditure that occurs with refeeding of women with anorexia nervosa to determine if it was related to bone mass increase. They found that prolonged nutritional rehabilitation may lead to recovery from osteopenia (a common finding in anorexia nervosa) and resumption of menses in the women who remain amenorrheic with low bone mineral density (BMD). The paper entitled "*Protective role of black tea extract (BTE) against non-alcoholic steatohepatitis (NASH)-induced skeletal dysfunction*" was aimed to examine the chemoprotective actions of aqueous BTE on decreased BMD induced by nonalcoholic steatohepatitis in the Wistar rats. Some previous studies have suggested that habitual tea consumption might have beneficial effect on BMD in adults. In this study, the authors confirmed this suggestion in rats and also found that BTE may influence levels of RANKL, osteoprotegerin, and bone turnover markers. The paper "*Bone health in patients with multiple sclerosis*" describes the up-to-date diagnostic criteria and includes detailed review of the common risk factors, pathophysiology, and treatment options of secondary osteoporosis in patients with multiple sclerosis. The paper "*A roadmap to the brittle bones of cystic fibrosis*" summarizes the current knowledge on the risk for osteoporosis in this autosomal recessive disorder. Unlike primary osteoporosis, bone disease in cystic fibrosis begins at a young age and is associated with significant morbidity due to fractures and decreased lung function. This paper reviews the pathophysiology, current clinical practice

Table 1: Causes of secondary osteoporosis.

Dietary
Anorexia nervosa
Excessive protein intake
Excess vitamin A
Inadequate vitamin D intake
Smoking
Excess alcohol intake
Parenteral nutrition
Lifestyle
Low physical activity
Prolonged immobilization
Endocrine
Adrenal insufficiency
Cushing's syndrome
Diabetes mellitus
Hyperthyroidism
Hypogonadism
Hypopituitarism
Pregnancy
Metabolic
Malabsorption syndrome
Chronic metabolic acidosis
Systematic diseases
End-stage renal disease
Primary biliary cirrhosis
Inflammatory bowel disease
Cystic fibrosis
Rheumatoid arthritis
Chronic obstructive pulmonary disease
Mastocytosis
Chronic inflammation
Surgery/transplantation
Bariatric surgery
Organ transplantation
Medications
Corticosteroids
Antiepileptics
Selective serotonin-reuptake inhibitors
Heparin

guidelines, and future therapies for treating bone disease associated with cystic fibrosis. Another paper in this issue, *"Are selective serotonin reuptake inhibitors a secondary cause of decreased bone density?"*, deals with severe complication of therapy with commonly prescribed selective serotonin-reuptake inhibitors (SSRIs). Although multiple consistent findings reveal a trend suggesting that SSRI use may negatively impact bone and result in lower BMD, a definitive causal relationship cannot be drawn. However, a growing body of evidence suggests an association between SSRI use and bone loss which seems sufficient to consider adding SSRIs to the list of medications that contribute to secondary osteoporosis. Another review paper in this issue, *"Secondary osteoporosis in patients with juvenile idiopathic arthritis,"* focuses on focal and systemic bone loss seen in juvenile idiopathic osteoporosis. Several clinical and epidemiological studies are reviewed in order to highlight putative factors that may contribute to bone loss in juvenile idiopathic osteoporosis, including low lean mass, growth retardation, impact of proinflammatory cytokines on bone remodeling, RANK/RANKL/osteoprotegerin imbalance, and abnormal Wnt signaling.

The papers presented in this special issue depict some novel and hitherto not intensively studied aspects of secondary osteoporosis, underscoring the complexity of mechanisms that predispose to bone mass deterioration induced by metabolic and endocrine risk factors. The editors thank the authors of all submissions and hope that the content of this special issue will be useful for clinical practice and future research.

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Review Article

Are Selective Serotonin Reuptake Inhibitors a Secondary Cause of Low Bone Density?

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Background. Osteoporosis is a chronic disease that can significantly impact numerous aspects of health and wellness. The individual consequences of osteoporosis can be devastating, often resulting in substantial loss of independence and sometimes death. One of the few illnesses with greater disease burden than low bone mineral density (BMD) is major depressive disorder (MDD). Both depression and antidepressant use have been identified as secondary causes of osteoporosis. The objective of this paper is to review and summarize the current findings on the relationship between antidepressant use and BMD. *Methods.* Relevant sources were identified from the Pubmed and MEDLINE databases, citing articles from the first relevant publication to September 1st, 2010. *Results.* 2001 articles initially met the search criteria, and 35 studies were thoroughly reviewed for evidence of an association between SSRI use and BMD, and 8 clinical studies were detailed and summarized in this paper. *Conclusions.* Current findings suggest a link between mental illness and osteoporosis that is of clinical relevance. Additional longitudinal studies and further research on possible mechanisms surrounding the association between SSRI use on bone metabolism need to be conducted. Treatment algorithms need to recognize this association to ensure that vulnerable populations are screened.

1. Introduction

Osteoporosis is a chronic disease that affects approximately 26% of women aged 65 years or older [1, 2]. A 50-year-old woman has approximately a 40% chance of sustaining an osteoporotic fracture [3, 4], and a 14-year-old girl has a 17% chance of sustaining a hip fracture at some point in her lifetime [5]. The individual consequences of osteoporosis can be devastating, often resulting in substantial loss of independence and sometimes death [6]. The burden on the health care system is also substantial, and it is estimated that the annual cost of hip fractures could exceed $2.4 billion by 2041 [7]. It is also an illness that is preventable if identified early and managed appropriately.

One of the few illnesses with greater disease burden than low bone mineral density (BMD) is major depressive disorder (MDD); it has been projected that MDD will be the biggest cause of disability world wide by 2020 [8]. Importantly, this is not simply attributed to psychiatric morbidity, and, in fact, MDD has been linked to a host of physical illnesses, mitigated in large extent by side effects of pharmacotherapy [9]. Recent evidence highlights the fact that impaired bone health may soon be joining this growing list.

1.1. Evidence of an Association between Antidepressant Use and Bone Health. Serotonin (5-hydroxytryptamine; 5-HT) is a neurotransmitter produced primarily in serotonergic neurons in the central nervous system (CNS). Its primarily role is to influence psychological and behavioural functions such as mood, anxiety, and sleep, and, as a consequence, it is a key player in the pathophysiology of MDD and other psychiatric illnesses [10–14]. Therefore, not surprisingly, a wide range of psychiatric disorders are treated with drugs that target this system [14–17].

Selective serotonin reuptake inhibitors (SSRIs) represent a class of medications that selectively and potently block the serotonin transporter (5-HTT) in the CNS to

effectively increase the extracellular levels of serotonin and relieve symptoms of depression [12]. The primary target of SSRIs are serotonin transporters (SERT), and the general principle of the SSRIs' mechanism of action is to boost the synaptic activity of serotonin by acute pharmacological inhibition of presynaptic SERTs, thereby increasing the synaptic concentration and activity of serotonin [12, 18–22]. As a result of elevated central serotonin, depressive symptoms are alleviated. The impact of serotonin 5-HT is not confined to the CNS, however, and a functional 5-HT signaling system in bone was identified in 2001 [23, 24]. Further investigation of this peripheral 5-HT system has also shown that SSRIs appear to affect both CNS and bone 5-HTT with similar potency [23]. While the specific biochemical nature of serotonergic pathways and their direct and/or indirect effects on bone metabolism are still unclear, existing data suggest an association between depression and increased risk of fracture and bone loss that may be mediated in part by antidepressants [16].

1.2. The Role of Serotonin in Bone Health. A functional role for 5HT in bone was first documented in 2001 when Bliziotes and colleagues demonstrated the presence of neurotransmitters, receptors, and transporters in osteoblasts and osteoclasts [23, 25, 26]. This group documented that the serotonergic system in bone plays a critical role in bone metabolism, a fact that was later confirmed by Westbroek and colleagues [23, 24]. Their findings also revealed that knockout mice without the serotonin transporter demonstrated significant decreases in bone density, impaired bone architecture, and bone mechanical properties [23]. Bliziotes' group also suggested that one possible mechanism to explain 5HT's negative effect on bone is the reduction in osteoblast activity, as a result of serotonin transporter inhibition, leading to lower BMD. This work provided evidence of the role serotonin in bone metabolism and a mechanism through which SSRIs may influence bone health [23]. Work on the link between depression, SSRI use, and BMD is equivocal; however, no association was noted between antidepressant use and BMD among men and women 17 years of age and older using data from the Third National Health and Nutrition Examination Survey (NHANES III) [27] or among postmenopausal women participating in the Women's Health Initiative (WHI) Observational Study [28]. In contrast, a recent meta-analysis identified depression, and especially depression in premenopausal women, as a significant risk factor for low BMD [29], while new work on novel mechanisms of serotonergic modulation of bone mass [30] highlights the biologic plausibility of an antidepressant mediated mechanism of decreased BMD.

The risks of low BMD associated with serotinergic antidepressant use and/or depression needs to be clarified. The scope of this problem cannot be denied; the use of antidepressants is ubiquitous, with combined American sales that exceeded $10 billion in 2004 [37]; a report published in 2000 ranked antidepressant drugs third among all drugs in the US prescription drug sales [38] and listed SSRIs as the most commonly prescribed class of antidepressants, accounting for approximately 62% of all antidepressants prescribed in the United States in 2002 [39]. Therefore, the goal of this paper is to summarize the literature on the association between SSRI use and bone health.

2. Materials and Methods

2.1. Study Selection. A comprehensive literature search using the computerized databases Pubmed and MEDLINE to identify relevant studies, covering the period ending September 2010, was performed using the medical subject headings "selective serotonin reuptake inhibitors," "depression," "major depressive disorder," "antidepressants," "bone mineral density," "osteoporosis," and "hypothalamic-pituary adrenal axis." A manual search of relevant reports was conducted by examining reference lists from original research papers and review articles. An initial screening was made of titles and abstracts of the articles, and simple relevant criteria for human participants, antidepressants, SSRIs, MDD, and BMD were used to exclude obviously irrelevant references. Inclusion criteria were (1) English-language journals, (2) full published studies with original data in peer-reviewed journals, (3) confirmation of depression with a standardized diagnostic tool, and (4) treatment with SSRIs. In total, eight relevant studies were identified that complied with these search criteria [16, 17, 31–36].

2.2. Data Extraction. All data were extracted independently by two investigators (MKC and VHT) using a standard protocol and data-collection form. Disagreements were resolved by discussion and, when necessary, by additional input from a third investigator. The extracted information included name of first author, year of publication, study design, country where the study was conducted, characteristics of study subjects (sample size, sampling methods, and distribution according to gender, mean age, race, weight, body mass index (BMI), menopausal status (women only), and antidepressant medication history), measures of BMD and of depression, confounding factors that were controlled by matching or multivariate adjustment, and mean BMD for depressed SSRI-treated, nontreated and nondespressed persons (if possible).

3. Results

Our initial search on PubMed identified 2001 potentially relevant and eligible studies; 35 full text articles were retrieved and screened for more detailed evaluation. Redundant references were eliminated, and studies that did not meet the eligibility criteria were excluded; therefore, a total of eight articles remained [16, 17, 31–36].

The eight articles reviewed in detail were human clinical studies [16, 17, 31–36]. Six studies examined BMD in SSRI users compared to nonusers and suggested an association between SSRI use and lower age- and gender- related BMD in humans [16, 17, 31, 32, 34, 35]. Richards et al. along with Ziere et al. also examined the fracture risk in SSRI users compared to nonusers. These studies suggested an

association between SSRI use and risk of fractions; however, only one study investigated BMD concomitantly with the risk of falls [17]. Of the eight studies identified, seven studies used fan-beam dual energy X-ray absorptiometry (DXA; QDR 4500 W, Hologic Inc.,) to measure BMD (g/cm2) [16, 17, 31–35] and one used the dual energy X-ray absorptiometry pencil beam (DPX-L, Lunar Corp., Madison, WI) [36].

There were various methods applied for assessing depressive symptoms. Five studies used the Diagnostic and Statistical Manual of Mental Disorder (DSM) depression assessment criteria [31–35], one study [36] used criteria for depression according to the Center for Epidemiologic Studies Depression Scale (CES-D), and others used the Mental Component Score (MCS), the Mental Health Inventory 5 (MHI-5) scales of the Medical Outcomes Study 36-Item Short-Form Health Survey questionnaire [17], or the Geriatric Depression Scale (GDS) [16].

Of the eight human clinical studies reviewed, five studies clearly provide support for an association between treatment with SSRI and lower BMD [16, 17, 32, 34, 36]. In contrast, three small studies demonstrated no connection between SSRI treatment and lower BMD [31, 33, 35]. Sample sizes in the studies selected ranged from 42 to 7983 and, and six studies were case-control studies and two contained data from prospective cohorts (Table 1).

4. Discussion

4.1. Clinical Studies Investigating an Association between SSRI Use and Bone Health. The possibility of an association between SSRI use and low BMD has sparked a recent rise in studies investigating the clinical implications of antidepressant treatment on bone health. In 2005, Cauley and colleagues conducted a population-based cross-sectional study of participants enrolled in The Osteoporotic Fractures Study in Men (aged 65 years) to determine the factors associated with BMD of the lumbar spine and proximal femur. The authors concluded that SSRI use was independently associated with a lower spine and hip BMD [32] but it was noted that the weight loss and poor diet in persons with depression could have confounded the results observed. A separate study was conducted by Diem et al. to determine whether SSRI use in a cohort of 2744 women (65 years) enrolled in the Study of Osteoporotic Fractures was associated with increased rates of bone loss, specifically in the hip. Patients in the study were divided into either "partial users" where SSRI use was recorded at one of the two visits only or "recurrent users" where SSRI use was recorded at both visits [16]. The study covered a period of 4.9 years, and BMD of the total hip and 2 subregions (femoral neck and trochanter) was assessed with serial measurements over 2 separate visits. The end result was that SSRI use in women was independently associated with increased rates of hip bone loss compared to nonusers [16]. An analysis of the Canadian Multicentre Osteoporosis Study (CaMos) cohort revealed an association between SSRI use and lower BMD that was related to increased clinical fragility fracture risk [17]. Consistent with the findings revealed by Richards et al. [17] and Diem et al. [16], the results from a large cohort study conducted in Rotterdam demonstrated that the use of SSRIs was associated with a 2.25-fold increase in fracture risk [36]. Of note, the authors in this study were able to distinctly define a direct correlation between treatment duration and greater fracture risk, which was detectable as early as 6 weeks following treatment. Furthermore, a similar trend was observed in an observational study conducted by Williams et al. to investigate the effect of SSRIs on BMD in women with a lifetime history of depressive disorder (SSRI-treated group and untreated) [34]. The results indicated that BMD among SSRI users was 5.6% ((0.977 (0.116) versus 0.922 (0.117) g/cm^2, $P = 0.03$)) lower at femoral neck, 6.2% (0.813 (0.105) versus 0.763 (0.107) g/cm^2, $P = 0.04$)) lower at the trochanter, and 4.4% ((0.745 (0.007) versus 0.712 (0.068) g/cm^2, $P = 0.03$)) lower at mid-forearm compared to SSRI nonusers [34]. Based on these findings, Williams and colleagues concluded that SSRIs negatively impacts BMD independent of the effect of depression on bone health. Of the above-mentioned studies, Williams et al. is the only study in which depressed patients were diagnosed according to the DSM-IV [34]. Interestingly, according to Bab and Yirmiya, the strength of an association is stronger, displaying significantly lower BMD, when patients were diagnosed with MDD by clinical assessment as opposed to being diagnosed by self-rated questionnaires [40].

Inconsistency exists in observations of an association between SSRI use and reduced BMD, with three small studies demonstrating a relationship [31, 33, 35]. In a study of the association between depression and BMD, 24 women with past or current MDD were matched with 24 healthy controls, with 15 of the depressed women reporting SSRI use [31]. Those women with current or past depression had lower trabecular bone density as compared to healthy controls, but, after controlling for age and BMI, BMD did not correlate with the duration of antidepressant drug therapy. The authors ultimately reported no association between a lifetime use of antidepressant drug treatment and bone density [31]. Similarly, Eskandari and colleagues conducted a prospective study in premenopasal women in which they examined the association between MDD and BMD using immune, pituitary-adrenal, and sympatheticbiomarkers to determine whether this population had a higher prevalence of osteopenia and osteoporosis and lower BMD than healthy controls [33]. While an association between premenopausal women with MDD and lower bone mass was confirmed, like the Michelson study, no association was reported between SSRIs use and BMD [33]. A limitation of this study as indicated by the authors is that women with MDD in their cohort had approximately 5 kg higher body mass than in other studies cohorts. This may have resulted in the lack of an association between SSRI use and BMD, given that higher body mass positively affects BMD [33]. Further support for these observations is found in the cross-sectional study of premenopausal women with unipolar depression matched with healthy controls who demonstrated significantly lower BMD. After adjusting for duration of drug exposure, however, it appeared that antidepressants had no impact on the osteodensitometric results [35]. In contrast to

Table 1: Studies reporting on antidepressant medications and BMD.

Reference	Study design, *n*, sample	*n* (%) Medication exposure and outcome	Findings	Limitations
Michelson et al. 1996 [31]	Cross-sectional analysis; $n = 48$ Women ($n = 24$) with past or current major depression; 24 nondepressed with age-matched controls; *n* SSRI users/nonusers = 15/33	Structured clinical interview for DSM-III-R 15 (62.5%) of 24 depressed women on SSRIs BMD (g/cm^2) of the anteroposterior and lateral lumbar (L1–L4) spine, total hip, and subregions (femoral neck and trochanter) were measured	The mean (SD) bone density in the women with past or current depression was 6.5% lower at the spine, 13.6% lower at the femoral neck, 10.8% lower at the trochanter compared to nondepressed women; but after controlling for BMI, no correlation between BMD and SSRI use	Sampling: small sample of subjects on SSRI therapy Some subjects were on concurrent drug therapy that may have affected the lack of an association between SSRI and BMD No report of dosage or duration of SSRI use
Cauley et al. 2005 [32]	Cross-sectional analysis; $n = 5995$ Men age 65 enrolled in the MrOS study; *n* SSRI users/nonusers = 160/5835	160 (2.6%) men were on SSRIs BMD (g/cm^2) of lumbar spine (L1–L4) and total hip and subregions (femoral neck and trochanter)	SSRI use resulted in 4-5% lower BMD at the hip and 6% lower at the spine	Sample: examined older population and only 10% were of minorities No mention of method of depression diagnosis in subjects No report of dosage or duration of SSRI use
Eskandari et al. 2007 [33]	Nested case-control analysis; $n = 89$ Premenopausal women (age 21–45 yrs) with MDD; 44 nondepressed women with age matched controls; *n* SSRI users/nonusers = 54/35	Structured clinical interview for DSM-IV and Global Assessment of Functioning Scale; Hamilton Depression Scale (24 questions) and the Hamilton Anxiety Scale (14 questions) 73 women with MDD were on antidepressants; 54 (61%) on SSRIs BMD (g/cm^2) of anteroposterior lumbar (L1–L4) spine, femoral neck, total hip, and mid-distal radius (CV 0.4%)	SSRI use did not result in lower BMD at the hip, spine, or radius after adjustment for BMI	Sample: women with MDD in this cohort had ~5 kg higher BMI and racial homogeneity Most depressed participants on SSRI were in remission No report of dosage or duration of SSRI use
Diem et al. 2007 [16]	Longitudinal analysis; $n = 2722$ Women (mean age 78.5 years) enrolled in the SOF study followed for 4.0 years; *n* SSRI users/nonusers = 198/2524	15-item Geriatric Depression Scale SSRIs 198 (7.2%) total participants, 65 (2%) at baseline, and 178 (6.5%) at followup BMD (g/cm^2) of the total hip and 2 sub-regions (femoral neck and trochanter) were measured; (mean ± SD, 4.9 ± 0.6 years between examinations)	SSRI use resulted in 1.7–2.6 greater rates of total hip bone loss, femoral neck, trochanterand 4% lower BMD at the hip (spine NS) after adjustment for confounders (0.82% for SSRI users versus 0.47% for nonusers)	Sample: cohort of only elderly women; thus, cannot generalize to other populations No report of dosage or duration of SSRI use

Table 1: Continued.

Reference	Study design, *n*, sample	*n* (%) Medication exposure and outcome	Findings	Limitations
Richards et al. 2007 [17]	Cross-sectionaland longitudinal analyses; *n* =5008 Men and women age 50 enrolled in the CaMOS study; *n* SSRI users/nonusers = 137/4871	MCS and MHI-5 ofMedical Outcomes Study 36-Item Short-Form Health Survey questionnaire 137 (2.7%) men were current daily SSRIs users and 609 (12.2%) men reported depressive symptoms BMD (g/cm^2) of the lumbar spine (L1–L4) and hip were measured	SSRI users had 4% decrease in BMD at the total hip (% difference between daily SSRI users and nonusers, −4.0 (95% CI, −6.6 to −1.4)) and 2.4% at the lumbar spine (% difference between daily SSRI users and nonusers, −2.4 (95% CI, −5.5 to 0.9))	Sample: cohort of only elderly men and racial homogeneity, thus, cannot extrapolate findings to other populations Subjects' depression was not diagnosed by a psychiatrist Duration of daily SSRI use was not reported
Williams et al. 2008 [34]	Cross-sectional analysis; *n* =607 Women age 40-65 yrs clinically diagnosed with depression; *n* SSRI users/nonusers = 26/581	Structured clinical interview for *DSM-IV-TR* research version, nonpatient edition 26 (20.3%) women were current users of SSRIs BMD (g/cm^2) was measured at the posterior-anterior (PA) spine, hip, total body, and forearm	BMD among SSRI users was 5.6% lower at the femoral neck, 6.2% lower at the trochanter and 4.4% lower at the mid-forearm than nonusers after controlling for confounders; no differences in BMD were detected at other sites.	Sample: relatively small number of SSRI users may have limited the power to detect significant differences in BMD, racial homogeneity No report of dosage or duration of SSRI use
Petronijević et al. 2008 [35]	Cross-sectional analysis; *n* = 73 Premenopausal women with unipolar depression compared with 47 healthy, age-and osteoporosis risk factors-matched premenopausal women; *n* SSRI users/nonusers = 32/41	Structured clinical interview for DSM-IV with at least 2 years of illness duration 32 (43.8%) women were current SSRI users BMD (g/cm^2) of the lumbar spine (L1–L4) and femoral neck were measured	BMD of the lumbarspine was 1.8% higher and 1.8% higher at the femoral neck compared SSRI nonusers; thus, BMD at lumbar spine and femoral neck NS	Sample: Absence of naive, untreated depressed women; small sample size No report of dosage or duration of SSRI use
Ziere et al. 2008 [36]	Prospective population-based Cohort study; *n* = 7983 Men and women age 55 years enrolled in the Rotterdam Study; *n* SSRI users/nonusers = 111/1061	Home interview using Center for Epidemiologic Studies Depression scale (CES-D) Total *n* = 7983; 111 (1.4%) SSRI users and 1061 (13.2%) nonusers BMD (g/cm^2) of the femoral neck was measured	BMD of femoral neck of SSRI users was 3-fold lower than SSRI nonusers (95% CI, 1.41–3.59); 2.25-fold risk increase of nonvertebral fracture for SSRI users	Sample: small number of SSRI users Depression diagnosis was not assessed by psychiatrist No report of dosage or duration of SSRI use

SSRI: Selective Serotonin Re-Uptake Inhibitors, CaMOS: Canadian Multicentre Osteoporosis Study, BMD: Bone Mineral Density, SOF: Study of Osteoporosis, OR: Odds Ratio, MDD: Major Depressive Disorder, NS: Not Significant, CI: Confidence Interval, MHI-5: Mental Health Inventory 5, DSM-IV-TR: Diagnostic and Statistical Manual of Mental Disorder, MCS: Mental Component Score, HDRS: Hamilton Depression Rating Scale.

these 3 studies, a study conducted by Kavuncu et al. using a similar sample of patients taking SSRIs reported greater bone resorption [41].

5. Limitations

MDD affects as many as 16% of adult in the US [42], with prevalence increasing as the population ages [43]. Depression has also been linked to a reduced BMD in some [16, 17, 32, 34, 36], but not all [31, 33, 35] studies. Therefore, a difficult task when defining the association between treatment with SSRI and lower BMD is controlling for confounding factors. Several clinical studies involving a population of depressed subjects to examine BMD in SSRI users and nonusers, however, have demonstrated that SSRIs may independently impact bone health, while physiologic and hormonal changes associated with depressive symptoms may magnify the adverse side effects of SSRI [16, 17, 32, 34, 44, 45]. Therefore, it is possible that depression, in combination with SSRI treatment, may have an additive negative effect on BMD. Accordingly, an investigation of the potential contribution of mental illness a subsequent SSRI treatment as a determinant of bone health is warranted.

6. Conclusion

The vastly growing body of research on SSRIs and its effect on bone health suggests that this relationship is complex and interpreting these findings has proved to be challenging. Although multiple consistent findings reveal a trend suggesting that SSRI use may negatively impact bone and result in lower BMD, a definitive causal relationship cannot be drawn. The distinct fact that depression itself, both as a consequence of innate biological changes that accompany the illness and secondary to lifestyle factors such as poor diet and lack of activity that often are linked to depression, has been shown to cause bone loss poses depression as a confounding variable in epidemiologic studies investigating the exact effects of SSRIs on bone health. While it may be too soon to infer causality, however, the burgeoning mountain of evidence consistently demonstrating an association between SSRI use and bone loss now seems sufficient to consider adding SSRIs to the list of medications that contribute to osteoporosis. This would imply that clinicians consider bone density testing for people on SSRIs, or those on SSRIs with certain additional risk factors, for their risk of fracture. Further investigations are needed to confirm the serotoninergic effects on bone to definitely guide physicians to provide clear recommendations to patients regarding the clinical implications associated with SSRI treatment. It is also necessary to continue future investigations to definitely prove a casual connection between SSRI use and bone, and furthermore, to confirm the recent promising animal findings that may potentially prevent and treat bone loss.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Clinical Study

Bone Mineral Density Accrual Determines Energy Expenditure with Refeeding in Anorexia Nervosa and Supersedes Return of Menses

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Osteopenia and osteoporosis are major complications of anorexia nervosa (AN). Since bone is a tissue requiring large amounts of energy, we examined the disproportionate increase in resting energy expenditure (REE) that occurs with refeeding of AN patients to determine if it was related to bone accretion. Thirty-seven AN patients aged 23.4 ± 4.8 years underwent a behavioral weight-gain protocol lasting a median of 66 days; 27 remained amenorrheic, and 10 regained menses. Sixteen controls aged 25.1 ± 4.7 years were age- and % IBW matched with patients. REE was measured using a respiratory chamber-indirect calorimeter. Significant correlations were found between REE and changes in spine ($r = 0.48$, $P < 0.02$) and leg ($r = 0.43$, $P < 0.05$) BMDs in AN patients. Further subgroup analysis of the amenorrheics revealed significant correlation between REE and change in spine BMD ($r = 0.59$, $P < 0.02$) and higher IGF-1 after weight gain compared to controls. Amenorrheics also had lower BMDs. These findings were absent in the regained menses group. The increase in REE seen in women with AN during nutritional rehabilitation may be related to active bone formation, which is not as prominent when menses have returned.

1. Introduction

Anorexia nervosa (AN) is an eating disorder characterized by severe voluntary restriction of food with resultant major weight loss affecting up to 1% of women in Western societies [1]. Patients with AN typically have primary or secondary amenorrhea likely due to suppression of the reproductive axis by means of inadequate energy stores [2].

Another consequence of the decreased caloric intake in AN patients is a consistently reported decrease in resting energy expenditure (REE). REE, also known as resting metabolic rate, represents the amount of calories required in a 24-hour period by the body during a nonactive period. Metabolic rate is mainly a function of the activity of lean body mass also known as fat-free mass (FFM). Although a reduced REE theoretically facilitates weight gain during refeeding, REE has also been consistently found to increase with nutritional rehabilitation, thereby potentially rendering weight gain more difficult for patients. Several small studies have proposed explanations for this phenomenon, including that the increase in REE is a reflection of lean body mass growth [3], a reversal of the initial adaptation to malnutrition [4], or a defense of low body weight [5]. Most puzzling is that others have found the increase in REE to be disproportionately greater than weight gain and deemed this clear evidence of a strong cellular "waste" phenomenon [6] or an energy drain of unknown source accounting for the use of extra calories. One possible hypothesis is that reversal of the metabolic consequences of caloric restriction necessitates activation of systems requiring energy.

Recently, bone has been recognized as a highly metabolically active tissue requiring energy [7] such that new bone formation is appropriately suppressed with inadequate nutrition. Indeed, reduction in bone mass to the degree of osteopenia and osteoporosis has been observed in over 90% of adolescents with AN who have been amenorrheic for more than 6 months [8]. Despite consideration of various therapies, weight restoration, which occurs prior to return of menses, along with menstrual recovery is regarded as the foundation of bone recovery [9]. Significant increases of

BMD have been noted prior to return of menses [10] with an increase in suppressed bone formation and a fall in bone resorption. Osteocalcin and *N*-telopeptide (NTX), established biochemical markers of bone formation and resorption, respectively, have been shown to appropriately increase and decrease, respectively, with weight recovery [10] accompanied with a 3-4% increase in BMD in as little as 4 months. In this longitudinal study in which we examine women with AN before and after weight normalization and compare them with healthy female control subjects, we propose that a substantial amount of the unexplained increase in REE during refeeding of AN patients is channeled towards BMD recovery such that significant bone rebuilding is required before energy stores become available for restoration of gonadal function.

2. Materials and Methods

2.1. Subjects. We studied 37 patients aged 23.4 ± 4.8 (range 18–36 years) with AN and 16 healthy control women aged 25.1 ± 4.7 (range 18–35 years). Patients were receiving inpatient treatment on the Eating Disorders Research Unit at the New York State Psychiatric Institute, Columbia University Medical Center (NYSPI, CUMC). All met criteria for AN from the 4th edition of the *Diagnostic and Statistical Manual of Mental Disorders*. Subjects were recruited, screened, and subjected to exclusion criteria as previously described [10].

The 16 healthy control women were recruited from the New York City area and the Columbia University campus by public advertisements. All were healthy, eumenorrheic, and matched with patients by age and percentage of ideal body weight (IBW) as previously described [10]. None of the control women had a history of an eating disorder or psychiatric or medical illness. Potential control subjects receiving hormonal or other medications known to affect reproductive function or bone metabolism were excluded. All of the control women exercised less than 3 h/wk.

All subjects provided written informed consent. The study protocol was approved by the IRB of the NYSPI, CUMC, and St. Luke's-Roosevelt Hospital Center. Procedures were followed according to approved ethical guidelines.

2.2. Bone Density, Body Fat, Fat-Free Mass Index (FFMI), and Fat-Free Mass (FFM). Spine, pelvis, leg, and total BMDs were determined using dual energy X-ray absorptiometry (DEXA) from a DPX scanner (Lunar Corp., Madison, WI) using version 3.6 software. The reports from the DPX-L scanner (GE Systems, Madison, WI) were analyzed with the use of version 3.6 software and were used to determine regional BMD of the hip and spine, total body bone mineral content, and total percentage of body fat. The CVs for BMD measurements ranged from 0.5% to 1.0% [11]. When measured by DXA, percentage of body fat is independent of BMD because this value is measured directly by recognized standard means in fat depots at sites where bone is not present [12]. FFM is calculated by the following: fat mass = weight × fat% and FFM = weight − fat mass. FFM is also known as lean body mass [13]. FFMI is calculated as FFM/height^2 [14].

2.3. REE. Each subject's REE was measured in a respiratory chamber-indirect calorimeter. The chamber was equipped with a high-precision gas (oxygen and carbon dioxide) exchange measurement system. A linear state space model converts gas exchange measurements to energy expenditure estimates. REE was measured early in the morning, with subjects in a fasted state for 12–15 h before the experiment. Subjects remained in the chamber for 1 h, and REE calculations were based on the average of 3 10-minute readings after 30 minutes had elapsed [15].

2.4. Biochemical Analyses. Serum osteocalcin was measured using a human immunoradiometric assay (Immunotopics International, San Clemente, CA) with a sensitivity of 0.5 ng/mL and an interassay CV of 5.5–6.7%. Urine NTX was measured with the use of an enzyme-linked immunoassay (Ostex International Inc, Seattle, WA) with a detection limit of 20 nmol bone collagen equivalent and an interassay CV of 4.1%. Assays for estradiol, FSH, LH, PRL, testosterone, DHEAS, T_3 and T_4 (both total and free), TSH, and cortisol were performed as previously described [10]. IGF-1 was assessed by RIA after alcohol extraction with an intra-assay coefficient of variation of 2.4–3.0% (Nichols Institute Diagnostics, San Juan Capistrano, CA). Leptin and total ghrelin levels were measured using commercial ELISA kits (Diagnostic Systems, Webster, TX). Assay sensitivity was 0.1 ng/mL for each. Both hormones were measured in nonfasting subjects. For leptin, the intra-assay coefficient of variation was 3.6%, and the interassay coefficient of variation was 4.9%. For ghrelin, the intra-assay coefficient of variation was 4.9%, and the interassay coefficient of variation was 5.5%, based on five assays. Blood and urine samples from patients were obtained at the initiation of hospitalization and at maintenance of weight gain to 90% IBW for ≥2 weeks except for one patient who reached 79% IBW. Venous samples from controls and menstruating subjects were obtained during the follicular phase of the menstrual cycle (days 3–10) as determined by a take-home ovulation test kit to confirm ovulatory cycles.

2.5. Treatment. Treatment for patients with AN followed a predominantly behavioral approach at the NYSPI aimed at normalizing weight and eating. Target weight was a minimum of 90% IBW based on 1959 Metropolitan Life Actuarial Tables. All but one patient reached a minimum of 90% IBW; that patient remained amenorrheic at 79% IBW. Median number of days of admission was 66. On admission, patients were fed a standard hospital diet of 1800 kcal (≈55% of energy from carbohydrates, 15% of energy from protein, and 30% of energy from fat), given as 3 meals/d and a snack. Patients were observed to eat 100% of the food prescribed and for 1 h afterwards. If patients did not gain weight, calories were increased in 400-kcal increments in food or liquid nutritional supplement (Ensure Plus; Abbott Laboratories, Abbott Park, IL). After a 1-2 wk medical stabilization period, patients began the active weight-gain treatment phase that

Table 1: Age and anthropometric measures (mean ± SD).

Characteristic	Patients with AN at admission (n)	Patients with AN at 90% IBW (n)	Amenorrheics at 90% IBW (n)	Regained menses at 90% IBW (n)	Controls (n)
Age[a] (years)	23.4 ± 4.8 (36)	23.6 ± 4.7 (36)	23.1 ± 4.2 (26)	24.8 ± 5.8 (10)	24.8 ± 4.7 (16)
Weight[a,b] (kg)	41.5 ± 5.4 (37)	53.8 ± 4.7 (37)	53.6 ± 5.0 (27)	54.5 ± 3.9 (10)	56.7 ± 4.4 (16)
BMI[a,b,c] (kg/m^2)	15.8 ± 1.6 (37)	20.4 ± 1.0 (37)	20.3 ± 1.1 (27)	20.8 ± 0.6 (10)	21.2 ± 0.9 (16)
REE[a] (kcal/d)	1087 ± 128 (23)	1378 ± 191 (23)	1395 ± 211 (17)	1327 ± 113 (6)	1451 ± 135 (12)
Fat free mass index[a] (kg/m^2)	14.4 ± 1.3 (36)	15.5 ± 1.1 (36)	15.6 ± 1.2 (27)	15.4 ± 0.8 (9)	15.6 ± 1.0 (16)

Significance set at $P < 0.05$ for all comparisons and is noted in the table.
[a]Significant difference between patients with AN at admission and patients with AN at 90% IBW.
[b]Significant difference between patients with AN at 90% IBW and controls.
[c]Significant difference between amenorrheics at 90% IBW and controls.

continued until patients reached 90% IBW with weight gain rates as previously described [10]. Formal exercise was not allowed during hospitalization although no effort was made to control for previous exercise load. Next, a 4–6 wk period of weight maintenance ensued during which patients gained independence and transitioned to outpatient care. Mean caloric intake on discharge was 2600 kcal. No calcium or vitamin D supplements were given.

2.6. Statistical Analysis. Data was analyzed using multiple t-tests, and the probability level was adjusted using the Bonferroni correction to compare patients at baseline and after weight gain, those with amenorrhea, those who regained menses, and controls. All data are expressed as mean ± SD. We used independent and dependent t-tests to measure differences between groups. Multiple comparisons were made with use of the Bonferroni method, available in SPSS software (version 12; SPSS Inc, Chicago, IL). Significance was set at $P < 0.05$ for all comparisons. Initially, patients were analyzed as one group with the use of independent t-tests; later, menstrual status was taken into account. For comparisons with controls, 2-factor repeated-measures analysis of variance with Bonferroni correction was conducted. Repeated-measures analyses were performed for LH, FSH, estradiol, testosterone, DHEAS, serum osteocalcin, urine NTX, and leptin after a log transformation that resulted in a SD < 20% value for each parameter. Correlations between REE and change in BMD and between REE and FFMI were tested using the Pearson linear correlation.

3. Results

Thirty-seven patients and 16 healthy control subjects entered and completed the study. Of the 37 study patients, 10 regained normal menstruation at 90% IBW. Age and anthropometric measures are shown in Table 1. Bone marker, BMD, and hormone data are shown in Table 2.

When the AN patients were refed to 90% IBW, significant increases were observed in BMI, REE, FFMI, osteocalcin, spine and pelvis BMDs, LH, FSH, estradiol, leptin, total T_3, and IGF-1 while cortisol and ghrelin decreased. As the metabolic rate is a function of metabolically active tissues, all of them contained in the FFM, we controlled for differences in FFM between patients with anorexia and controls by using REE/FFM and REE/FFMI [16]. At 90% IBW, significant correlations were observed between REE and changes in spine ($r = 0.48$, $P < 0.02$) and leg ($r = 0.43$, $P < 0.05$) BMDs as shown in Figures 1 and 2, respectively. At 90% IBW, both BMI and weight correlated with change in leg BMD, and total T_3 correlated with change in spine BMD ($P < 0.05$). The REE correlations with spine and leg BMDs were even more significant after controlling for weight gain, an important predictor of BMD ($r = 0.65$, $P < 0.005$) and ($r = 0.56$, $P < 0.02$), respectively. The correlations remained significant after controlling for T_3. No significant correlations were found between REE and FFMI at baseline or at 90% IBW or between REE and the hormonal parameters including leptin, ghrelin, and IGF-1.

Comparison of the patients with AN at 90% IBW to controls showed significantly lower BMI, spine, pelvis, leg, and total BMDs, LH, FSH, estradiol, and free T_4 and higher urine NTX and IGF-1. Osteocalcin was higher in patients with AN but not significantly so. In contrast to the patients with AN, controls exhibited high correlation between REE and FFMI ($r = 0.71$, $P < 0.01$).

As a further analysis, patients were divided into two groups according to menstrual status at the time of treatment after 90% IBW testing: those who remained amenorrheic and those who regained menses. Of note, there was no difference in baseline weight (41.1 kg versus 42.3 kg, amenorrheic versus regained menses). After weight rehabilitation, no differences were observed between the groups in weight, BMI, or REE, but the amenorrheic group had higher urine NTX ($P < 0.02$) and lower estradiol ($P < 0.006$) than those

Table 2: Bone marker, BMD, and hormone data (mean ± SD).

Characteristic	Patients with AN at admission (n)	Patients with AN at 90% IBW (n)	Amenorrheics at 90% IBW (n)	Regained menses at 90% IBW (n)	Controls (n)
Osteocalcin[a] (ng/mL)	8.1 ± 3.0 (33)	11.1 ± 5.9 (34)	11.4 ± 6.6 (25)	10.1 ± 3.6 (9)	8.7 ± 3.6 (12)
Urine NTX[b,d] (nmol/mmol Cr)	72.4 ± 32.7 (34)	70.9 ± 46.8 (30)	80.0 ± 50.3 (22)	45.9 ± 22.7 (8)	48.3 ± 14.4 (11)
Total BMD[b,c] (g/cm^2)	1.05 ± 0.09 (37)	1.05 ± 0.09 (37)	1.04 ± 0.09 (27)	1.07 ± 0.06 (10)	1.13 ± 0.05 (16)
Spine BMD[a,b,c] (g/cm^2)	0.89 ± 0.15 (37)	0.94 ± 0.14 (37)	0.92 ± 0.14 (27)	0.98 ± 0.13 (10)	1.07 ± 0.09 (16)
Pelvis BMD[a,b,c] (g/cm^2)	0.91 ± 0.13 (37)	0.94 ± 0.12 (37)	0.93 ± 0.12 (27)	0.98 ± 0.09 (10)	1.07 ± 0.06 (16)
Legs BMD[b,c] (g/cm^2)	1.10 ± 0.15 (37)	1.09 ± 0.13 (37)	1.07 ± 0.14 (27)	1.12 ± 0.09 (10)	1.19 ± 0.07 (16)
IGF-1[a,c] (ng/ml)	244.4 ± 103.1 (34)	343.6 ± 120.1 (35)	345.7 ± 121.4 (26)	337.6 ± 123.2 (9)	247.9 ± 76.5 (12)
Leptin[a] (ng/ml)	2.9 ± 2.7 (28)	14.7 ± 17.8 (31)	13.1 ± 15.6 (23)	19.3 ± 23.8 (8)	11.5 ± 5.9 (10)
Ghrelin[a] (pg/ml)	2025 ± 748 (24)	1567 ± 669 (25)	1588 ± 654 (21)	1835 ± 1213 (4)	1738 ± 542 (11)
LH[a,b] (mIU/ml)	1.4 ± 2.9 (30)	2.8 ± 2.8 (35)	2.5 ± 2.7 (25)	3.6 ± 3.1 (10)	4.0 ± 2.3 (13)
FSH[a,b](mIU/ml)	1.8 ± 2.1 (29)	2.7 ± 1.5 (34)	2.6 ± 1.3 (24)	3.0 ± 1.8 (10)	3.9 ± 1.3 (13)
Estradiol[a,b,c,d] (pg/ml)	23.7 ± 6.4 (32)	32.4 ± 14.0 (35)	28.2 ± 10.1 (25)	42.7 ± 17.3 (10)	52.4 ± 31.7 (14)
Testosterone (ng/dl)	76.2 ± 36.7 (26)	81.8 ± 36.7 (31)	79.3 ± 38.6 (23)	89.1 ± 31.8 (8)	63.1 ± 21.0 (16)
DHEAS (μg/dl)	153.1 ± 59.6 (25)	142.0 ± 81.1 (29)	139.8 ± 86.9 (23)	150.2 ± 59.3 (6)	175.8 ± 78.3 (16)
Cortisol[a] (μg/dl)	20.5 ± 5.5 (24)	16.7 ± 6.3 (27)	16.6 ± 5.9 (21)	16.2 ± 7.6 (6)	12.0 ± 4.3 (10)
TSH (mIU/L)	2.0 ± 1.2 (23)	1.7 ± 1.0 (26)	1.9 ± 1.0 (21)	1.2 ± 0.4 (5)	1.9 ± 1.0 (12)
Total T_3[a] (ng/dl)	76.8 ± 18.3 (27)	106.0 ± 28.3 (32)	103.8 ± 27.8 (25)	113.9 ± 30.9 (7)	120.1 ± 22.9 (12)
Free T_4[b] (ng/dl)	1.0 ± 0.2 (27)	1.0 ± 0.2 (32)	1.0 ± 0.2 (25)	1.0 ± 0.1 (7)	1.1 ± 0.1 (12)

Significance set at $P < 0.05$ for all comparisons and is noted in the table.
[a]Significant difference between patients with AN at admission and patients with AN at 90% IBW.
[b]Significant difference between patients with AN at 90% IBW and controls.
[c]Significant difference between amenorrheics at 90% IBW and controls.
[d]Significant difference between amenorrheics at 90% IBW and regained menses at 90% IBW.

who regained menses. Osteocalcin was also highest in this group, but not significantly so. Compared to controls, the amenorrheic group at 90% IBW had lower BMI ($P < 0.03$), estradiol ($P < 0.001$), spine ($P < 0.001$), pelvis ($P < 0.0005$), leg ($P < 0.008$), and total ($P < 0.001$) BMDs and higher IGF-1 ($P < 0.05$). Significant correlation between REE and change in spine BMD ($r = 0.59$, $P < 0.02$) was exhibited in the group who remained amenorrheic without correlation between REE and change in leg BMD ($P < 0.09$). Controlling for weight gain was limited by the number of patients in the group. No difference in BMI, estradiol, IGF-1, or BMDs was exhibited in the group who regained menses compared with

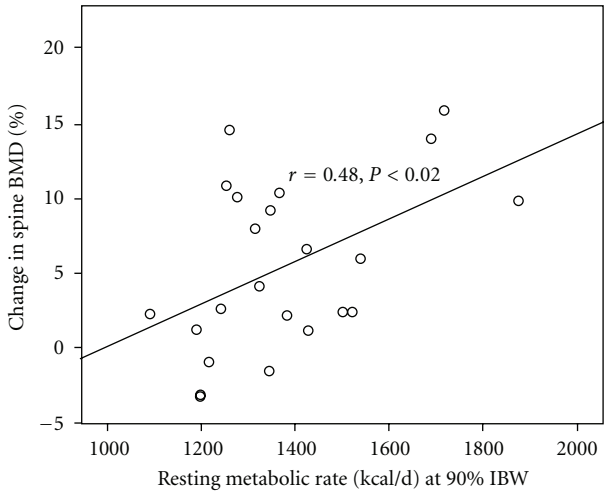

Figure 1: Correlation between resting metabolic rate of patients at 90% IBW and % change in spine BMD. Each circle represents the data point of a patient.

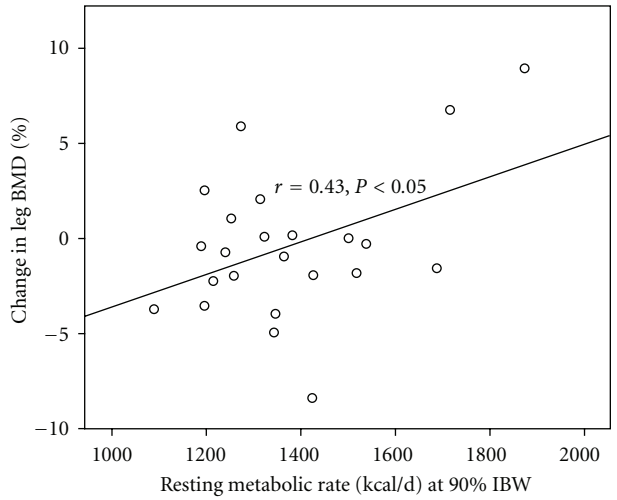

Figure 2: Correlation between resting metabolic rate of patients at 90% IBW and % change in leg BMD. Each circle represents the data point of a patient.

controls, indicating near recovery of bone mass and gonadal function. Interestingly, no significant correlation between REE and changes in BMD was found in this group.

4. Discussion

The etiology of the increase in REE with refeeding in AN patients has been the subject of much debate. This is the first report showing significant correlations between REE and changes in BMD with nutritional rehabilitation. This correlation is apparent in only women who have not yet regained menses, suggesting a physiologic event with bone as a possible metabolic sink or energy drain.

Previous studies have not attempted to correlate REE and FFMI in AN patients although REE was disproportionately elevated compared to increases in lean body mass [5]. This is the first study to show no correlation between REE and height-normalized FFM indices in AN patients despite high correlation in the normal population [17]. The absence of correlation found in our study along with the disproportionate REE elevations previously reported suggest that the increase in REE with refeeding is not accounted for by lean body mass but by another physiologic system undergoing marked energy utilization.

During refeeding, the significant correlations between REE and changes in BMD along with the elevated osteocalcin and the increase in IGF-1 to induce bone collagen formation suggest bone recovery from the osteopenic or osteoporotic state as a source of the increased REE. The observed correlation of REE with change in BMD in amenorrheic subjects with low BMDs and the lack of correlation in the group with resumed menses and near-normal BMDs suggest that the latter group no longer needs to channel the energy towards bone. Indeed, whereas the group who remained amenorrheic continued to exhibit elevated urine NTX, the group who regained menses demonstrated normalization of urine NTX, indicating a return to the normal state of bone resorption. The recent discovery of bone remodeling as a physiologic phenomenon requiring a large amount of energy but serving an evolutionary advantage [7] further supports the notion of bone as a metabolic sink, for the utilization of REE for bone recovery would serve a survival purpose. Indeed, the endocrine manifestations of AN typically result from mechanisms designed to preserve energy [18].

Many studies relate osteopenia and osteoporosis in AN to nutrition, but observations linking resumption of menses to bone recovery [19–21] may be explained by the notion that only after substantial bone recovery occurs are sufficient energy stores available for gonadal recovery. The etiology of bone loss in AN is multifactorial, and nutritional factors, particularly IGF-1, have been recognized to likely have an important role [22]. The dramatic increases in REE seen with nutritional rehabilitation and weight gain appear to be related to bone recovery. Indeed, a comprehensive study in AN following bone indices showed the powerful anabolic effect of nutritional rehabilitation on bone [10]. However, in this study, the authors state that menstrual resumption was required to suppress bone resorption and allow maximal appropriate bone recovery. Yet a quantitative effect of regained menses on bone mass remains unclear. In our study, the group who regained menses had near-normal BMDs and no correlation between REE and changes in BMD, suggesting that the majority of bone recovery had already occurred such that resumption of menses would not lead to further significant change. Instead of favorably predicting BMD recovery, menstrual return may indicate adequate replacement of energy stores.

One potential regulator linking disordered eating, amenorrhea, and bone is leptin, a fat cell hormone disproportionately lowered by fasting. Leptin thresholds have been associated with increased gonadotropins [2] and leptin administration with resumption of ovulation in hypothalamic amenorrheic women [23]. Recently, leptin receptors were also

discovered in bone [24], thereby providing a possible mechanism for interaction between nutritional and metabolic bone axes and resumption of menses.

Our data are consistent with other studies examining the bone and hormonal changes that occur with refeeding in AN [2, 9]. In this present study, however, we searched for and found significant correlations between REE and changes in BMD during nutritional rehabilitation. The main limitation was the size of the study population, for a larger sample would allow for control of various variables while examining correlations and would facilitate detection of differences between those who remained amenorrheic versus those who regained menses. Of note, it would have been interesting to follow the bone markers of the amenorrheics at 90% IBW to see if continuation of adequate nutritional intake would reverse bone loss and amenorrhea, two of the many important complications of AN [25].

In conclusion, we explore here an alternate hypothesis of the increase in REE during refeeding of AN patients to relate the increased energy expenditure to bone formation such that only after substantial BMD recovery are sufficient energy stores available for gonadal recovery. Bone recovery from osteopenic and osteoporotic states may take priority because of its survival advantage, a notion consistent with the reproductive then bone loss that occurs in AN. Further research is necessary to identify why one group of AN patients recovers bone mass and menses while undergoing the same nutritional rehabilitation as another group yet to recover either. Nonetheless, our hypothesis that bone recovery utilizes a vast amount of energy offers the exciting possibility that prolonged nutritional rehabilitation may lead to recovery from osteopenia and osteoporosis and resumption of menses in the women who remain amenorrheic with low BMD.

Disclosure

M. Sum and L. Mayer have nothing to disclose. M. P. Warren : Consultant/Advisory Board: Pfizer, QuatRx, Yoplait; Grants/Research Support: Ferrring, Pfizer; Speaker's Bureau: Amgen, Upsher Smith, Warner Chilcott.

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Research Article

Protective Role of Black Tea Extract against Nonalcoholic Steatohepatitis-Induced Skeletal Dysfunction

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Aim. This paper aimed to examine the chemoprotective actions of aqueous black tea extract (BTE) against nonalcoholic steatohepatitis- (NASH-) induced skeletal changes in rats. *Material*. Wistar rats (body wt. 155–175 g) of both sexes, aged 4–5 months, were randomly assigned to 3 groups; Group A (control), Group B (60% high-fat diet; HFD), and Group C (HFD + 2.5% BTE). *Methods*. Several urinary (calcium, phosphate, creatinine, and calcium-to-creatinine ratio) serum (alkaline phosphatase and serum tartrate-resistant acid phosphatase), and molecular markers of bone turnover (receptor activator of NF-κB ligand (RANKL), osteoprotegerin (OPG), and estrogen) were tested. Also, several bone parameters (bone density, bone tensile strength, bone mineral content, and bone histology) and calcium homeostasis were checked. *Results*. Results indicated that HFD-induced alterations in urinary, serum, and bone parameters as well as calcium homeostasis, all could be significantly ameliorated by BTE supplementation. *Conclusion*. Results suggest a potential role of BTE as a protective agent against NASH-induced changes in bone metabolism in rats.

1. Introduction

Nonalcoholic steatohepatitis (NASH) is a condition, which is described as accumulation of fat in liver with inflammation. This disorder may be observed in patients with no history of significant alcohol consumption, though histological examination may resemble alcohol-induced liver injury. It indeed may be the first clinical indication of insulin resistance, with complication of high blood pressure, coronary heart disease (CHD), and type 2 diabetes [1, 2]. It is claimed that, after cancer, cirrhosis from NASH is now the second most common age-related cause of death in type II diabetes [3]. Similar alarming observation is the association between human bone diseases with liver diseases, like viral liver cirrhosis is a risk factor for increased loss of minerals from bone [4, 5]. In an identical manner, patients with cholestatic liver disease have low bone turnover [6]. Even earlier [7, 8], it was reported that hepatic diseases are frequently associated with metabolic bone disorders. Hepatic osteodystrophy was reported to occur in up to 50% of patients with chronic liver disease (CLD). The results of this report suggested that increased bone resorption and less bone formation are the predominant cause of hepatic osteodystrophy in patients with CLD [9]. Osteopenia and osteoporosis are common complications of chronic liver disease. Patients with chronic liver disease may have other complications, such as malnutrition, loss of muscle mass, hypogonadism, low calcium intake, and vitamin D deficiency, all of which alter normal bone metabolism [10]. Several earlier studies had indicated the possible role of lipids in osteoporosis. High lipid levels were found closely related with decrease in bone mineral density (BMD) coupled with osteoporosis [11–13] as well as other disorders that may be a consequence of high lipids, including cardiovascular calcification [14–16] and atherosclerosis [11]. Consistent with these findings are the results of two in vivo studies that have shown

that mice [17] and chickens [18] fed on a high-fat, high-cholesterol diet have reduced bone mineral density. An earlier study also had indicated that diets with high saturated fat content can produce deleterious effects on the absorption of dietary calcium and consequently an adverse effect on bone mineralization in growing animals [19]. Perhaps even more significant are data reporting that the loss in bone mineral density by high lipid diet can be revived with antioxidants, suggesting a possible mechanism of action through suppression of lipid oxidation levels [18].

Tea (*Camellia sinensis*) is rich in polyphenolic compounds, collectively known as the tea flavonoids. Its therapeutic value come forefront with numerous reports on tea as antioxidants, hypolipidemic, antineoplastic, and hypocholesterolemic [20–23]. Tea also has been reported to possess a cardiovascular-protective effect [24, 25]. In an epidemiological report it has been suggested that skeletal health is better preserved in aged women who drank tea than nontea drinkers [26]. Similar epidemiological studies have confirmed that tea drinking may be a possible preventive measure to reduce risk of bone loss in postmenopausal women as well as in men by increasing BMD [27, 28]. Estrogenic activity of naturally occurring isoflavones by virtue of their ability to bind nuclear estrogen receptor was reported earlier [29], and, recently, soy isoflavones with weak estrogen-like activities have been reported to modulate bone metabolism and serum lipids in perimenopausal women [30]. A recent study has further indicated that soy isoflavone can reduce the risk of obesity and preserve bone health in menopausal women [31]. Moderate consumption of tea, which contains flavonoids closely related to soy isoflavones, has been reported to be associated with higher BMD in men and women [26, 27] and lower rates of fracture [32, 33]. Results of series of studies from our laboratory with aqueous black tea extract [34–36] further support the idea that phytocompounds have efficacy in preventing bone loss. This study aimed to examine the protective role of black tea extract (BTE) against nonalcoholic steatohepatitis- (NASH-) induced skeletal dysfunction.

2. Methods

2.1. Animals. As NASH does not have any gender specificity and is seen in both sexes, animals from both sexes were selected for this study. Wistar rats, both male and female, aged 4-5 months, weighing 155–170 g were procured from a local authorized breeder registered under the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Upon arrival at our institute, all animals were kept under standard conditions (12 hours light/dark schedule and at $25 \pm 2^{\circ}$C room temperature throughout the experimental period) for a week with free access to drinking water and standard laboratory diet composed of 71% carbohydrate, 18% protein, 7% fat, and 4% salt mixture [37]. All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

2.2. Diet and Treatment. Eighteen animals were randomly assigned to three groups: Group A (regular diet), Group B (high-fat diet), and Group C (high-fat diet + BTE). Each group contained six animals, three male and three female. Group A animals were fed standard laboratory diet [37] containing 71% carbohydrate, 18% protein, 7% fat, and 4% salt mixture. Group B animals were fed high-fat diet [38] containing carbohydrate: 22%, fat: 60% containing corn oil (palmitic acid: 6.3–18.2%, stearic acid: 0.9–4.5%, oleic acid: 18.5–46.1%, linoleic acid: 36.6–66.8%, linolenic acid: 0–2%, and arachidonic acid: 0–1.4%) [39], and protein: 18%. Animals of group C, in addition to high fat diet, were fed 2.5% (25 g/L) BTE by gavage technique for 30 days at a single dose of 10 mL/kg/day [40]. Animals of group A and B were given deionized water by gavage technique, 10 mL/kg/day as vehicle. In our preliminary studies, a fourth group (Group D) of animals was included containing six animals (male 3, female 3), which were given standard laboratory diet simultaneously with 2.5% BTE by gavage technique for 30 days at a single dose of 10 mL/kg/day. As aqueous black tea extract in Group D animals independently could not produce any significant variation of results in marker parameters of NASH, compared to control, this group was not considered for further detailed study to restrict animal use as per recommendation of the Animal Ethics Committee.

2.3. Preparation of Aqueous BTE. The black tea (*Camellia sinensis*) extract was prepared from CTC (Curl, Tear and Crush) BOP- (Broken Orange Pickoe-) grade black clonal tea. It was processed and supplied by Tocklai Experimental Station, Jorhat, Assam, India to the Drug Development Division, Indian Institute of Chemical Biology, Jadavpur, Kolkata, India. We received a generous gift from that institute. A fresh 2.5% aqueous BTE was prepared everyday following the method of Wei et al. [40]. Twenty-five gram of black tea was added to 500 mL of boiling water and was steeped for 15 min. The infusion was cooled to room temperature and then filtered. The tea leaves were extracted a second time with 500 mL of boiling water and filtered, and the two filtrates were combined to obtain a 2.5% aqueous black tea extract (2.5 g of tea leaf/100 mL water). The resulting clear solution is similar to tea brews consumed by humans.

2.4. Selection of Effective Dose of High Fat Diet and Black Tea Extract. For determination of effective dose (ED) of high fat diet (HFD) and black tea extract (BTE), dose response studies were undertaken. In case of high fat, it was observed that although significant changes could be seen from 30% of high fat onward, but both biochemical and histological changes were most prominent with 60% HFD. Similar changes with 60% HFD were reported earlier [38]. As for BTE, a submaximal dose response study revealed that significant recovery responses were seen with doses between

2-3% (w/v) aqueous BTE. Recovery responses with similar dose of BTE were also reported earlier [40].

2.5. Urine Collection. Fasting urine was collected for 24 hours (9 AM to next day 9 AM) according to the standard laboratory procedure [41] as described elsewhere by Chanda et al. [37]. Care was taken so that no urine was lost through evaporation. Total volume of urine was measured, and the following parameters were assessed.

2.6. Estimation of Urinary Parameters

2.6.1. Urinary Calcium. Urinary calcium was measured according to the method of Adeniyi et al. [42]. The assay of calcium was based on the principle that metal complexing dye orthocresopthalein complexone (CPC) forms a chromophore with calcium in alkaline solution. Diethylamine (DEA) were added to enhance the colour intensity. For urinary calcium estimation, to 20 μL of urine 1 mL CPC reagent and 1 mL DEA buffer was added. Colour intensity was measured at 575 nm against a blank by using a UV-double beam spectrophotometer (Shimadzu 160 A; Shimadzu Corporation, Kyoto, Japan). Calcium carbonate ($CaCO_3$) solution pH-3.0 was used as standard. All the reagents were prepared with calcium-free triple distilled water.

2.6.2. Urinary Phosphate. Urinary phosphate was measured according to the method of Lowry and Lopez [43]. For urinary phosphate estimation, 10 μL of urine was diluted 50 times with distilled water and to it 1 mL molybdate reagent and 200 μL amino naphthol sulfonic acid (ANSA) was added, mixed well, and kept in dark for 10 minutes. Reading was taken at 630 nm against blank. The concentration of phosphate in urine was calculated using a standard curve of monopotassium phosphate (KH_2PO_4).

2.6.3. Urinary Creatinine. Urinary creatinine was measured according to the method of R. L. Nath and R. K. Nath [44]. For urinary creatinine estimation, 50 μL of urine was diluted 10 times and to it 2 mL saturated picric acid and 150 μL 10% sodium hydroxide solution were added, mixed and kept for 15 minutes at 37°C incubator. To it, 5 mL of water was added. Reading was taken at 520 nm against blank. The concentration of creatinine in urine was calculated using a standard curve of creatinine.

2.7. Serum Collection. Blood was collected directly from the heart under urethane anesthesia (1.7 mg/g body weight). Serum was obtained by using standard laboratory protocol.

2.8. Estimation of Serum Alkaline Phosphatase (AP) Activity. Serum alkaline phosphatase was measured using p-nitrophenyl phosphate as substrate [45]. Alkaline phosphatase activity was measured by the hydrolysis of p-NPP (paranitrophenyl phosphate) at pH-10.8 using glycine-NaOH buffer at 37°C. In brief, 0.5 mL of 5.5 mM p-NPP in 0.4 mL of glycine-NaOH buffer (pH-10.8) solution were pipetted in a test tube and incubated at 37°C for 10 minutes. After incubation, 0.05 mL of sample was added, mixed, and incubated at 37°C for 30 minutes. Reaction was stopped by adding 4 mL of 0.1 N NaOH, and reading was taken at 410 nm against a blank in a spectrophotometer (Shimadzu 160 A; Shimadzu Corporation, Kyoto, Japan).

2.9. Estimation of Serum Tartrate-Resistant Acid Phosphatase (TRAP) Activity. Serum TRAP activity was estimated by kinetic method by using reagent kit (LABKIT, Spain). Briefly, unhemolyzed serum was mixed with the test reagent (10 mM α-naphthyl phosphate, 6 mM Fast Red TR, and 2 mM sodium tartrate in 50 mM sodium citrate buffer, pH-5.2) and the absorbance of the sample was read at 405 nm at 1 minute interval thereafter for 3 minutes. The difference of absorbance and the average absorbance difference per minute (ΔA/min) was calculated [46].

2.10. Estimation of Serum Estradiol. Serum was obtained by using standard laboratory protocol. Serum estrogen level (pg/mL) was determined by using the ELISA EIAgen Estradiol kit (Adaltis Italia, Italy). All samples were assayed in duplicate. The intra-assay coefficient of variation was 9.08%. To avoid inter-assay variation all samples were run at one time.

2.11. Estimation of Serum RANKL. Serum RANKL was estimated by using the ELISA kits (Quantikine, R & D Systems Inc., Minneapolis, MN, USA). All samples were assayed in duplicate. The intra-assay variation was 11.75%. All samples were run at one time to avoid inter-assay variation. Optical density of each well was determined by using a microplate reader (Thermo Labsystems, Finland).

2.12. Estimation of Serum Osteoprotegerin (OPG). Serum OPG was estimated by using the ELISA kits (Quantikine, R & D Systems Inc., Minneapolis, MN, USA). All samples were assayed in duplicate. The intra-assay variation was 10.09%. All samples were run at one time to avoid inter-assay variation. Optical density of each well was determined by using a microplate reader (Thermo Labsystems, Finland).

2.13. Measurement of Bone Density. The right femur, eighth thoracic rib, eighth thoracic vertebra, and fourth lumbar vertebra were freed off soft tissue and cleaned using small scissors, tweezers, and cotton gauze. Before measurement of the density, femur was cut at the middiaphysis and the marrow was washed out. Bone density was measured according to the method described by Arjmandi et al. [47] by using Archimedes' principle. Briefly, each bone was put in an unstoppered vial filled with deionized water, and the vial was placed under a vacuum for 90 minutes to ensure that all the trapped air diffused out of the bone. Each bone was removed from the vial, blotted with gauze sponge, weighed, and returned to the vial containing deionized water. The bone was reweighed in water and density was calculated (g/cm^3 bone volume).

2.14. Estimation of Bone Tensile Strength. After sacrifice of the animal, left femur was excised and cleaned off adhering soft tissues. Bone tensile strength was measured as described by Shapiro and Heaney [48] using a hand-held force meter (Excel Enterprises, India). Briefly, the femur was supported latitudinally on each end, and pressure was placed directly onto the middle of the bone until it fractured. The breaking force (kg) was recorded.

2.15. Estimation of Bone Calcium and Bone Phosphate Level. Right femur, eighth thoracic rib, eighth thoracic vertebra, and fourth lumbar vertebra were removed and cleaned of adhering tissue. The whole bone was extracted two times with a 1 : 1 mixture of ethanol and diethyl ether for 48 hours and one time with diethyl ether for 24 hours. The dehydrated and defatted bones were turned into ash at 600∘C for 48 hours and hydrolyzed in 6 N HCl for determination of calcium and phosphate [49]. Calcium and phosphate were estimated according to the method as described, respectively, by Adeniyi et al. [42] and Lowry and Lopez [43].

2.16. Preparation of Intestinal Loops. After the experimental period was over, the animals of all groups were fasted for 16 hours. The preparation of animals and intestinal loops for the study of calcium transference in situ was made by following the method as described elsewhere by Islam et al. [50]. Briefly, the animal was anesthetized (urethane, 1.7 mg/kg body weight), the abdomen of each animal was opened through a midline longitudinal incision, the bile duct was ligated and duodenal, jejunal, and ileal segments were located. Two ligatures, one proximal and the other distal, were applied tightly in each loop measuring about 8 cm in all duodenal, jejunal, and ileal segments. Loops were so selected that each contained 8–10 vessels, and care was taken so that no major blood vessels were occluded by the ligature.

2.17. Measurement of Intestinal Calcium Transference. For the measurement of intestinal calcium transference, 1 mL of prewarmed (37°C) Tris-HCl buffer solution containing 0.2 mM $CaCl_2$ was injected with a 25-gauge needle in each ligated segment. The intestinal loops were placed in their usual position and the abdomen was closed. After one hour, animals were sacrificed, the preselected loops were removed and the fluid from each loop was collected separately, together with a few washings of the lumen with triple distilled water. The collected fluid was then increased to a definite volume with calcium free triple distilled water. A fraction of this fluid was then used for the estimation of Ca^{2+} using a double beam spectrophotometer (Shimadzu, UV-160 A) according to the method described by Adeniyi et al. [42]. The difference between the amount of Ca^{2+} introduced and the amount left unabsorbed was used to estimate the amount of Ca^{2+} absorbed. The intestinal part constituting the loop was dried on a watch glass in an electric oven at 90°C to attain a constant weight, which was recorded as the weight of the dried loop. Intestinal transference of calcium was expressed as μM of calcium/g of dry weight/hour.

2.18. Preparation of Intestinal Mucosal Extract. After sacrificing the animal and opening of the abdomen, the whole of the small intestine was quickly removed. The portion comprised of the duodenum, jejunum, and ileum were separated and chilled in ice. Intestinal mucosa was collected as described by Maenz and Cheeseman [51], and the scrapings were homogenized according to the method of Koyama et al. [52].

2.19. Estimation of Intestinal Mucosal Alkaline Phosphatase Activity. The activity of alkaline phosphatase of intestinal mucosa was estimated by using p-nitrophenyl phosphate as substrate [45]. The protein content of the homogenate used for the study was determined using the method of Lowry et al. [53].

2.20. Estimation of Intestinal Mucosal Ca^{2+} Activated ATPase Activity. The activity of the enzyme Ca^{2+}-ATPase was also studied from the mucosal extract using the method of Rorive and Kleinzellar [54]. The assay was based on the principle that the release of inorganic phosphate (Pi) from ATP is measured in presence of either Ca^{2+} or Mg^{2+}. For the assay of mucosal Ca^{2+}, activated ATPase, 250 μL 0.4 M Tris-HCl buffer pH-7.4 and 250 μL 40 mM ATP (Tris-salt, Sigma) were added into three of the test tubes placed on ice. To tubes 1 and 3, 250 μL of 40 mM $CaCl_2$ were added. At time zero, the reaction was started by addition of 250 μL of mucosal homogenate to tubes 1 and 2. The volumes in all tubes were adjusted to 2 mL with calcium free distilled water. The three tubes were then incubated at 37°C with gentle shaking for 30 minutes. Under this condition the release of Pi is linear unto 60 mins. The reaction was stopped by transfer of the tubes to ice and addition of 0.4 mL ice-cold 35% (w/v) trichloro acetic acid (TCA). The tubes were then centrifuged for 10 minutes at 10,000 rpm in a refrigerated centrifuge. The protein pellet was dissolved in 1 mL of 1 N sodium hydroxide (NaOH), and protein content was determined following the method of Lowry et al. [53]. Phosphate liberated during Ca^{2+} ATPase enzyme activity was estimated by the method of Lowry and Lopez [43].

2.21. Bone Tissue Collection and Processing. The left proximal tibia and fourth lumbar vertebra were removed, dissected free of soft tissue, and fixed with 4% paraformaldehyde for 16–18 hours at 4°C. After fixation, specimens were washed for 12 hours at 5°C in each of the following series of solutions: 0.01 M PBS containing 5% glycerol, 0.01 M PBS containing 10% glycerol, and 0.01 M PBS containing 15% glycerol. The specimens were then decalcified in EDTA-G solution (14.5 g EDTA, 1.25 g NaOH, and 15 mL glycerol, pH-7.3) for 10–14 days. The decalcified tissues were washed sequentially at 5°C for 12 hours in (a) 15% sucrose and 15% glycerol in PBS, (b) 20% sucrose and 10% glycerol in PBS, (c) 20% sucrose and 5% glycerol in PBS, (d) 20% sucrose in PBS; 10% sucrose in PBS, (e) 5% sucrose in PBS, and (f) 100% PBS. Then the tissues were washed with PBS and dehydrated in graded series of alcohols, followed by clearing in xylene, and finally embedded in paraffin [55]. The specimen were cut into 5-6 μm sections and stained with

haematoxylin-eosin. Representative sections were observed and photomicrography was performed with the help of a bright field microscope equipped with a digital camera (Carl Zeiss, Germany).

2.22. Statistical Analysis. Data were expressed as mean ± S.E.M. obtained from a particular group comprising both male and female animals. Data were compared using Kruskal-Wallis nonparametric ANOVA followed by Mann-Whitney "U" multiple comparison test (software version 2.6.5, StatsDirect, UK). Differences were considered significant if $P < .05$.

3. Results

3.1. Urinary Calcium, Phosphate, and Creatinine Excretion Profiles and Calcium-to-Creatinine Ratio. The urinary calcium, phosphate, and creatinine excretion profiles together with Ca : Cr ratio of control group (Group A), HFD (Group B), and HFD + BTE (Group C) are shown in Table 1. Compared to control group, animals of HFD-fed group showed a significant increase in all the urinary parameters studied, namely, calcium, phosphate, creatinine, and Ca : Cr ratio ($P < .01$). Elevated response of all these parameters was significantly counter regulated in the HFD + BTE-supplemented group of rats (Group C).

3.2. Serum Alkaline Phosphatase (AP) and Tartrate-Resistant Acid Phosphatase (TRAP) Activity Profiles. The serum alkaline phosphatase (AP) activity profile of rats of control, HFD, and HFD + BTE supplemented groups are shown in Table 2. Rats of HFD-fed group (Group B) showed a significant increase in serum alkaline phosphatase activity when compared to animals of control group ($P < .01$) (Group A). This increase in AP activity was significantly lowered ($P < .01$) in rats receiving BTE (Group C). Likewise, the significant increase ($P < .01$) in TRAP activity in HFD-fed group (Group B), compared to control (Group A), could be effectively reduced by aqueous BTE supplementation (Group C; Table 2).

3.3. Bone Density Profiles. Animals in the HFD-fed group (Group B) had significantly lower densities of the right femur ($P < .01$), eighth thoracic rib ($P < .01$), eighth thoracic vertebra ($P < .01$), and fourth lumbar vertebra ($P < .01$), compared with the control group (Group A). BTE supplementation could produce significant increase ($P < .01$) in bone density of all bones: right femur ($P < .01$), eighth thoracic rib ($P < .01$), eighth thoracic vertebra ($P < .05$), and fourth lumbar vertebra ($P < .01$; Table 3).

3.4. Bone Tensile Strength. Figure 1 shows that compared to control, bone tensile strength in HFD-fed animals was significantly reduced ($P < .01$). BTE supplementation in these animals was found effective in recovering ($P < .01$) this tensile strength back to control group level.

3.5. Bone Calcium and Bone Phosphate Levels. Results of bone calcium and phosphate levels are shown in Table 4.

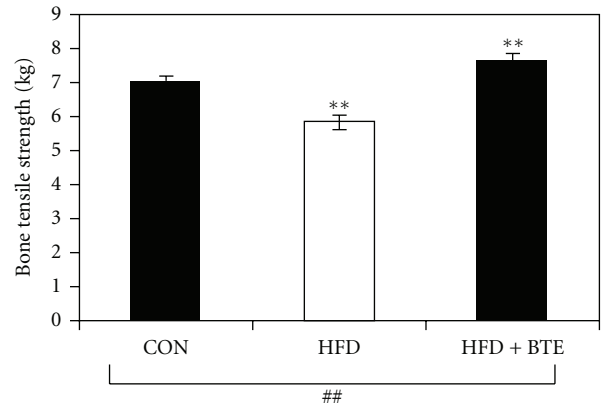

Figure 1: Graphical presentation of bone tensile strength of control (CON; Group A), HFD (Group B), and HFD + BTE supplemented (Group C) groups of animals. Values are expressed as mean ± S.E.M. ($n = 6$). $^{\#\#}P < .01$ denotes significance based on Kruskal-Wallis non-parametric ANOVA test, $^{**}P < .01$ denotes significance based on Mann-Whitney "U" multiple comparison test.

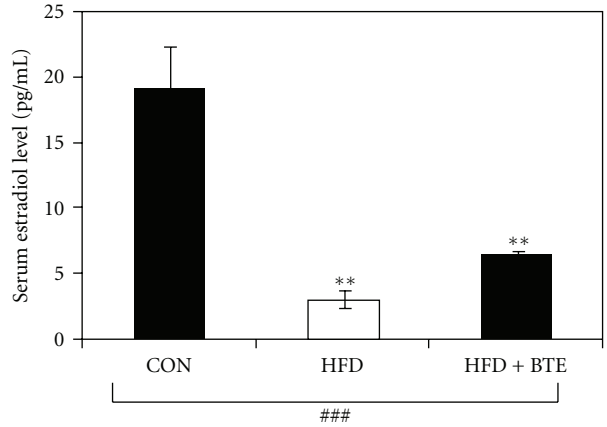

Figure 2: Serum estradiol level of control (CON; Group A), HFD treated (Group B), and HFD + BTE (Group C) supplemented female groups of rats. Values are expressed as mean ± S.E.M. ($n = 6$, female data only). $^{\#\#\#}P < .001$ denotes significance level based on Kruskal-Wallis nonparametric ANOVA test and $^{**}P < .01$ denotes significance level based on Mann-Whitney "U" multiple comparison test.

Animals of HFD-fed group (Group B), compared to control group (Group A), showed a marked decrease in calcium and phosphate levels of right femur (calcium: $P < .01$; phosphate: $P < .01$), eighth thoracic rib (calcium: $P < .01$; phosphate: $P < .01$), eighth thoracic vertebra (calcium: $P < .01$; phosphate: $P < .01$), and fourth lumbar vertebra (calcium: $P < .01$; phosphate: $P < .01$). When these HFD-fed animals were supplemented with BTE (Group C), significant recovery in content of both minerals was seen.

3.6. Serum Estradiol Level. Figure 2 shows the effects of supplementation of BTE on serum estradiol level of HFD, fed rats. Compared to control (Group A), a significant decrease in estradiol level was seen in HFD-fed (Group B) animals

Table 1: Urinary excretion of calcium, phosphate, creatinine, and calcium : creatinine (Ca : Cr) ratio in control (Group A), HFD (Group B) and HFD + BTE (Group C) supplemented groups of rats.

Parameters	Control (Group A)	High-fat (Group B)	High-fat + BTE (Group C)	Significance level##	Significance level*	
					Gr. A versus Gr. B	Gr. B versus Gr. C
Urinary calcium (mg/24 h)	0.81 ± 0.24	3.21 ± 0.56	1.01 ± 0.11	$P < .01$	$P < .01$	$P < .01$
Urinary phosphate (mg/24 h)	1.13 ± 0.084	3.21 ± 0.085	1.32 ± 0.206	$P < .01$	$P < .01$	$P < .01$
Urinary creatinine (mg/24 h)	0.45 ± 0.16	0.97 ± 0.021	0.65 ± 0.09	$P < .01$	$P < .01$	$P < .01$
Ca : Cr	1.132 ± 0.16	3.37 ± 0.54	1.48 ± 0.14	$P < .01$	$P < .01$	$P < .01$

Values are expressed as mean ± S.E.M. ($n = 6$). ##Denotes significance level based on Kruskal-Wallis nonparametric ANOVA test and * denotes significance level based on Mann-Whitney "U" multiple comparison test.

Table 2: Serum alkaline phosphatase (AP) activity and serum tartrate-resistant acid phosphatase (TRAP) activity in control (Group A), HFD (Group B) and HFD + BTE (Group C) supplemented groups of rats.

Serum enzyme activity	Control (Group A)	High-fat (Group B)	High-fat + BTE (Group C)	Significance level##	Significance level*	
					Gr. A versus Gr. B	Gr. B versus Gr. C
Alkaline phosphatase (U/L)	9.41 ± 0.22	16.48 ± 3.2	9.97 ± 0.24	$P < .01$	$P < .01$	$P < .01$
TRAP (U/L)	2.1 ± 0.32	4.29 ± 0.26	2.04 ± 0.28	$P < .01$	$P < .01$	$P < .01$

Values are expressed as mean ± S.E.M. ($n = 6$). ##Denotes significance level based on Kruskal-Wallis nonparametric ANOVA test and * denotes significance level based on Mann-Whitney "U" multiple comparison test.

Table 3: Density of femur, eighth thoracic rib, eighth thoracic vertebra, and fourth lumbar vertebra in control group (Group A), HFD group (Group B), and HFD + BTE supplemented group (Group C) of rats.

Parameters	Control (Group A)	High-fat (Group B)	High-fat + BTE (Group C)	Significance level##	Significance level*	
					Gr. A versus Gr. B	Gr. B versus Gr. C
Bone density (gm/cm^3)						
Right femur	1.39 ± 0.02	1.22 ± 0.01	1.34 ± 0.007	$P < .01$	$P < .01$	$P < .01$
Eighth Thoracic Rib	2.57 ± 0.28	1.44 ± 0.03	2.15 ± 0.11	$P < .01$	$P < .01$	$P < .01$
Eighth Thoracic Vertebra	1.33 ± 0.03	1.23 ± 0.02	1.299 ± 0.01	$P < .05$	$P < .05$	$P < .01$
Fourth Lumbar Vertebra	1.272 ± 0.28	1.44 ± 0.03	2.15 ± 0.11	$P < .01$	$P < .01$	$P < .01$

Values are expressed as mean ± S.E.M. ($n = 6$). ##Denotes significance level based on Kruskal-Wallis nonparametric ANOVA test and * denotes significance level based on Mann-Whitney "U" multiple comparison test.

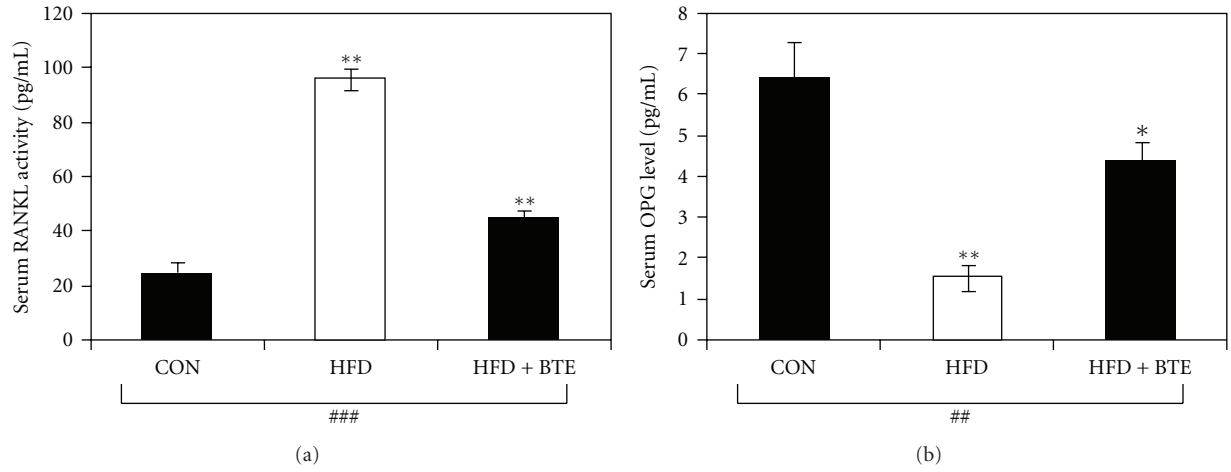

Figure 3: (a) Serum RANKL activity and (b) OPG level of control (CON; Group A), HFD treated (Group B), and HFD + BTE supplemented (Group C) groups of rats. Values are expressed as mean ± S.E.M. ($n = 6$). ###$P < .001$ denotes significance level based on Kruskal-Wallis nonparametric ANOVA test, ##$P < .01$ denotes significance level based on Kruskal-Wallis nonparametric ANOVA test, **$P < .001$ denotes significance level based on Mann-Whitney "U" multiple comparison test, and *$P < .05$ denotes significance level based on Mann-Whitney "U" multiple comparison test.

Table 4: Bone mineral content of femur, eighth thoracic rib, eighth thoracic vertebra and fourth lumbar vertebra in control group (Group A), HFD group (Group B), and HFD + BTE supplemented groups (Group C) of rats.

Parameters	Control (Group A)	High-fat (Group B)	High-fat + BTE (Group C)	Significance level##	Significance level*	
					Gr. A versus Gr. B	Gr. B versus Gr. C
Bone calcium (% of ash weight)						
Femur	19.39 ± 1.09	9.73 ± 0.53	13.75 ± 0.64	$P < .001$	$P < .01$	$P < .01$
Eighth thoracic rib	23.39 ± 0.84	15.49 ± 0.92	23.01 ± 1.38	$P < .01$	$P < .01$	$P < .01$
Eighth thoracic vertebra	24.3 ± 1.39	13.7 ± 0.7	21.52 ± 1.02	$P < .01$	$P < .01$	$P < .01$
Fourth lumbar vertebra	25.68 ± 0.57	13.65 ± 0.78	18.56 ± 0.8	$P < .001$	$P < .01$	$P < .01$
Bone phosphate (% of ash Weight)						
Femur	8.6 ± 0.49	3.88 ± 0.46	7.81 ± 0.62	$P < .01$	$P < .01$	$P < .01$
Eighth thoracic rib	11.89 ± 0.11	6.24 ± 0.45	11.67 ± 0.16	$P < .01$	$P < .01$	$P < .01$
Eighth thoracic vertebra	10.67 ± 0.36	7.21 ± 0.23	10.72 ± 0.43	$P < .01$	$P < .01$	$P < .01$
Fourth lumbar vertebra	11.38 ± 0.39	5.34 ± 0.28	11.56 ± 0.12	$P < .01$	$P < .01$	$P < .01$

Values are expressed as mean ± S.E.M ($n = 6$). ##denotes significance level based on Kruskal-Wallis nonparametric ANOVA test and * denotes significance level based on Mann-Whitney "U" multiple comparison test.

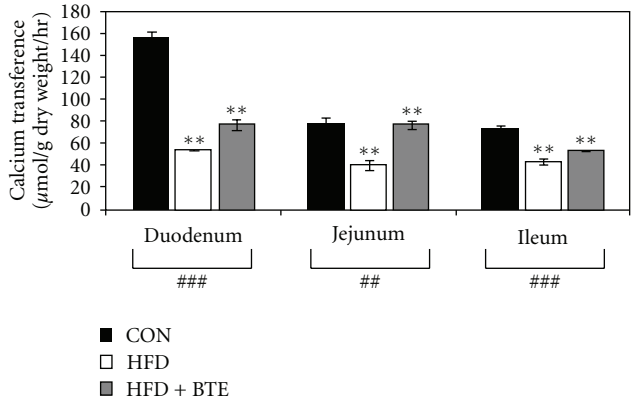

Figure 4: Transference of calcium in duodenal, jejunal, and ileal segments of control (CON; Group A), HFD treated (Group B), and HFD + BTE supplemented (Group C) groups of rats. Values are expressed as mean ± S.E.M. ($n = 6$). ### and ## denote significance level $P < .001$ and $P < .01$, respectively, based on Kruskal-Wallis nonparametric ANOVA test and **$P < .01$ denotes significance level based on Mann-Whitney "U" multiple comparison test.

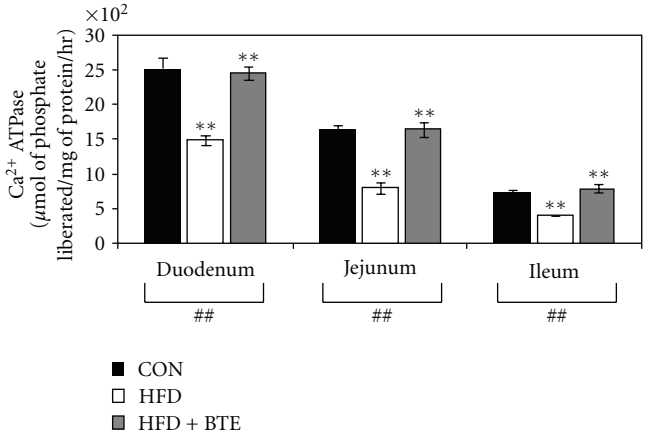

Figure 5: Ca^{2+}-ATPase activity of intestinal mucosal extracts in duodenal, jejunal, and ileal segments of control (CON; Group A), HFD treated (Group B), and HFD + BTE supplemented (Group C) groups of rats. Values are expressed as mean ± S.E.M. ($n = 6$). ##$P < .01$ denotes significance level based on Kruskal-Wallis nonparametric ANOVA test and **$P < .01$ denotes significance level based on Mann-Whitney "U" multiple comparison test.

($P < .01$). This could be recovered significantly ($P < .01$) when BTE was supplemented (Group C).

3.7. Serum RANKL and OPG Activity. Figure 3 depicts serum levels of RANKL (Figure 3(a)) and OPG (Figure 3(b)) of different groups of rats. Compared to control, HFD-fed rats showed a decrease in serum OPG ($P < .01$) level, which was found significantly ($P < .05$) counter regulated on BTE supplementation. In contrast, compared to control, serum RANKL level was increased significantly ($P < .01$) in HFD-fed animals. On BTE supplementation, a significant decrease ($P < .01$) in the RANKL level was noted.

3.8. Intestinal Transference of Calcium. Results in Figure 4 depicts that, compared to control, HFD-fed rats (Group B) showed a segment-wise reduction in intestinal transference of calcium ($P < .01$ for duodenum, jejunum, and ileum). BTE supplementation could significantly correct such alterations in calcium transference in HFD-fed rats ($P < .01$ for duodenum, jejunum, and ileum).

3.9. Calcium ATPase Activity. Figure 5 shows the effects of feeding of HFD on the mucosal calcium ATPase activity of different segments of small intestine of rats. Results show that, compared to control rats, HFD feeding caused a significant reduction in the activity of this enzyme ($P < .01$ for duodenum, jejunum, and ileum), while BTE supplementation could significantly increase this enzyme activity ($P < .01$ for duodenum, jejunum, and ileum).

3.10. Intestinal Alkaline Phosphatase Activity. Figure 6 depicts that, compared to control group, HFD-fed rats

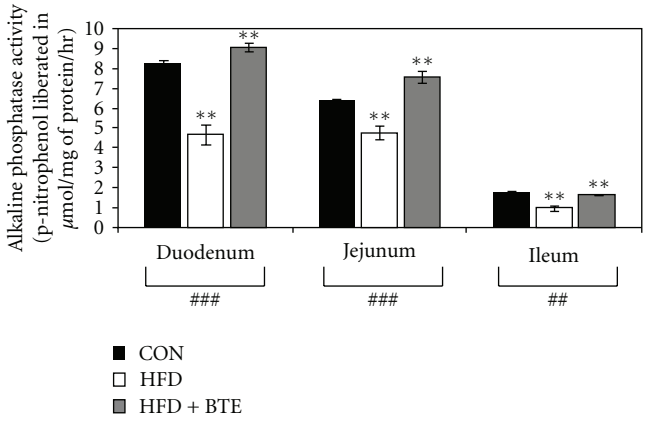

Figure 6: Alkaline phosphatase activity of intestinal mucosal extracts in duodenal, jejunal, and ileal segments of control (CON; Group A), HFD treated (Group B), and HFD + BTE supplemented (Group C) groups of rats. Values are expressed as mean ± S.E.M. ($n = 6$). $^{###}$ and $^{##}$ denote significance level $P < .001$ and $P < .01$, respectively, based on Kruskal-Wallis nonparametric ANOVA test and $^{**}P < .01$ denotes significance level based on Mann-Whitney "U" multiple comparison test.

showed a significant reduction in the activity of alkaline phosphatase in all segments of small intestine ($P < .01$ for duodenum, jejunum, and ileum). BTE supplementation was found significantly effective in restoring this enzyme activity in all these segments ($P < .01$ for duodenum, jejunum and ileum).

3.11. Histological Analysis of Cancellous and Cortical Bones. Histological studies of two different types of bones were conducted to determine the *in vivo* effects of BTE in the HFD-fed animals. HFD-fed rats (Group B) showed a decrease in cortical thickness and empty bone marrow space at the proximal tibia (Figures 7(a)–7(c)) and fourth lumbar vertebra (Figures 7(d)–7(f)), as compared to control (Group A). Additionally, there was a decrease in trabecular bone volume and thickness at the fourth lumbar vertebra. However, on BTE supplementation, bone repairing actions were seen in these HFD-fed animals.

4. Discussion

The present study examined the chemoprotective actions of aqueous black tea extract (BTE) against HFD fed (60%) NASH skeletal changes. Bone manifestations are well-known as extrahepatic complications of chronic liver disease. Patients with chronic liver disease are at increased risk of developing hepatic osteodystrophy manifested as osteomalacia or osteoporosis [56]. Osteoporosis is a frequent complication of end-stage liver disease irrespective of its etiology [57].

Recent research also suggests that there is a strong positive correlation between osteoporosis and hyperlipidemia. Excess lipid consumption may lead to lipid accumulation and subsequent oxidation within bone vasculature, wherein osteoblastic differentiation may be altered. In addition to their inhibitory role in osteoblastic differentiation, it is believed that because oxidized lipids induce endothelial expression of monocyte chemotactic factors, as well as other potent inducers of osteoclastic differentiation, oxidized lipids may also promote bone resorption [58].

Biochemical markers of bone turnover have been widely used as research tool to measure the effects of drugs on bone remodeling [59]. Elevated levels of bone markers are associated with rapid bone loss and may predict a greater risk of fracture independently of bone mineral density (BMD) variability [10]. In our study, compared to control, HFD treated rats showed an increase in urinary loss of calcium, phosphate, and creatinine (Table 1), which could be significantly corrected by BTE supplementation, suggesting BTE possibly has some positive role in preventing bone resorption and/or increase bone formation or both.

Results of studies of two other specific markers of bone turnover, namely, urinary calcium-to-creatinine (Ca : Cr) ratio and serum alkaline phosphatase (AP) further support our speculation (Tables 1 and 2, resp.). It was observed that BTE was effective in reducing HFD-induced increase in serum AP and urinary Ca : Cr ratio. Since a rise in AP and Ca : Cr ratio has been linked with collagen degradation, bone resorption and osteoporosis [60–63], it appeared that BTE was possibly effective in preventing bone loss. A close association of increased serum concentrations of TRAP as a potential index for osteoclastic activity is well established [64]. In the present study, compared to control, HFD-fed animals showed an increase in serum TRAP level (Table 2). However, this response was found well regulated by BTE supplementation, indicating that BTE possibly was effective even in controlling osteoclastic activity to preserve skeletal health.

Such speculations were cross-examined in our studies by measuring parameters like bone density (Table 3) and bone mineral content (Table 4). Rats in the HFD fed-group had significantly lower bone densities and mineral content, compared to control rats, which could be significantly regained by BTE supplementation, suggesting again the protective role of BTE against HFD-fed skeletal dysfunctional changes. Further confirmatory evidence in favor of our speculation was obtained in our experiment for bone tensile strength or breaking force, which is required to break the bone. Compared to control, HFD-fed animals showed lower tensile strength (Figure 1), which could be significantly elevated (increase in breaking force) on BTE supplementation.

Chronic liver disease accelerates the development of hypogonadism due to both reduced hypothalamic release of gonadotrophins and primary gonadal failure. A decline in circulating estrogen may be a mediator of bone loss in women and men with chronic liver disease [10]. In the present study, HFD-fed female rats showed a significant decrease in serum estradiol level (Figure 2), which could be considerably restored by BTE supplementation. This result showed that BTE has efficacy as a potent phytoestrogenic compound. The estrogen-enhancing property of flavonoids [65, 66] from various extragonadal sites like mesenchymal cells of adipose tissue, osteoblasts, and chondriocytes of bone, numerous sites in the brain, breast, and vasculature

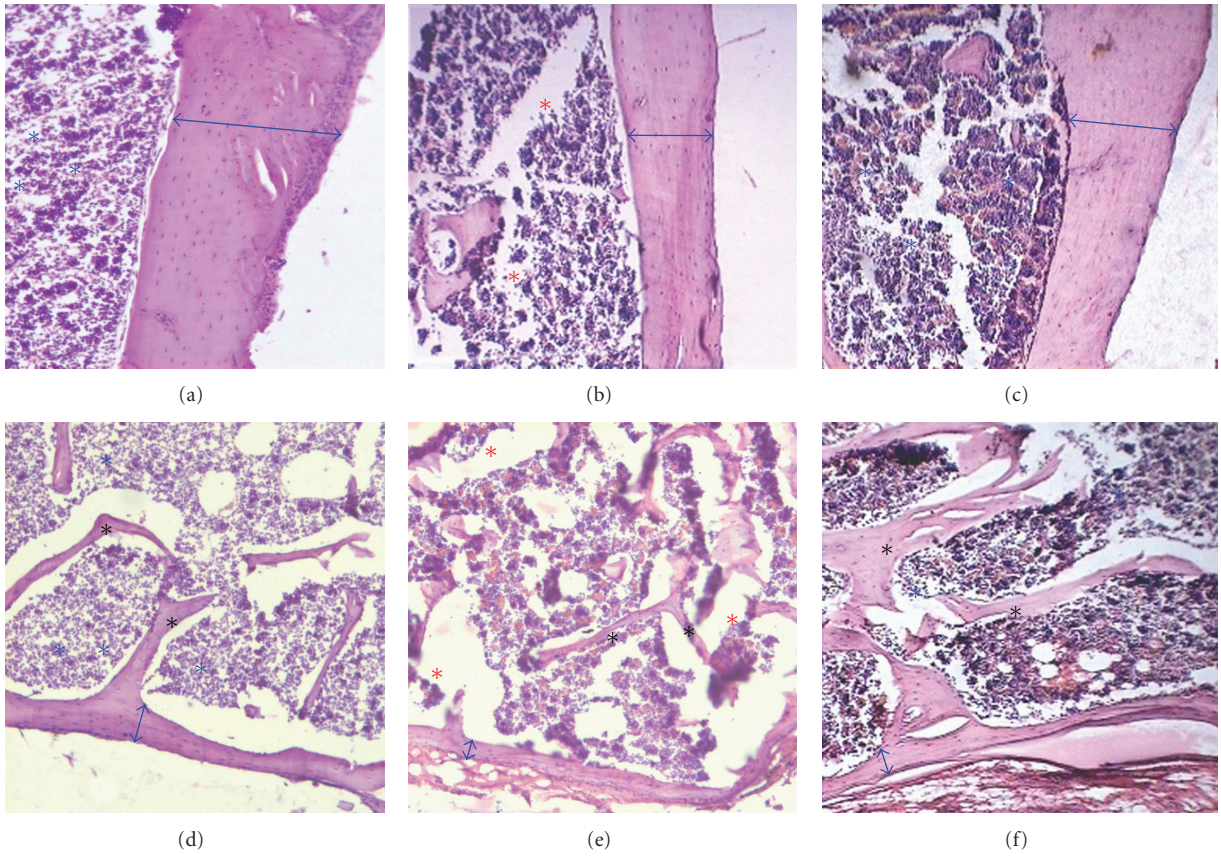

Figure 7: Histological analysis of tibia ((a)–(c)) and lumbar vertebra ((d)–(f)) of rats (hematoxylin-eosin staining, X 100). Representative photomicrograph from HFD group (b) with reduction in cortical thickness and empty bone marrow of the tibia than CON (a) and HFD + BTE (c). Photomicrograph of lumbar vertebra of HFD group (e) with reduction in cortical thickness, empty bone marrow and less trabecular bone compared with CON (d) and HFD + BTE group (f). Blue line, cortical thickness; blue star, bone marrow; red star, empty bone marrow; black star, trabecular thickness.

[67, 68] possibly was responsible for such an increase in serum estradiol level by modulating the aromatase activity [68].

Osteoprotegerin (OPG) is a novel receptor that blocks osteoclast formation. Either in a cell membrane-bound or in a soluble form, RANKL, receptor activator of NF-kappa β (RANK), stimulates osteoclastogenesis and osteoclastic bone resorption. Factors that stimulate bone resorption increase RANKL expression and, with some exceptions, decrease OPG expression [69]. In the present study, animals of HFD-fed group showed a significant decrease in serum level of OPG (Figure 3(b)) and a significant increase in serum level of RANKL (Figure 3(a)), compared to control group of rats. BTE supplementation, however, could significantly restore altered levels of serum OPG and RANKL, suggesting a more confirmatory protective role of BTE against osteoclastic differentiation and activity in HFD-fed rats.

Calcium absorption has been reported to occur in body throughout the length of the small intestine, the absorption being greater in the duodenum and proximal jejunum than in the ileum [70]. The involvement of two intestinal enzymes, alkaline phosphatase, and Ca^{2+}-ATPase, has been proposed frequently in this phenomenon because the activity of these enzymes correlates with the degree of calcium absorption in different parts of intestinal tract under different circumstances [50, 71, 72]. Results also indicate that Ca^{2+}-induced ATP hydrolysis in the intestine is the result of two enzymatic activities, namely, alkaline phosphatase present in brush border as well as basolateral membranes and a more specific Ca^{2+}-ATPase exclusively located in basolateral plasma membrane [73]. Evidence further indicates that estrogen is more directly involved in determining intestinal calcium absorption because decreased calcium absorption due to ovarian hormone insufficiency is corrected by hormone replacement therapy [74, 75]. In the present study, intestinal transference of Ca^{2+} was reduced in HFD-fed rats. Result indicated that deficiency of estrogen had a negative influence upon intestinal transference of calcium, as these animals showed a greater degree of decrease in the transference of calcium than the control animals (Figure 4). Such observation finds its support from earlier findings that estrogen may have direct role via the estrogen receptors in regulating intestinal calcium absorption *in vitro* and *in vivo* [76, 77]. BTE supplementation in the present study was found effective in correcting such reduction in calcium transference, indicating that BTE also possibly had positive influence upon mucosal transference of calcium.

To ascertain the mechanism of such decrease in intestinal transference of calcium, the activities of two most relevant mucosal calcium transferring enzymes, namely, alkaline phosphatase and calcium-ATPase were examined. Activities of both of these enzymes were found inhibited in HFD-fed group of animals (Figures 5 and 6). This supports well the earlier observations that both the enzymes are involved in calcium absorption as the activities of these enzymes correlate with the degree of calcium absorption in different parts of the intestinal tract under different circumstances [50, 71, 72]. BTE supplementation could well restore the activities of both of these enzymes indicating that the observed positive influence of BTE upon intestinal transference of calcium is thus mediated through modulation of activities of these transferring enzymes.

Histological (Figure 7) observation of serial section of tibia and lumbar vertebrae revealed that, compared to control, significant reduction in cortical thickness and bone marrow content was seen in HFD fed rats. On BTE administration, these changes were significantly corrected, suggesting a potential bone repairing action of BTE.

It is recognized that animal studies contain some inherent design limitations. First, restrictions of animal use and therefore sample size; second, the doses required for demonstrating the disease prevention effects are usually higher than the amounts consumed by humans. Furthermore, because of infrastructure limitations it was necessary to rely on biochemical and histological measures rather than using peer reviewed DEXA-derived indices for examining bone profiles. To minimize the error associated with such limitations, we took extreme care while selecting the parameters, collecting data and analyzing them by well-recognized statistical methods followed by us [34, 35] earlier.

5. Conclusions

In conclusion, results of this study strongly suggest a protective role of BTE against NASH-induced changes in bone metabolism in rats.

Acknowledgment

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Review Article

Bone Health in Patients with Multiple Sclerosis

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Multiple sclerosis (MS) is a gait disorder characterized by acute episodes of neurological defects leading to progressive disability. Patients with MS have multiple risk factors for osteoporotic fractures, such as progressive immobilization, long-term glucocorticoids (GCs) treatment or vitamin D deficiency. The duration of motor disability appears to be a major contributor to the reduction of bone strength. The long term immobilization causes a marked imbalance between bone formation and resorption with depressed bone formation and a marked disruption of mechanosensory network of tightly connected osteocytes due to increase of osteocyte apoptosis. Patients with higher level of disability have also higher risk of falls that combined with a bone loss increases the frequency of bone fractures. There are currently no recommendations how to best prevent and treat osteoporosis in patients with MS. However, devastating effect of immobilization on the skeleton in patients with MS underscores the importance of adequate mechanical stimuli for maintaining the bone structure and its mechanical competence. The physical as well as pharmacological interventions which can counteract the bone remodeling imbalance, particularly osteocyte apoptosis, will be promising for prevention and treatment of osteoporosis in patients with MS.

1. Introduction

Osteoporosis is a condition of impaired bone strength which leads to increased risk of fracture [1]. The enhanced bone fragility reflects the integration of the amount of bone (bone mass) and bone quality. Bone quality depends on its macro- and microarchitecture and on the intrinsic properties of the materials that comprise it (e.g., matrix mineralization, microdamage accumulation, or collagen quality) [2]. Bone is continually adapting to changes in its mechanical and hormonal environment via the process of bone remodeling. Bone remodeling maintains bone structure and its mechanical competence by removing damaged bone and replacing it with new bone and thus restores bone's material composition, micro-, and macroarchitecture. This process depends on the normal production, work and lifespan of osteoclasts, osteoblasts, and osteocytes. Thus, diseases and drugs that have an impact on bone cells and bone remodeling will influence bone's structure and its resistance to fracture [3]. Multiple sclerosis (MS) is a chronic progressive disease affecting the myelin sheath covering of nerve fibers in the brain and spinal cord, leading to functional impairments such as visual impairment, abnormal walking mechanics, poor balance, muscle weakness, fatigue, and progressive immobilization [4]. The resultant functional impairments lead to frequently falls [5]. The disease affects mainly young adults (20 to 40 years) and its incidence is more frequent in women (approximately 2 : 1) [6]. Its prevalence ranges from 2 to 150 patients at 100 000 [7]. Impaired mobility or lack of weight-bearing physical activity reduced mechanical stress on bone, which causes a marked imbalance in bone remodeling with a disruption of osteocytes network [8]. Management of MS requires long-term disease-modifying therapy, such as glucocorticoids (GCs) with a further negative effect on bone remodeling and bone strength. Secondary osteoporosis may develop and low-trauma fractures occurring in patients with MS more frequently than in healthy controls [9–15]. Fractures and their sequelae can have important personal as well as (economic) implications for society. Therefore, the attention on the issue of bone health among patients with MS is warranted. This paper examines the underlying pathogenic mechanisms of osteoporosis in patients with MS as well as its management. Understanding the causes associated with decreased bone strength in patients with MS will help in the optimal therapeutic intervention.

2. Prevalence of Osteoporosis in Patients with Multiple Sclerosis

The analysis of a registry of 9029 patients with MS in the USA found that 27.2% responders reported low bone mass, and more than 15% of responders reported a history of fracture [9]. Most studies in patients with MS evaluated BMD in comparison with the control group of healthy subjects and showed significantly lower BMD in patients with MS than in controls [11–16]. Several of these studies were shown that vertebral BMD is affected to a lesser degree than femoral BMD [11, 12, 16]. The low BMD in MS patients involve both sexes [15]. Interestingly, one study in men with MS reported low BMD (osteoporosis) in 37.5% (15 out of 40) and 21% (8 out of 38 patients) had vertebral, rib, or extremities fractures [15]. Patients with progressive forms of MS showed a more severe loss of BMD than those with relapsing-remitting MS [12]. Fracture incidence in patients with MS evaluated only few studies. Cosman group found fracture rates of 22% in patients with MS compared with 2% in controls [11]. It remains unexplained whether all patients with MS are more susceptible to osteoporosis and fractures; for example, there is evidence that patients with a low expanded disability status scale (EDSS) score did not show any significant difference in BMD with comparison with healthy control subjects [17, 18]. Therefore, further elucidation is needed to qualify which risk factors are most responsible for a bone loss in patients with MS.

3. Pathogenic Mechanisms

Bone remodeling is under way throughout life and maintains bone strength by removing damaged bone and replacing it with new bone and thus restores bone's micro- and macroarchitecture. This process depends on the normal production, work, and lifespan of osteoclasts, osteoblasts, and osteocytes [19]. Chronic diseases, such as MS, may significantly disturb the process of bone modeling and remodeling with resulting bone loss, deterioration of bone's quality and increased frequency of fractures [20, 21]. Secondary osteoporosis and low-trauma fractures occur in patients with MS more frequently than in healthy controls [9, 11]. The underlying pathogenic mechanisms of the osteoporosis in patients with MS are probably based on the progressive immobilization, long-term GCs treatment, vitamin D deficiency, skeletal muscle atrophy and possibly on the presence of various cytokines involved in the pathogenesis of MS [10]. In addition, chronic use of other drugs, such as antidepressants may contribute to the development of osteoporosis and fractures [22]. The functional impairments also leads to an increased risk of falling that, combined with bone loss and impaired quality of bone mass, can increase the frequency of bone fracture in individuals with MS [11].

3.1. Disability. Mechanical loading is an important factor controlling bone mass. Increased bone loss in immobilized subjects is well-recognized complication in patients after spinal cord injury with tetraplegia [23, 24], in bedridden patients, or in astronauts [25], whereas localized bone loss is well documented in patients with regional disuse, for example, after fracture itself. Immobilization causes an overall progressive bone loss at a similar rate to osteoporosis caused by estrogen deficiency, but at the same amount of induced bone loss, disuse led to more deteriorated bone structure and mechanical properties than estrogen deficiency [26]. The available studies showed that cortical thinning and substantial decline of trabecular bone density account for increased bone fragility [27–29]. The duration and degree of motor disability appears to be a major contributor to the pathogenesis of secondary osteoporosis in patients with MS. The degree of disability measured by the Kurtzke EDSS score significantly correlated with BMD in patients with MS [11]. Specifically, site-specific effects of motor disability were documented in MS patients, and EDSS correlated mainly with BMD in the hip but not in the lumbar spine [14]. In wheelchair-bound patients, an atrophy of hip muscles affects proximal femur, while BMD of lumbar spine is not decreased because of its adequate mechanical stimulation by the trunk and back muscles in the upright position. Similarly, patients with spinal cord injury lose BMD mainly at femoral sites [30]. Also, hemiplegic patients showed a significant loss of BMD in both trabecular and cortical bone at the forearm and at the neck and great trochanter on the paretic hip [31]. Higher total body bone mineral content was documented in ambulatory patients (EDSS score ≤ 6.5) compared with nonambulatory patients (EDSS score ≥ 7.0) [12, 13]. Also, higher prevalence of osteoporosis was found in nonambulatory patients [32]. In male patients, a positive correlation has been observed between BMD and both EDSS score (correlation with femoral and also vertebral BMD) and BMI (correlation with femoral BMD only). There was shown that also EDSS score and BMI two years prior to the study could be used as future indicators of low BMD [15].

A reduced mechanical stress on bone causes a marked imbalance in bone remodeling with a transient increase in bone resorption (which occurs initially) and a decrease in bone formation (which is sustained for a longer duration) [25, 33, 34] (Table 1). The mechanism causing this decrease in bone formation probably lies in the reduction of mechanical stress during immobilization which results in a marked disruption of osteocytes network due to increase of osteocyte apoptosis. Osteocytes represent 95% of all bone cells and form a mechanosensory system which is based on a three-dimensional network of tightly interconnected osteocytes entombed in mineralized bone matrix [35]. Disruption of this system affects probably several aspects of bone homeostatic system, such as mechanosensitivity, mechanotransduction, and basic multicellular units responsible for bone remodeling [36]. The immobilization-induced osteocyte apoptosis is followed by osteoclastogenesis and increased bone resorption [37]. While molecular mechanisms of disuse osteoporosis are not well understood, recent evidence found that mechanical unloading caused upregulation of *Sost* gene in osteocytes and increased levels of sclerostin (product of *Sost* gene) [38]. Sclerostin is responsible for the inhibition of Wnt/beta-catenin signaling in vivo and for the suppressed viability of osteoblasts and osteocytes. Interestingly, sclerostin-deficient mice (*Sost* $-/-$) were resistant to

Table 1: Changes in bone cells metabolism and in bone mass/structure in patients with long-term immobilization.

	Increased	Decreased
Osteocytes	Apoptosis	Metabolism and function
		Repair of microdamage
Osteoblasts	Apoptosis	Activity
		Synthesis of type I collagen
Osteoclasts	Activity	Apoptosis
Bone homeostasis	Remodeling rate	
	Bone resorption	Bone formation
		Bone mineral density*
		Cortical thickness*
		Cortical density*
		Trabecular density*

*The anatomic location and function of the bone in the skeleton account for the magnitude of skeletal response to immobilization.

mechanical unloading-induced bone loss [38]. Importantly, the administration of sclerostin neutralizing antibody in experimental model of immobilization resulted in a dramatic increase in bone formation and a decrease in bone resorption that led to increased trabecular and cortical bone mass [39]. Osteocytes are also necessary for targeted bone remodeling to avoid microdamage accumulation, which could lead to whole-bone failure. Recently, Waldorff et al. showed that osteocyte apoptosis may be insufficient for repair of microdamage without the stimulation provided through physiologic loading [40]. MS affects a wide range of neurological function and most of patients with MS have abnormal muscle strength, impaired balance, and gait control which leads to frequent falls [5, 41] that combined with a bone loss increase the frequency of bone fractures. Imbalance is also often the initial symptom of MS. The pathogenesis is not completely understood yet. It was demonstrated that changes in postural control in most patients with MS are probably the result of slowed afferent proprioceptive conduction in the spinal cord [5]. Disuse, inflammatory changes, as well as GCs treatment or vitamin D deficiency, may also contribute to weakness and loss of muscle strength and thus to frequent falling.

3.2. Glucocorticoids (GCs). GCs are frequently used to control MS relapses. Oral GCs treatment in patients with MS may increase the risk of osteoporosis. Epidemiological studies showed that fracture risk is increased rapidly after starting oral GCs treatment and is related to the dose and duration of GCs exposure [42]. Doses as low as 2.5–5 mg of prednisolone equivalents per day can be associated with a 2.5-fold increase in vertebral fractures, and the risk is greater with higher doses used for prolonged periods [43]. Bone loss due to GCs treatment is steep during the first 12 months and more gradual but continuous in subsequent years. However, the fracture risk returns towards baseline levels after discontinuation of oral GCs treatment [44]. The mechanism of osteoporosis in patients on GC treatment is complex [45] (Table 2). However, the contribution of other risk factors, such as vitamin D insufficiency and physical disability confounds the assessment of GCs effects on bone in patients with MS.

Repeated pulses of high-dose methyprednisolone in MS patients did not result in a subsequent decrease in BMD [18]; however, the risk of osteoporotic fractures remains slightly increased in patients undergoing cyclic GCs treatment at high doses [47]. High-dose, short-term intravenous GC regimens cause an immediate and persistent decrease in bone formation and a rapid and transient increase of bone resorption [48]. In fact, GCs may increase proresorptive IL-6 signaling as well as increase the expression of receptor activator of NF-κB ligand (RANKL) and decrease the expression of its soluble decoy receptor, osteoprotegerin (OPG), in stromal and osteoblastic cells [49]. Moreover, GCs may directly decrease apoptosis of mature osteoclasts [50]. However, discontinuation of such regimens is followed by a high bone turnover phase [48]. In physically active patients with MS treated with low-dose steroids, the bone turnover markers were not different from controls [51]. Addressing the question of whether duration of low-dose GCs use in combination with other immunomodulators in patients with MS increases risk of osteoporosis requires further prospective study by taking into account other risk factors, particularly the level of disability.

3.3. The Effect of Other Immunomodulatory Drugs. Although no harm effect of low-dose methotrexate was observed in patients with MS, several case reports have described associations between pathological nonvertebral fractures and low-dose methotrexate (MTX) in rheumatoid arthritis (RA) patients [52]. In addition, methotrexate osteopathy, characterized by pain, osteoporosis, and microfractures, has been very rarely observed in patients with low-dose MTX treatment [53]. Other immune-modifying drugs, such as interferon-beta or azathioprine, which are used in conjunction with GCs have not been shown to promote bone loss experimentally or clinically. On the contrary, interferon-beta may have favorable effect on bone metabolism in patients with MS [54], probably due to the inhibitory effect of interferon-beta on osteoclasts development [55]. Experimentally, also treatment with the S1P(1) agonist FTY720, a new and promising drug for the treatment of MS, relieved ovariectomy-induced osteoporosis in mice by reducing the number of mature osteoclasts attached to the bone surface [56]. However, further investigation with regard to their effects on bone health is needed.

3.4. Vitamin D Insufficiency. The role of vitamin D in bone homeostasis is well understood, and the use of vitamin D to prevent and treat osteoporosis was recently reviewed [57]. There is also evidence from both observational studies and clinical trials that hypovitaminosis D are predisposing conditions for various common chronic diseases. In addition to skeletal disorders, vitamin D deficiency is associated with increase the risk of malignancies, particularly of colon, breast, and prostate gland cancer, of chronic inflammatory and autoimmune diseases (e.g., insulin-dependent diabetes mellitus, inflammatory bowel disease, or multiple sclerosis),

Table 2: The mechanisms of bone loss during long-term GCs treatment.

	Inhibition	Stimulation
Bone cells direct effects		
Bone marrow/stromal cells	differentiation into osteoblasts	differentiation into adipocytes
Osteoblasts	differentiation, activity	—
	synthesis of type I collagen	apoptosis
Osteocytes	metabolism and function	apoptosis
Osteoclasts	apoptosis	stimulation
Indirect effects		
Gut	Ca^{2+} absorption	—
Renal tubule	Ca^{2+} reabsorption	—
Parathyroid-PTH rate*	Tonic secretory rate*	Pulse secretory rate*
		Fractional pulsatile secretion*
Pituitary	Growth hormone/IGF-1	—
	FSH, LH	—
Testes, ovaries	Testosterone, estradiol	—

* Data from Bonadonna et al. [46]; abbreviations: FSH: follicle stimulating hormone; LH: luteinizing hormone; IGF-1: insulin like growth factor 1.

as well as of metabolic disorders (metabolic syndrome and hypertension) [58]. Vitamin D intake, decreasing latitude, increased sun exposure, and high serum vitamin D levels have all been shown to be associated with a decreased risk of MS [59]. Patients with MS have more often vitamin D deficiency due to its low intake as well as limited sunlight exposure [12]. Mean 25-hydroxyvitamin D_3 (25OHD) levels in patients with MS are more often lower (below the level of 20 ng/mL) than in age-matched controls [11, 14]. There was no significant correlation between 25(OH)D and BMD in patients with MS [11, 14]. Thus, while patients with MS are susceptible to low 25OHD levels, the evidence implicating linking levels to reduced BMD and osteoporosis in patients with MS is unclear. Only a few studies have investigated this link [11, 12, 14]. A low vitamin D state, from inadequate diet intake and decreased exposure to sunlight, contributes to malabsorption of calcium and vitamin D insufficiency in MS patients. Secondary hyperparathyroidism may develop, which can contribute to bone remodeling imbalance and bone loss in patients with MS. Moreover, patients with MS treated with GCs will be at greater risk for an imbalance between bone formation and bone resorption and, therefore, more susceptible to development of osteoporosis due to vitamin D insufficiency/deficiency. GCs treatment is associated with reduced calcium absorption from the gastrointestinal tract by opposing vitamin D action. Furthermore, renal tubular calcium reabsorption is also inhibited by GCs. In addition, GCs may affect PTH secretory dynamic, with a decrease in the tonic release of PTH and an increase in pulsatile burst of the hormone [46].

3.5. The Chronic Inflammatory Process of Multiple Sclerosis. MS is an inflammatory disease of the central nervous system (CNS) with a prominent role of immune cells and cytokines in degradation of the myelin sheaths [60]. Recent evidence has indicated that a number of additional cell types, such as T cells, play a key role in bone loss [61]. In inflammatory or autoimmune disease states, activated T-cells produce receptor activator of nuclear factor kappaB ligand (RANKL) and proinflammatory cytokines, such as TNF-α, IL-1, or IL-11, all of which can induce RANKL expression in osteoblasts and bone marrow stromal cells. The systemic or local activation of T-cells may, therefore, trigger bone loss via the expression of RANKL [61]. Osteoprotegerin (OPG), a protein member of the tumor necrosis factor (TNF) receptor family and its ligand RANKL were identified as a key cytokines that regulate osteoclastogenesis [61]. Significantly, higher levels of RANKL and OPG were found in the patients with MS with low mean EDSS as compared to the age-matched controls [62]. Among other cytokines, osteopontin (OPN) has been studied in the shared pathogenesis of MS and osteoporosis. OPN is a member of the SIBLING (small integrin-binding ligand N-binding glycoprotein) family of noncollagenous matricellular proteins [63]. OPN was identified as the most abundantly expressed cytokine in MS lesions, and OPN levels were found to be increased in cerebrospinal fluid of MS patients [64, 65] and in the plasma in patients with relapsing-remitting MS [66]. However, other studies found that OPN circulating levels are low in patients with MS [67]. It seems likely that further future studies experiments will uncover the role of OPN and additional molecules mediating bone loss in inflammatory diseases, such as MS.

3.6. Use of Antiepileptic and Antidepressant Drugs. Antiepileptic drug treatment can lead to osteoporosis [68, 69]. Meta-analyses have revealed that barbiturate, antidepressant, antipsychotic, and benzodiazepine treatment increases patient's risk of osteoporosis [70]. More recently, current

use of antidepressant drugs with a high affinity for the 5-hydroxytryptamine reuptake transporter (5-HTT) was associated with a higher risk of osteoporotic fractures compared to use of antidepressants with a medium or low affinity [71]. BMD was lower among those reporting current selective serotonin reuptake inhibitors (SSRI) use but not among users of other antidepressants [72, 73]. In vivo studies have found that 5-HT could alter bone architecture and could reduce bone mass and density [74]. The 5-HTT has been located in osteoclasts, osteoblasts, and osteocytes, and the the inhibition of 5-HTT using a SSRI (fluoxetine hydrochloride) had antianabolic skeletal effects in rats [74]. Further research is needed to confirm this finding in light of widespread SSRI use and potentially important clinical implications.

4. Diagnosis and Management of Osteoporosis in Patients with MS

Despite the fact that patients with MS can develop osteoporosis and fractures more often than their age-matched healthy controls, many patients with MS are not evaluated for their bone status, and there are no clinical guidelines for prevention and treatment of osteoporosis in patients with MS. Patients with MS are also at a higher risk of falls that can increase the frequency of bone fracture combined with bone loss and impaired bone's quality.

Clinical evaluation in all patients with MS should include the assessment of the clinical risk factors for osteoporosis and fractures, such as the hereditary disposition of osteoporosis, previous low trauma fractures, and smoking or alcohol habits. The specific risk factors of the osteoporosis in patients with MS are the level of disability (specifically motor disability) and possibly a long-term GCs treatment, vitamin D deficiency, skeletal muscle atrophy, and increased risk of falling. The examination of the motor function using the EDSS score could provide a useful indicator for further evaluation. Cutoff EDSS 6 represents reasonable end of motor performance of the patient; 6.5 means only several meters with bilateral support, and 7 is only the ability of transfer to wheelchair from the bed. The EDSS scores of 6 or greater has been found to correlate well with decreased BMD [12, 15], and BMD should be routinely measured in these patients. On the other hand, patients with a good physical activity and low EDSS score (<5) may have normal BMD [14] as well as markers of bone turnover [51]. BMD measurement should be also performed in all patients who are receiving 5 mg of prednisone equivalents daily for more than 3 months.

BMD testing using dual-energy X-ray absorptiometry (DXA) should be conducted at the lumbar spine and hip. This measure provides an assessment of fracture risk prior to the occurrence of a fragility fracture as well as monitors the course of the disease and response to therapy. No consensus exists as to how frequently patients at risk osteoporosis should have followup scans. However, BMD should be remeasured after 1 or 2 years to ascertain that it is stable or to identify the patient with ongoing bone loss, especially in patients treated with long-term GCs treatment. In the presence of clinical risk factors, fracture risk may be increased independently from BMD. Therefore, combination of BMD with clinical risk factors is recommended to identify a risk patient and to target pharmacologic therapy. In postmenopausal women and men (between 40 and 90), the assessment of individualized 10-year absolute fracture risk (FRAX, fracture prediction algorithm) is recommended [75].

The identification of previous low-trauma fractures, especially vertebral fractures is important for the decision-making process as a previous vertebral fracture is a particularly strong risk factor. Importantly, vertebral fractures may occur in 30–50% of patients receiving chronic GCs therapy [76] and up to 50% of vertebral fractures are asymptomatic and, therefore, do not come to the attention of physicians. Spinal X-rays should be performed in those with localized back pain or a loss of more than 3 cm in height in order to detect prevalent vertebral fractures. Alternatively, the vertebral fracture assessment tool of the bone densitometer, which is associated with low radiation, may be useful screening test for vertebral fractures assessment.

Laboratory tests are indicated to exclude other secondary causes of osteoporosis, such as vitamin D deficiency, renal insufficiency, malabsorption, and hypogonadism. Useful biochemical tests include routine standard tests to exclude renal or hepatic impairment, blood count, serum calcium, 24-hour urinary calcium, 25-hydroxyvitamin D_3 (to exclude vitamin D deficiency), and gonadal hormones (to exclude hypogonadism).

5. Treatment Options

5.1. Nonpharmacological Considerations. Prevention is more effective than treatment of established osteoporosis. For all patients, nonpharmacological therapies should be considered for prevention of skeletal fragility, including adequate weight-bearing exercise, nutrition (protein, calcium, vitamin D), and lifestyle modifications. As reviewed above, disability is the most often cause of bone loss in patients with MS, and mechanical loading and exercise interventions can prevent osteocyte apoptosis and bone loss [77, 78]. Exercises have beneficial effects on strength, physical endurance, mobility-related activities (transfer, balance, and walking), and on mood, without any evidence of detrimental effects [79]; however, there was no evidence that any particular exercise programs were more effective in improving or maintaining function. Whole-body vibration is a new approach to improve neuromuscular functions and bone strength, but there is limited evidence that whole body vibration provides any additional improvements [80]. Further experimental studies are necessary to identify optimal physical activities for the prevention of osteocyte apoptosis and bone loss. Recurrent falls may be an important risk factor for fracture in disabled patients with MS. In patients with MS, falls are related to the level of disability [81], and possibly other factors may contribute to muscle weakness and imbalance, such as vitamin D deficiency or GCs treatment.

5.2. Calcium and Vitamin D. Calcium and vitamin D supplementation has been routinely provided in most clinical trials of bone protective therapy for both primary and

secondary osteoporosis, for example, in glucocorticoid-induced osteoporosis (GIO). The effect of calcium and vitamin D supplementation is maximized in patients whom baseline intake is low. As patients with MS are at a higher risk of calcium and vitamin D deficiency should have their calcium and vitamin D status checked and intake must be individualized. Those with a personal or family history of nephrolithiasis must be screened with 24-h urinary calcium. In immobilized patients, an increase in serum calcium is provoked by bed rest alone and additional calcium intake would not be helpful and might be harmful and provoke an increased risk of kidney stone formation. However, calcium and vitamin D should be used as an adjunct treatment, because a low calcium intake may exacerbate calcium loss during low mechanical loading [82]. In general, the amount of vitamin D supplementation should aim at achieving serum 25OHD levels above 50 nmol/l in >95% of adults without causing vitamin D toxicity. A daily dose of 800–1000 IU of vitamin D3 should be able to obtain this minimal 25OHD target. Due to vitamin D resistance in patients receiving GCs, those patients may require amounts of 1000–2000 IU of vitamin D3 daily [83]. Measurement of serum 25OH vitamin D is recommended, especially in GCs-treated patients. Although some evidence suggests that daily supplemental intake of 2000–4000 IU colecalciferol is required to obtain at least 75 nmol/l 25OHD, which may be optimum for many health outcomes [84], prospective trials showing that higher 25OHD levels (>75–80 nmol/l) are conveying additional benefits without new risk are needed.

5.3. Pharmacological Interventions. The ultimate goal of all pharmacological interventions is prevention of fractures. Although a number of drugs have been evaluated for the prevention and treatment of postmenopausal osteoporosis and GIO, the evidence of their efficacy in patients with MS, especially in premenopausal women and younger men is less strong. As osteoporosis in MS patients have multiple pathogenesis, medical interventions used in women with postmenopausal osteoporosis may not be similarly efficient. Patients requiring long-term GCs treatment and those being immobilized may require pharmacological therapy to prevent excessive bone loss and fractures. Options for treatment include antiresorptive drugs, such as estrogen, or aminobisphosphonates, or anabolic agents such as teriparatide.

Aminobisphosphonates (BPs). Although the use of BPs may be appropriate, the etiology of osteoporosis in patients with MS is fundamentally different from the osteoporosis commonly found in the postmenopausal women for whom these drugs were originally developed. As immobilization in patients with MS can cause substantial bone loss and increase in the risk of fractures [20], BPs may be option for treatment for those patients. Although BPs have not been systematically evaluated in the therapy of these conditions, some studies support the potential benefit of BPs in the management of bone loss associated with immobilization [24, 85, 86]. In immobilized patients, BPs is known to reduce immobilization-induced hypercalcaemia by inhibiting bone resorption of calcium. An immobilization-related elevated serum calcium level may inhibit parathyroid hormone (PTH) secretion, and hence renal 1, $25(OH)_2D_3$ production, in disabled long-standing MS patients. If oral therapy of BPs cannot be tolerated or excluded due to gastroesophageal disease, intravenous route of administration of ibandronate or zolendronate may be applied. However, acute phase reaction with fever, particularly after the first application of BP, may occur. BPs (alendronate, risendronate, or zolendronate) were also approved for the treatment of GIO. These drugs were shown to improve BMD, whereas the data on fractures were scanty in GIO, particularly in premenopausal or younger men. The mechanism by which BPs reduce the adverse skeletal effects of GCs have not been elucidated. The disadvantage of long-term BPs treatment is that it may lead to a reduction in bone turnover to a level inadequate to support normal bone remodeling. Although experimental data showed that BPs also prevents osteocyte apoptosis, there is also experimental evidence of increased accumulation of microdamage with long-term BPs therapy [87]. Also, as BPs accumulate in the skeleton (with a long-term residual time), they cross the placenta, accumulate in fetal skeleton, and cause toxic effects in pregnant rats. Therefore, BPs should be used with caution in women who may become pregnant.

5.4. Anabolic Drugs. Drugs, such as BPs, that suppress bone resorption have been proposed as interventions for prevention of GIO as well as disuse osteoporosis. The disadvantage of this approach is that it may lead to a reduction in bone turnover to a level inadequate to support normal bone remodeling. An alternative approach is to maintain a normal level of bone formation using a bone anabolic agent such as PTH. The human recombinant N-terminal parathyroid hormone (PTH 1–34 or teriparatide) is a potent osteoanabolic agent, which decreases osteoblast and osteocyte apoptosis and increases bone formation and bone strength. Because of GCs-induced decrease in the number of osteoblasts and rate of bone remodeling, anabolic, and antiapoptotic treatment with teriparatide may directly counteracts the key pathogenetic mechanisms of GCs excess on bone, thus, it may be a more effective treatment than BPs [88]. The same rationale applies to immobilization-induced osteoporosis, as progressive immobilization as well as long-term GCs exposure results in osteocyte apoptosis and reduced bone formation [89].

5.5. Future Options. As sclerostin augments osteocyte apoptosis, the antibody-mediated blockade of sclerostin represents a promising new therapeutic approach for the anabolic treatment of immobilization-induced osteoporosis and probably also for GCs-induced osteoporosis. Indeed, more recently, experimental data showed that administration of sclerostin neutralizing antibody in rat model of right hindlimb immobilization resulted in a dramatic increase in bone formation and a decrease in bone resorption that led to increased trabecular and cortical bone mass [39].

6. Summary

We have described a spectrum of pathogenetic factors which may contribute to the development of osteoporosis and

low-trauma fractures in patients with MS. Whilst there is evidence to support an important role for many of the risk factors, the most significant etiology of bone loss in patients with MS seems to be the level of motor disability and reduced bone load within individual patients. Other risk factors, such as long-term GCs treatment, hypovitaminosis D, or inflammation, may also play an important part in subset of patients with MS; however, further examinations in prospective studies are required. With regard to diagnostic as well as therapeutic interventions, there are currently no specific recommendations in patients with MS; however, identification and treatment of underlying cause should be the goal of therapeutic management. Optimally, the patients in a higher risk of osteoporosis should be early identified and preventively promptly treated to avoid the bone loss and fractures. Because the long-term disability and long-term GCs are probably two most significant etiologic risk factors for osteoporosis development in the majority of the patients with MS, the interventions which can counteract the osteocyte apoptosis as well as loss of muscle mass and muscle weakness will be promising.

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Review Article

Secondary Osteoporosis in Patients with Juvenile Idiopathic Arthritis

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Bone disease in patients with juvenile idiopathic arthritis (JIA) is associated with focal (joint erosion and juxtaarticular osteopenia) and systemic bone loss (generalized osteopenia or reduction of bone mass density). Pathophysiology of bone loss is multifactorial and involves particularly proinflammatory cytokines and deleterious effects of glucocorticoid therapy. Clinical studies in patients with JIA indicate excessive activation of osteoclastogenesis and reduction of bone formation. Reduction of physical activity, muscle atrophy caused by high disease activity, and compulsory restriction in movements are also associated with bone loss. In patients with JIA, the disease can be complicated by growth cartilage involvement and systemic or local growth retardation. In the absence of preventive measures, fragility fractures can occur even at an early age.

1. Introduction

Juvenile idiopathic arthritis (JIA) is a systemic autoimmune chronic inflammatory joint disease beginning until 16 years of age. JIA is the most frequent rheumatic systemic disease in the childhood. In the industrial countries, the incidence of JIA is 5–18 and prevalence of JIA is 30–150/100 000 children until 16 years old. In the Czech Republic, the annual incidence of JIA is 13/100 000 and prevalence of JIA is 140/100 000 children until 16 years old [1]. The initial cause of the chronic inflammatory processes targeting the synovial lining of joints is not known. Many of the proinflammatory factors stimulate the differentiation of osteoclasts from the hematopoietic precursor. Generalized bone loss of bone mass is a common feature in JIA.

2. Low Bone Mass and Increased Risk of Fracture

During childhood and adolescence in patients with JIA, when the peak of bone mass is attained in the healthy people, the accrual of bone mass is suppressed through direct and indirect mechanisms, namely by the inflammatory disease, by drug therapy and immobilization [2, 3]. In JIA, both synovial-derived and soluble cytokines are involved. Osteopenia or osteoporosis occurs in all of the JIA forms, most typically in systemic and polyarticular forms of disease. The low bone mass is associated with the high activity of the disease and with the number of involved joints in JIA patients [4–9], also with the reduction of bone formation [4, 6]. Reduced bone mineral density (BMD) is observed at all sites of the skeleton in the children and adolescents with JIA and also in adults with JIA. In the cross-sectional study, the low BMD in lumber spine and hip was found in 40–52% adult patients with JIA [10]. However, even the full remission of the disease in young adults is not able to completely normalize BMD at all skeletal sites. In 229 young adults with a past history of JIA, persistently low BMD was observed at the femoral neck and on total body [11]. In another study, 41% of adults with a history of JIA had osteopenia [12].

Therapy with glucocorticoids can prevent the acquisition of an optimal peak bone mass in young patients. The lower

peak of bone mass is associated with the increased risk of osteoporosis and the increased risk of fracture in the adult age [13–15].

Drug therapy with glucocorticoids may increase the risk of developing osteopenia and osteoporosis. Vertebral collapse is more common in children receiving a cumulative dose of at least 5 g of prednisone equivalents, prolonged periods of bed rest, and with low BMD and low serum concentrations of 25-hydroxyvitamin D [13, 16]. In a study in 103 patients with JIA, 23% had at least one fracture in the presence of growth failure, articular erosions and high cumulative dose of glucocorticoids; 56% of these fractures were vertebral [17] (Figure 1).

3. Fractures and Low Lean Mass

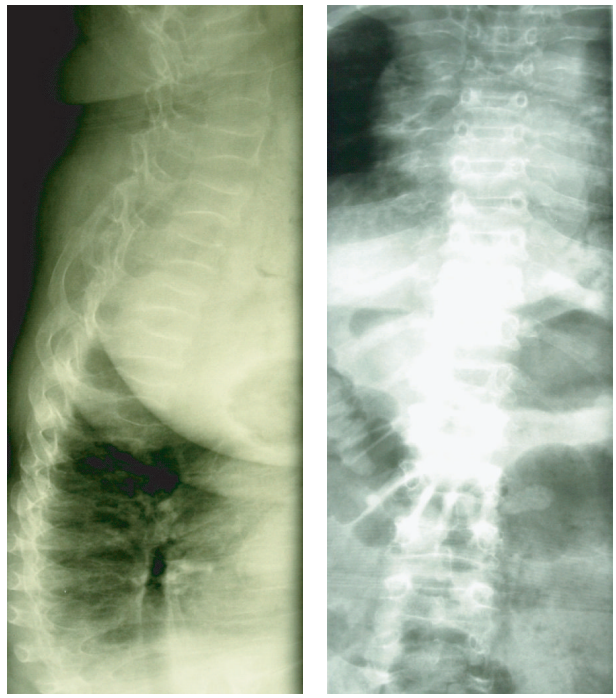
The increased risk of fracture has been proven especially in patients with erosive arthritis, growth retardation and high cumulative dose of glucocorticoids [2, 3].

Myopathy caused by the autoimmune inflammation is one of reasons of the low lean mass in patients with JIA. Proinflammatory cytokines (especially TNF-α) stimulate protein degradation, inhibit myocyte differentiation, and cause myocyte apoptosis [18, 19], as demonstrated by the muscle biopsies [20]. Glucocorticoid myopathy can also induce a low lean mass [21]. Patients with JIA are less physically active than healthy population, their physical condition is impaired, and risk of fracture is increased [22, 23].

In patients with JIA, there is an important relationship between risk of fracture, bone mineral density and quantity of lean mass. The lean mass corelates with BMD at the various skeletal sites. Bone status in children with JIA (and also in healthy children) markedly depends on the muscle force affecting the skeleton [4, 18]. Reduction of whole body lean mass and higher accrual of fat mass was established at the early phase of the disease [8]. The increased risk of forearm fracture was demonstrated also in healthy children and adolescents with low whole body lean mass and high fat mass [24]. BMD at the cortical and trabecular bone of the forearm and bone and lean muscle geometry was measured in 57 children with JIA using the peripheral quantitative computed tomography (QCT) [18]. Patients with JIA had significantly reduced cross-sectional area of the lean muscle mass (CSA). This reduction notably corelated with lean mucle force and bone geometry abnormalities and with significant reduction of the cortical bone thickness. All of these can be associated with increased risk of fracture. The peripheral QCT was also used to demonstrate a reduced calf lean muscle mass and tibial trabecular BMD and cortical thickness in patients with JIA [25].

4. Growth Retardation

The important difference between JIA and rheumatoid arthritis in adults lies in growth retardation of children with JIA, deceleration of growth rate, low stature in adult age and local growth retardation at sites of joints involved in arthritis [2, 3]. A significantly low stature (the final height below −2 standard deviation, SD) was demonstrated



(a) (b)

Figure 1: Multiple compressive vertebral fractures and rib fractures in a 22-year-old women diagnosed with JIA at 3 years of age. Bone densitometry (DXA, GE Prodigy): total femur BMD of 0,453 g/cm^2, T-score −4,5; femur neck BMD of 0,536 g/cm^2, T-score −4,2.

in 11% of children with JIA, and in 41% children with systemic form of disease [26]. Sources of growth retardation in JIA are multifactorial; chronic inflammation and long-term glucocorticoid treatment are the most relevant. The linear growth can be improved with remission of the disease.

The growth hormone (GH) and insulin-like growth factor I (IGF-I) are the most important regulators of postnatal growth. In children with JIA and serious growth retardation, the normal pulsative secretion of growth hormone, but low levels of IGF-I were described (resistance to growth hormone). Proinflammatory cytokines influence linear growth of children by their systemic effects and by local effects on growth cartilage of long bones [26]. Increased production of interleukin 6 (IL-6) and interleukin 1 beta (IL-1β) accelerates degradation of insulin-like growth factor I binding protein 3 (IGFBP-3) resulting in reduction of IGF-I levels and growth retardation. Chondrocyte apoptosis induced by the tumor necrosis factor α (TNFα) through the medium Fas-associated death domain (FADD) acts the important part in growth abnormalities [26]. Growth retardation caused by chronic inflammation and glucocorticoid treatment can be positively influenced by growth hormone treatment [27].

5. Biochemical Markers of Bone Turnover

Studies of bone formation and bone resorption markers are not unambiguous; however, most studies indicated prevalence of bone resorption over bone formation [5],

the others conversely indicate reduction of the bone formation [4, 6, 28]. Decreased bone formation in the course of adolescent growth spurt obstructs achievement of peak of bone mass and increase risk of fracture in the adult age [4, 13, 14]. Successful treatment of the disease is associated with elevation of bone formation markers [6].

In prepubertal children with active JIA, prevalence of bone resorption markers over reduced markers of bone formation corelated with laboratory indices of disease activity, namely in children with polyarticular phase of JIA [4, 6, 28, 29]. Except for the high disease activity, reduced concentrations of bone turnover markers and low BMD were found in patients with joint destruction and longer disease duration [29]. According to Pereira et al., bone formation in patients with JIA was suppressed from early to middle puberty, while at older patients with JIA, the main factor of bone loss was the elevation of bone resorption [30]. Thus, similarly to adults with rheumatoid arthritis, the proinflammatory cytokines (TNF-α, IL-1) produced by synovial membrane are responsible for excessive bone resorption also in adult patients with JIA [31].

6. Cellular and Molecular Mechanisms of Bone Remodeling in Patients with JIA

6.1. Influence of Proinflammatory Cytokines on Bone Remodeling. Proinflammatory cytokines, such as tumor necrosis factor α (TNFα), interleukin 1 (IL-1), interleukin 6 (IL-6) a interleukin 17 (IL-17), present in arthritic joint can cause an excessive osteoclastogenesis [32–35]. TNFα significantly elevates bone resorption [36] and attenuates osteoblastogenesis and bone formation [37–40]. IL-1 also accelerates osteoclast maturation [41]. In patients with rheumatoid arthritis, treatment with TNFα antibodies demonstrably reduces joint erosions [32]. IL-17, which is produced by T-lymfocytes [34, 42, 43] induces osteoclast differentiation by increasing expression of RANKL and RANK. On the contrary, IL-17 suppresses expression of osteoprotegerin in osteoblasts resulting in prevalence of bone resorption over bone formation and bone loss [6, 34, 44–46].

6.2. RANKL/RANK/OPG Triad in Patients with JIA. Receptor activator nuclear factor kappa B (RANK) and its ligand (ŔANKL) have essential importance for osteoclastogenesis and osteoclast function [47–50]. Osteoprotegerin (OPG) is a soluble decoy receptor for RANKL produced by osteoblasts. OPG binding to RANKL prevents RANKL activation of RANK and thus activation of the osteoclastogenesis [48, 51]. High RANKL/OPG ratio results in prevalence of bone resorption over bone formation [52].

Increased production of RANKL in synovial fluid and increased concentrations of RANKL in serum are found in adults with rheumatoid arthritis [53]. An increased RANKL/OPG ratio was observed in children with juvenile dermatomyositis, and in patients with juvenile idiopathic arthritis [54, 55]. In the later study in patients with JIA, increased serum OPG concentrations were not sufficient to compensate for increased levels of RANKL [54, 55]. Serum concentrations of RANKL were increased in all the forms of JIA [55]. An increased RANKL/OPG ratio was also found in a subset of 30 girls with polyarticular course of highly active JIA and joint erosions [56], and in synovia in patients with polyarticular and enthesitis-related forms of JIA [57].

6.3. Wingless Proteins (Wnt) Signalling Pathway. The extent of the bone involvement in the rheumatic inflammatory processes depended on age and bone maturity [34]. In children with JIA, decreased osteoblastic formation and function may contribute to the bone loss [30, 34, 58, 59]. With this respect, the proinflammatory cytokines (especially TNFα), stimulate production of inhibitors of the Wnt proteins signalling pathway, especially sclerostin and Dickkopf 1 (DKK-1) and consequently inhibit osteoblast differentiation [60–63] (Figure 1).

The Wnt proteins are large family of factors that bind to cell-surface receptors of the Frizzled family, causing the receptors to activate Dishevelled family proteins and ultimately resulting in a change in the amount of β-catenin that reaches the nucleus and interacts with T-cell factor/lymhoid enhancer factor (TCF/LEF) family transcription factors to promote specific gene expression [64]. Decreased production of DKK-1, an inhibitor of the Wnt signaling pathway, is associated with reverse of osteoresorptive pattern in mouse model of rheumatoid arthritis to pattern of osteoarthritis with increased bone formation and osteophyte formation [61]. The DKK-1 blockade is associated with stimulation of OPG production by osteoblasts and consequent decrease in bone resorption [61, 64]. The importance of Wnt proteins for susceptibility to JIA was confirmed in the study of Wnt-1 inducible signaling pathway protein 3 (WISP3) polymorphism [65].

6.4. Matrix Metalloproteinases and Their Inhibitors in Patients with JIA. Matrix metalloproteinases (MMPs) are responsible for cartilage destruction and periarticular bone erosions in juvenile idiopathic arthritis [21, 55, 66–68]. MMP1/tissue inhibitor of metalloproteinases 1 (TIMP1) and MMP3/TIMP1 ratios are significantly higher in all the forms of JIA in comparison to healthy controls. These ratios significantly correlate with disease activity and could be efficacious biomarkers for monitoring of disease development [55].

7. Effects of Glucocorticoids, and Disease-Modifying Antirheumatic Drugs (DMARDs)

In children, a negative correlation between bone mass and cumulative dose of glucocorticoids was found [69–71]. During the childhood and adolescence, glucocorticoids can impair physiological process of bone mass accumulation and can cause deterioration of peak of bone mass resulting in increased risk of fracture in future life.

Methotrexate, which is the most frequent DMARDs in children, can cause osteopenia in children patients with malignancies, but low-dose methotrexate used in inflammatory diseases did not influence negatively the bone mass [72, 73].

8. Therapeutic Options to Improve Bone Status in JIA

8.1. TNFα Antibodies Treatments. Biological treatment with infliximab and etanercept in children with JIA is associated with decrease in disease activity [74–76]. The positive influence of treatment with TNFα antibodies was also documented upon the skeleton. Simonini was the first to demostrate increased bone mass after 1-year etanercept treatment in children with JIA; reduction of bone loss was associated with therapeutic response with decreased disease activity [75]. Etanercept also improves the linear growth in children with JIA [76].

8.2. Antiresorption Treatments. Aminobisphosphonates represent an effective option in patients with documented prevalence of bone resorption over bone formation [77]. In JIA, calcium and vitamin D, calcitonin, and aminobisphosphonates have been studied [78–80]. However, studies treated small numbers of patients with different characteristics of JIA, and controlled studies on both preventive strategies and treatment in augmenting bone mass and reducing the fracture risk are still lacking.

8.3. Bone Anabolic Treatments. Growth hormone was effective in stimulating collagen production and improving liner growth in JIA [81–83]. However, long-term controlled studies are needed to determine impact on bone mass and bone turnover and the risks of growth hormone therapy [84].

In adult patients with closed linear growth and severe osteoporosis, an intermittent administration of PTH 1–34 (teriparatide) or PTH 1–84 represents an effective option to restore bone structure that has previously been lost [85].

9. Conclusion

Well-timed and efficient treatment of JIA in children and adolescents can improve bone status. However, patients who suffered from JIA during childhood and adolescence may attain decreased bone mass and have an increased risk of fragility fractures. It is important to identify the subjects with an increased risk of fracture as early as possible. In adult patients with closed linear growth, it is necessary not only to reduce bone resorption, but also to support formation of new healthy bone mass. Several new therapeutical procedures are under investigation.

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Disclosure Policy

The authors declare no conflict of interests.

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Review Article

A Roadmap to the Brittle Bones of Cystic Fibrosis

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Cystic fibrosis (CF) is an autosomal recessive disorder which despite advances in medical care continues to be a life-limiting and often fatal disease. With increase in life expectancy of the CF population, bone disease has emerged as a common complication. Unlike the osteoporosis seen in postmenopausal population, bone disease in CF begins at a young age and is associated with significant morbidity due to fractures, kyphosis, increased pain, and decreased lung function. The maintenance of bone health is essential for the CF population during their lives to prevent pain and fractures but also as they approach lung transplantation since severe bone disease can lead to exclusion from lung transplantation. Early recognition, prevention, and treatment are key to maintaining optimal bone health in CF patients and often require a multidisciplinary approach. This article will review the pathophysiology, current clinical practice guidelines, and potential future therapies for treating CF-related bone disease.

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder caused by defects in the cystic fibrosis transmembrane conductance regulator protein (CFTR), a chloride channel found in the epithelial tissues in the lungs, sinuses, pancreas, skin, and gastrointestinal tract. CF most commonly affects Caucasians and occurs with a frequency of 1 in 2000 to 3000 live births in the United States each year [1]. The defects in CFTR leads to alterations in the sodium, chloride, and water transport in the epithelial cells and in turn to changes in the viscosity and hydration of the fluids overlying the epithelial cells. The change in the fluid composition is partially responsible for several of the complications associated with the progression of CF such as chronic respiratory infections, pancreatic duct obstruction, pancreatic insufficiency, biliary obstruction, cirrhosis as well as distal intestinal obstruction syndrome. In addition to expression of CFTR in a variety of epithelial cells, its expression has been found in osteoblasts but its precise role in these cells remains to be elucidated [2, 3]. The respiratory disease is hallmarked by bronchiectasis caused by cycles of infection, inflammation, and destruction of the airways. Airway clearance and aerosolized therapies have been a staple in the care of the CF patients for the past several decades and have lead to an improvement in the lifespan of the patients leading some to consider it now a life-limiting instead of a fatal disease. However, CF continues to lead to premature respiratory failure from repeated exacerbations, chronic infection by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Burkholderia cepacia*, or other pathogenic organisms.

The rapid advancements in medical therapy and patient care discussed above have increased the median predicted survival age for patients with CF to 37.4 years (http://www.cff.org/). In the coming years, the number of patients with CF who are over the age of 18 will surpass those who are in the pediatric age group; currently approximately forty-six percent of the CF population in the United States is over 18 years of age. As a consequence, the various disease-related complications that were only seen in a subset of the CF population are now being seen in a larger number of patients, such as osteoporosis and osteopenia. The secondary bone loss seen in the CF population is multifactorial, however can cause significant morbidity in the adult population.

2. Bone Disease in CF

Bone disease in patients with cystic fibrosis was first described over 3 decades ago and is characterized by decreased mineral density, increased fracture rates, and kyphosis [4, 5]. Unlike bone loss seen in postmenopausal women, bone loss in the CF population begins at a young age and continues as the patient ages. The prevalence of bone disease in the CF population increases with age and has been correlated with severity of lung disease [6–11]. However, one is more likely to see an adult CF patient with low bone mineral density than with normal bone mineral density even with normal lung function.

A recent meta-analysis by Paccou et al. reported that the prevalence of osteoporosis and osteopenia in young adults with CF was 23.5% and 38%, respectively [12]. Multiple cross-sectional studies have demonstrated an increased incidence of fractures in individuals with CF with vertebral fractures being the most common followed by rib fractures [4, 5, 13–15]. Another common skeletal problem seen in the CF population seen as early as the third decade of life is kyphosis [13, 16, 17]. Some studies have noted it to be between 10%–40% CF patients [16, 17]. The development of bone disease in the CF population can lead to significant issues with loss of lung function, deformities, and also increased pain issues. One can imagine that a vertebral or rib fracture would make it difficult to maintain a regimen of airway clearance that is needed for the prevention of CF exacerbations and the maintenance of lung function.

Although most of the pathologic consequences occur during adulthood in the CF population, there have been several studies that indicate that during childhood and puberty the CF patients achieve approximately half of the bone density than their healthy counterparts [18–22]. Puberty is especially important for the development of bone density and is a time where there is both peak growth velocity and bone density accrual. The reduction in the bone formation in the CF patients may be due to a combination of their delay in puberty, chronic infections, and hormonal imbalances. Numerous cross-sectional and longitudinal studies suggest that children and adolescents with cystic fibrosis fail to achieve adequate bone mass as compared to healthy controls during their pubertal growth spurt [18–20, 22]. To compound this decrease in bone density in puberty and adolescence, clinical studies show annualized rates of loss in BMD in the CF patients approach those seen in postmenopausal women [23–25]. Lower baseline bone density coupled with increased losses puts CF individuals at higher risk of low BMD.

The maintenance of bone health is also essential for the CF population as they approach the time for lung transplantation. Severe bone disease can lead to exclusion from lung transplantation, which is often a life-saving treatment for many CF patients. The most effective strategies have been found to be early recognition, prevention, and treatment in this population. This article will discuss the pathogenesis, the current guidelines, and potential therapies for the CF population.

3. Pathogenesis

3.1. Histologically. Three of the studies that have looked at bone histomorphometric data in CF adults with low BMD have shown a decrease in cortical as well as trabecular bone volume and a decrease in connectivity [26–28]. At a cellular level, there is a disruption in the delicate balance between osteoblast and osteoclast activities. The osteoblastic activity is decreased in the bones of the CF patients due to decreased osteoblast number and their biosynthetic potential that is compounded by osteoclast activity which is increased, primarily through increased osteoclast number. The rise in bone resorption when compared to new bone formation leads to low bone density in this population even in patients who are clinically stable [26, 28]. The exact cause of the disruption in this balance has not been completely been elucidated; however, there are studies to suggest that mutations in CFTR itself may play a role in a portion of the CF patients. Interestingly, even though vitamin D deficiency is very common in individuals with CF and is an important etiologic factor for the low BMD observed in this condition, osteomalacia is usually not a feature of CF-related bone disease [28].

4. Possible Role of CFTR

There are three observations that indicate that CFTR has a direct association with the loss in bone density in the CF patients. First, CFTR has been shown to be expressed in human osteoblasts, osteocytes, and osteoclasts [3]. Second, CF patients with at least one ΔF508 allele had significantly lower Z-scores than those with other genotypes, which suggests a direct association between CF-related bone disease and the ΔF508 mutation [29]. Finally, experiments in CFTR-deficient mice demonstrated a decreased BMD with more cortical bone thinning and altered trabecular architecture as compared to control mice despite lacking other overt manifestations of CF (e.g., lung disease and pancreatic insufficiency) [30]. Together these findings suggest that loss of CFTR function may adversely affect bone density. However, the exact role of CFTR, specifically the ΔF508 allele in CF-related bone disease, is yet unclear.

In addition, the loss or reduction in CFTR activity has been shown to lead to chronic inflammation through a decrease in osteoprotegerin and a concomitant increase in prostaglandin E2 [2]. The alteration of osteoprotegerin and prostaglandin E2 increases the inflammation-driven bone resorption [2]. This would further indicate that there is a direct association between CFTR and the BMD in the CF patients. These studies have increased the interest in the possible role of CFTR gene in bone development and pathophysiological processes. Once again however, it is not known whether CFTR has a direct or indirect effect on bone formation.

5. Factors Influencing Bone Loss

The development of bone disease in the CF patients is thought also to be secondary to their chronic illness and is

Table 1: Causes of decreased bone density in cystic fibrosis.

(i) Pancreatic insufficiency
(ii) Malnutrition and poor growth
(iii) Vitamin D, vitamin K, and calcium insufficiency
(iv) CF-related diabetes
(v) Glucocorticoids
(vi) Sex steroid deficiency and delayed puberty
(vii) Chronic inflammation
(viii) Tobacco, alcohol, and caffeine use
(ix) Moderate to severe lung disease
(x) Lack of exercise, especially weight bearing
(xi) Organ transplant and immunosuppressive therapy
(xii) Medications (Depo-Provera, Megestrol Acetate, and Aluminium containing-antiacids)
(xiii) Possible role of CFTR.

influenced by multiple factors. It is essential to remember that nutrition, lung disease, and bone health are all related in this population. (Table 1).

6. Malnutrition and Pancreatic Insufficiency

Optimum nutrition is vital not only for pulmonary health but also for bone health [17, 31–33]. Malnutrition seen in the adult CF patients is contributed by pancreatic insufficiency, increased catabolism due to chronic infections, and body images issues which afflicts many of the CF patients. A large portion of CF patients are pancreatic insufficient which leads to inadequate secretion of pancreatic enzymes and malabsorption of fat soluble vitamins (A, D, E, and K), calcium, and other macronutrients which play a key role in bone formation [34]. Despite pancreatic enzyme replacement therapy, the majority of CF patients have body mass index that are in the low normal or malnutrition range. A great deal of energy is spent on dietary education and nutritional support in an attempt to improve the body mass index of the CF population. Low body mass index has been linked to low bone mineral density, especially in the adolescent and young adults [35, 36].

6.1. Vitamin Deficiencies. One vitamin deficiency which is prevalent in the CF population is Vitamin D deficiency, regardless of season or latitude [37–40]. In addition to pancreatic insufficiency and malabsorption described above, multiple other factors such as inactivity and decreased sunlight exposure, low body fat (reduced Vitamin D stores), reduced 25 hydroxylation, increased metabolic degradation, and decreased vitamin D binding protein have all been implicated as potential etiologies for the low vitamin D reserves.

In non-CF individuals, Vitamin D deficiency in children results in failure to achieve optimal height and peak bone mass or even overt rickets while in adults it is associated with impaired bone density due to decreased calcium absorption and hyperparathyroidism as well as osteomalacia. Although Vitamin D deficiency is commonly seen in CF population, osteomalacia in not a feature of CF-related bone disease, suggesting that other etiologies play an equally important role in pathogenesis [28].

Even with replacement therapy, vitamin K is known to be depleted in up to 40% of the CF patients. The significance of vitamin K to bone health has been linked to osteocalcin. A depletion of vitamin K has been associated with higher levels of undercarboxylated osteocalcin. Several studies have looked beneficial role of vitamin K supplementation specifically on the increased carboxylation of osteocalcin and improvement in bone formation [38, 41–44].

7. Cystic Fibrosis-Related Diabetes

Patients with CF can develop impaired glucose tolerance (IGT) or even frank diabetes due to the progressive pancreatic damage caused by defective acinar and ductular secretions. This type of pancreatic damage may not be similar to classic Type I or Type II diabetes; however, the resultant abnormalities in glucose and insulin regulation can affect bone health [45–47]. Just as in patients without CF, cystic fibrosis-related diabetes and IGT may play an important role in the development of bone loss [45].

8. Gonadal Steroid Deficiency

Puberty is a crucial period in which peak bone mass accrual occurs. CF individuals are frequently noted to have delayed puberty and decreased overall bone mineral accretion. Adolescents with CF have lower sex hormone levels as compared to healthy age matched controls, but most have normal values when adjusted for Tanner stage [48, 49]. Hypogonadism can cause accelerated bone loss. The lower peak bone mass combined with increased bone losses may contribute to bone disease, but observational studies have not observed a consistent association [48, 49].

9. Physical Inactivity

Just as with some of the general population, the CF population is not as physically active as they should be. Although there are limitations with many of the patients with severe lung disease and there is social stigmata attached to the chronic cough that the CF patients have especially when exercising, any reduction in activity or weight bearing activity in this population has negative impact on their BMD. The CF patients are often burdened with a significant treatment burden and in the adult population frequent pulmonary or sinus exacerbation. For all of these reasons, there is a decrease in total activity hours and weight-bearing activities in this population even in light of the fact that it

has been shown not only to improve bone health but also pulmonary health [32, 50–53].

10. Recurrent Infections

As stated previously, there is an association between the severity of lung disease and CF-related bone disease [5–10]. During acute exacerbations of lung disease, markers of bone turnover tend to rise and their levels drop after appropriate treatment with antibiotics, chest physical therapy, improved nutrition, and other supportive measures [54]. Systemic hormones, inflammatory cytokines, and localized growth factors can all affect bone remodeling [55]. Elevated levels of cytokines and growth factors like tumor necrosis factor-α (TNF-α), vascular endothelial growth factor (VEGF) and IL-1, IL-6 and IL-11 found in the serum and respiratory tract of CF patients may stimulate osteoclast-mediated bone resorption and inhibit bone formation [56].

11. Glucocorticoids

It is well established that glucocorticoids negatively impact bone health. Their primary effect on bone is to suppress formation of osteoblasts and promote apoptosis of osteoclasts and osteocytes. Systemic glucocorticoids can cause a rapid bone loss within the first few months of treatment, followed by a slower 2%–5% loss per year with chronic use [57]. CF patients with symptoms and signs of asthma or allergic bronchopulmonary aspergillosis are often treated with systemic or oral steroids to improve lung function. In most studies, the dose and duration of systemic steroid use impacted the decreased bone mineral density [58–62]. The use of inhaled corticosteroids, although associated with short term changes in markers of bone turnover, has not been shown to affect BMD or fracture risk, independent of severity of underlying lung disease [58, 62].

12. Lung Transplantation and Immunosuppressive Therapy

Lung transplantation may be potentially lifesaving in CF patients with end stage lung disease. Patients awaiting lung transplants almost always have a low BMD due to factors discussed above [63, 64]. Immediately following the transplant, a rapid decline in BMD is noted and may be attributed to limited mobility, chronic steroid use, and use of other immunosuppressive agents [65–70]. This decreased BMD is associated with an increased fracture risk. Though BMD may stabilize or even increase a year or more after transplant, the fracture risk appears unchanged [65].

13. Guidelines to Improve Bone Health in CF

The clinical practice guidelines were outlined by the CF foundation committee for optimizing bone health in CF patients in The Journal of Clinical Endocrinology & Metabolism in 2005 and are scheduled to be revised within the next year [13].

13.1. Screening for Bone Disease. The CF foundation bone health committee recommended that all adults with CF should have a baseline BMD at age 18 years. Children age 8 years or greater with other risk factors (i.e., ideal body weight <90%, FEV1 <50% predicted, glucocorticoids 5 mg/day or more for 90 days/year or longer, delayed puberty, or previous history of fractures) should also be screened.

The committee cautioned that while interpreting DEXA scan results, Z-scores should be used up to age 18, Z- or T-scores are nearly equivalent between ages 18 and 30, while T-scores should be used above the age of 30. The recommendations are summarized in Table 2.

13.2. Preventive Strategies. The preservation of optimal bone health in CF requires a multisystemic approach. Good nutrition is critical in maintaining lean body mass and overall bone health. Vitamin and macronutrient deficiencies should be adequately repleted, and a BMI >50% percentile should be targeted. Pulmonary infections should be treated aggressively, and every effort should be made to maintain adequate glycemic control and to minimize the use of steroids whenever possible. Weight bearing exercises are well known to increase BMD in healthy subjects and are highly recommended in preventing bone loss in CF individuals.

14. Therapeutic Considerations

14.1. Vitamin D Supplementation. As discussed previously, Vitamin D insufficiency is common among CF patients and is associated with bone disease. An optimum level has not been established but based on observations in non-CF patients that parathyroid hormone levels start to increase when 25OH Vit D levels fall below 30 ng/mL, the CF foundation committee's consensus guidelines recommend a target 25OH Vit D level of 30 ng/mL or 75 nmol/L. It should also be noted that the recommended daily allowance of Vit D for non-CF individuals will not be adequate to maintain target levels in CF patients. Various possible replacement strategies have been suggested, but the optimal Vitamin D analog and dose (D2, D3, calcifediol, calcitriol, and UV therapy) remains to be determined.

14.2. Calcium Supplementation. Calcium is an important component of bone infrastructure and is essential to the maintenance of skeletal health. Schulze et al. showed that increased calcium absorption in young women with CF was associated with increased rates of bone calcium deposition [34]. Since there are no long-term randomized controlled trials in CF patients, appropriate supplemental doses are not defined and national dietary reference intakes should be followed. The CF foundation bone health consensus committee recommends supplementation with 1300 mg to 1500 mg of elemental calcium daily.

14.3. Vitamin K Supplementation. Vitamin K is necessary for posttranslational activation of osteocalcin, which in turn is important for bone formation and mineralization [42, 43].

Table 2: Cystic fibrosis foundation guidelines for treatment of osteoporosis.

	General Recommendations		
Vitamin D goal	25OH Vit D level >75 nmol/L or 30 ng/mL		
Calcium	1300–1500mg/day		
Vitamin K	0.3–0.5 mg/day		
Target BMI	>50th percentile BMI adult females 23 BMI adult males 22		
Exercise	Encourage outdoor and weight bearing exercise		
Infections	Aggressive treatment of pulmonary infections		
	Specific Recommendations by T/Z Score		
	T/Z score ≤ -2	**$-1 \leq$ T/Z score ≤ -2**	**T/Z scores ≥ -1.0**
Repeat DEXA	Annual	Repeated every 2–4 years	Repeated every 5 years
Steroid	Minimize steroid doses		No recommendation
Endocrine	Recognize and treat CF related diabetes, delayed puberty, hypogonadism, and consider endocrine consult		No recommendation
Bisphosphonate	Start bisphosphonate	If fragility fracture, patient awaiting transplant or accelerated BMD loss >3-5% per year start bisphosphonate	No recommendation

Clinical practice guidelines recommend supplementation with 0.3 to 0.5 mg daily.

14.4. Gonadal Steroid Replacement Therapy. While adolescents with CF have lower sex hormone levels as compared to healthy age matched controls, most have normal values when adjusted for Tanner stage [48, 49]. The risk/benefit ratio of sex steroid replacement has not been adequately studied. Gonadal hormone replacement is controversial and should be limited only to those patients who have persistently low levels [13, 71]. Delayed puberty should be addressed by an experienced endocrinologist.

14.5. Antiresorptive Agents. Bisphosphonates are a class of drugs that chemically bind to calcium hydroxyl-apatite in bone and inhibit bone resorption through their inhibitory action on osteoclasts function and survival. Observational and randomized controlled trials (RCTs) on adults with CF have shown significant improvement in BMD with IV pamidronate and IV zoledronic acid as well as PO alendronate, but trials of these drugs in children with CF have not been performed [23–25, 65, 72, 73]. Oral agents are currently considered the first-line therapy for CF-related bone disease. The IV agents are frequently associated with side effects like fever, severe body aches, and bone pains [25, 65, 72], but concomitant use of steroids, acetaminophen, and/or nonsteroidals may help reduce this pain and fever syndrome.

The current guidelines strongly recommend consideration of bisphosphonate therapy in all CF patients with T/Z-scores less than -2.0 and also in patients with T/Z score between -1.0 and -2.0, with a history of fragility fractures, those awaiting lung transplant, or who experience a BMD loss of >3%–5% per year. It is essential that when a bisphosphonate is used that the Vitamin D and calcium are adequately replaced prior to and during therapy.

14.6. Recombinant Human Growth Hormone. Children with CF are noted to have poor linear growth and inadequate weight gain as well as low levels of IGF-1. The use of growth hormone in children with CF has been shown to have a beneficial effect on linear growth and weight gain. There is evidence to suggest that treatment with recombinant human growth hormone results in improvement in clinical status with decreased hospitalizations and courses of intravenous antibiotics, improvement in exercise tolerance, and bone accumulation [74–79]. Human recombinant growth hormone has not been studied as a treatment for low BMD in adults with cystic fibrosis [74, 75, 77, 78, 80].

14.7. Teriparatide. Human parathyroid hormone has been shown to have both anabolic and catabolic effects on bone, depending on the dose and duration of use. While continuous exposure to high doses leads to bone resorption, daily low dose injections may actually increase osteoblast formation and bone growth [81–84]. Teriparatide (PTH 1–34) is the only anabolic agent available in the US for use in patients with advanced osteoporosis at high risk for fractures. Recombinant human (PTH 1–84) is available in Europe. It is contraindicated in children with open epiphyses but holds promise for CF adults with severe osteoporosis or prior history of fractures. There are currently no published studies

of teriparatide in CF; however, this maybe a therapeutic option that should be considered and studied in the future for the adult population.

14.8. Newer Agents. Over the past few decades, researchers have made significant strides in the field of osteoporosis, which has led to an improved understanding of the regulation of bone remodeling. Exciting new treatment strategies have emerged and may potentially broaden our options for treatment of CF-related bone disease. Several of the therapies discussed are not clinically available currently but are under investigation and show promise to improve the bone health of the CF patients in the future.

14.8.1. Antiresorptive Agents. Denosumab, a human monoclonal antibody to RANKL (receptor activator of nuclear factor kappaB ligand), was recently approved by FDA for treatment of osteoporosis in postmenopausal women. Denosumab inhibits the maturation of osteoclasts by binding to RANKL [85]. The inhibition of osteoclast formation, function, and survival are responsible for decrease in osteoclast-mediated bone resorption. The improvement in lumbar and hip BMD seen with denosumab is at least comparable to bisphosphonates, if not superior. Denosumab also improves distal radius (cortical) bone density, an effect not seen with bisphosphonates [85]. Side effects noted with bisphosphonates such as delayed fracture healing, osteonecrosis of jaw, and femoral shaft fractures have not been observed in denosumab-treated individuals to date but further trials would be needed to assess the long-term effects of this medication [85]. The beneficial effect on both trabecular as well as cortical bone, combined with fewer side effects and the prospect of improved patient compliance due to twice-yearly dosing makes this an attractive consideration in CF-related bone disease.

Cathepsins are a family of proteases with collagen as their main target. Cathepsin K (CAT k) is unique to osteoclasts and plays a role in degradation of bone matrix proteins, including collagen. CATk inhibitors have an antiresorptive effect, with lesser inhibition of bone formation than bisphosphonates. CATk inhibitors have been shown in animal studies to increase cortical thickness and periosteal bone formation and are currently undergoing phase 3 trials. They may have the potential to improve long bone strength and prevent nonvertebral fractures [86, 87].

14.8.2. Anabolic Agents

PTH ligands and PTHrP. Antiresorptive agents can only increase bone mass but anabolic agents can also improve bone quality and strength, in addition to increasing bone mass. Currently, PTH [1–34] is the only approved anabolic agent in the US, and its duration of use is limited for 2 years. There is thus an unmet need for development of more anabolic agents.

Researchers have looked at various PTH ligands (PTH 1–31, PTH 1–28), which appear to have a more potent anabolic effect than the hormone itself (PTH 1–34) [88, 89]. Parathyroid hormone-related protein (PTHrP) is a hormone produced by mature osteoblasts and is structurally similar to PTH. Unlike PTH, which has both anabolic and catabolic effects on bone, PTHrP appears to be purely anabolic when administered intermittently. Studies in animals have shown an increase in trabecular bone volume, osteoblast number, bone mineralization rates, biomechanical strength, and BMD [90]. Human studies show increase in BMD similar to that with PTH, but without significant hypercalcemia or other adverse effects as observed with PTH. There is no activation of bone resorption at therapeutically effective doses [91–93].

14.8.3. Calcilytic Agents. Allosteric modulations of the G-protein coupled calcium-sensing receptor in the parathyroid gland can affect PTH release. Positive allosteric modulators decrease PTH production, while negative modulators increase it. Calcilytics or calcium sensing channel antagonists are negative modulators, which when administered orally, can cause a pulsatile increase in PTH production. Animal studies look promising; unfortunately, a human trial of ronacaleret was discontinued due to a poor effect on BMD. Another agent is currently in phase 2 trials [94].

14.8.4. Modulation of the Wnt Signaling Pathway

Antisclerostin Antibody. A large family of extracellular glycoproteins, called Wnt proteins, are key regulators of bone remodeling and other cellular processes [95–97]. Sclerosteosis, an autosomal recessive disorder characterized by increased bone mass, mainly in the skull and in long bones, results from a mutation in the *SOST* gene, which codes for sclerostin [98]. A deficiency of sclerostin results in increased Wnt signaling and high bone mass. Transgenic mice that overexpress sclerostin are seen to have low bone mass and increased susceptibility to fractures [99] while mice deficient in sclerostin show increased bone density [100]. Treatment with sclerostin antibody has been associated with an increase in bone mass and strength in various animal models [101–103]. In a phase I trial in 72 healthy men and postmenopausal women, a single subcutaneous dose of sclerostin antibody was associated with a statistically significant increase in the levels of bone formation markers propeptide of type I procollagen (P1NP), osteocalcin and bone-specific alkaline phosphatase (BSAP), a dose-dependent decrease in bone resorption marker C-telopeptide, and a 5.3% increase in lumbar spine BMD [104]. Antisclerostin antibody seems promising as a potentially effective anabolic agent for the treatment of low bone mass in individuals with CF.

Anti-Dkk1 Antibody. Dickoppf1 (DKK1) is a naturally occurring Wnt pathway antagonist which works by preventing the interaction between two key Wnt pathway coreceptors, LRP5/6 and the frizzled Wnt pathway receptor, which results in inhibition of Wnt signal transduction and impaired bone formation [105]. DKK1 inhibition increases trabecular bone volume and bone formation in rats [106, 107]. Antibodies to dickkopf-1 could be used as an anabolic agent for the treatment of patients with low bone mass.

Activin Inhibitors. Activin is a member of the bone morphogenic protein (BMP)/transforming growth factor (TGF)β superfamily of polypeptides and stimulates the release of FSH by the pituitary gland [108]. In bone, activin binds to activin receptor IIA, increases osteoclastogenesis, and is a negative regulator of bone mass. Its effects on bone formation are more complex and unclear [109].

ACE-011, a protein formed by fusing soluble activin receptor type II to IgG-Fc, was shown to decreases bone resorption in ovariectomized mice and enhance bone formation in intact animals [110]. Data in cynomolgus monkeys shows a significantly higher BMD and trabecular bone volume after injection of ACE-011 [111]. In phase I trials, the administration of a single dose to 48 postmenopausal women resulted in an increase in levels of bone-specific alkaline phosphatase and a decrease in C-telopeptides [112]. Activin inhibitors hold promise as new anabolic therapy for patients with low bone mass and increased susceptibility for fractures.

15. Conclusion

CF-related bone disease is a common complication in the aging CF population. It is multifactorial in origin, affects young individuals, and is associated with significant morbidity. Despite increasing research in the field, numerous questions about pathogenesis and appropriate therapy still remain. A multisystemic, multidisciplinary approach is required to tackle bone disease in the CF population. Various therapies are currently available for treatment of postmenopausal osteoporosis but these have not been studied in individuals with CF. Clinical trial data is especially limited in the pediatric age group. The new Cystic Fibrosis Foundation guidelines for the treatment of bone disease are expected to be published in the next year. Exciting new therapies are currently under development, and investigation and hold promise for reducing the burden of secondary bone disease in individuals with CF.

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