Bone in women with premature ovarian insufficiency: a review

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ABSTRACT

Premature ovarian insufficiency (POI) is a condition characterized by amenorrhea occurring before the age of 40, accompanied by elevated FSH levels measured on at least two occasions, with a minimum interval of 4 weeks. While idiopathic cases are prevalent, autoimmune or iatrogenic factors may also contribute to the condition. POI presents clinically as menopausal symptoms and failure of fertility, often resulting from hypoestrogenism, which adversely affects bone health. Assessing peak bone mass is crucial in order to evaluate fracture and osteoporosis risk, as it is determined by genetics, and also lifestyle factors such as nutrition and physical activity exerted during childhood and adolescence. In adult women, maintaining hormonal balance is vital for optimal bone health. Sex hormones exert their effects on bone tissue through various receptors, with estrogens demonstrating a protective role by preserving adequate bone mineral density. Hormonal replacement therapy plays a pivotal role in the management of POI by maintaining bone mass and preventing osteoporosis and other complications arising from hypoestrogenism. Extensive research has evaluated different treatment schemes and administration routes to determine the most effective approach. Additionally, recent studies have highlighted the impact of other hormones on bone health, including pituitary hormones (TSH, FSH, GH), incretins, and hunger hormones. This review explores the pathways through which these hormones may influence bone, highlighting their potential contribution for the development of novel therapeutic options for osteoporosis treatment.

KEYWORDS

Premature ovarian insufficiency, bone, osteoporosis, POI, bone mineralization, peak bone mass, hormone replacement therapy.

1. Premature ovarian insufficiency: diagnosis, characteristics, and clinical features

Premature ovarian insufficiency (POI) presents a complex challenge for both clinicians and patients. This condition is characterized by the absence of menstruation before the age of 40 [1]. The ovarian function gradually decreases, leading to its eventual cessation, which manifests in both morphological changes in the ovaries and various clinical symptoms experienced by affected individuals. Globally, POI affects approximately 1% of women, with the incidence varying across different age groups. The likelihood of POI is lower in younger patients, with only 0.1% of healthy women aged 30 experiencing this condition [2]. While most cases of POI are idiopathic, there are several potential causes, including genetic, metabolic, infectious, and autoimmune factors.

Genetic abnormalities, such as trisomy X (47,XXX), partial or total X chromosome deletions, and Turner syndrome, are commonly associated with POI. Furthermore, it has been observed that 10-30% of first-degree relatives of women diagnosed with idiopathic POI also develop the condition, providing strong evidence for a genetic link [3]. Non-genetic causes of POI include autoimmune disorders, accounting for an estimated 5-30% of cases. In these cases, the persistent presence of elevated inflammation mediators in the patient's system disrupts ovarian function. The primary target of autoimmunity-mediated aggression is steroidogenic cells, which leads to an imbalance in hormone levels and therefore also ovarian homeostasis [3]. Common autoimmune diseases associated with POI include hypothyroidism, autoimmune adrenal insufficiency, autoimmune polyglandular syndrome, and autoimmune Addison's disease [3].

POI can be also classified as iatrogenic, which includes procedures such as extensive ovary surgeries (e.g. benign or endometriotic cystectomy) and chemotherapy [4,5]. The diagnosis of POI typically involves a comprehensive approach that encompasses the patient's medical history and laboratory tests.

When assessing a patient's medical history, indicators of POI include symptoms of ovarian dysfunction and hypoestrogenism. These symptoms consist of menstrual irregularities leading up to eventual amenorrhea as ovarian function declines, as well as vasomotor symptoms commonly experienced by menopausal individuals. Hot flushes, in particular, are a prevalent symptom reported by patients and can occur within 2 years prior to

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menstrual disturbances that meet the laboratory criteria for POI [5]. Additionally, patients often present with sleeplessness, headaches, and irritability. Impaired fertility and pregnancy failure are frequently reported by individuals with POI [4]. Vaginal dryness and dyspareunia may also be observed as possible symptoms of the condition. The combination of these symptoms significantly impairs patients' quality of life and mental well-being. The psychological distress and emotional trauma experienced by patients can lead to depression [5]. Laboratory testing plays a vital role in the diagnostic process. The criteria proposed by the European Society of Human Reproduction and Embryology (ESHRE) for diagnosing POI are as follows: oligo/amenorrhea for at least 4 months and an elevated FSH level exceeding 25 IU/L on two occasions, at least 4 weeks apart [6].

Although the ovarian reserve, indicated by the anti-Mullerian hormone (AMH), is typically low in individuals with POI, this is not sufficient for the diagnostic confirmation [6]. A combination of AMH levels below 0.5–1.0 ng/mL and antral follicle count (AFC) less than five to seven follicles is defined as poor ovarian response (POR) and is commonly observed in POI patients that undergo ovulation stimulation in attempts to achieve pregnancy [5].

Ultrasound examination may reveal certain ovarian abnormalities. Some patients exhibit small ovaries with a low follicle count, most of which demonstrate histological abnormalities. However, in some cases, the morphology of the ovary and follicle maturation appear normal [3]. Therefore, while ultrasound is a useful diagnostic tool, ultrasound findings cannot be considered as definitive criterion for the diagnosis of POI.

2. Definition and importance of peak bone mass and bone mass development

Peak bone mass (PBM) refers to the amount of bony tissue present at the end of skeletal maturation [7]. While the human skeleton is generally considered fully developed by the end of the second decade of life, the period of most significant increases in bone mass occurs around 12.5 ± 0.90 years in girls and 14.1 ± 0.95 years in boys of European ancestry [8].

The development of PBM results from the linear growth and consolidation of cortical and trabecular components. The acquisition of bone mass is particularly rapid during the later stages of puberty, coinciding with the peak secretion of growth hormone (GH), elevated levels of serum insulin-like growth factor 1 (IGF-1), and increasing levels of estradiol and testosterone [9].

PBM is a crucial parameter for assessing fracture risk. This is exemplified by the higher incidence of bone fractures in pre-pubertal children as the bone mass in this age group is lower compared to adolescents and adults who have reached PBM [8]. Similarly, in elderly individuals, the risk of fractures is elevated as bone density decreases over time [9]. PBM is also a key factor in the incidence of osteoporosis, a systemic skeletal disease characterized by decreased bone mass and gradual destruction of bone tissue, resulting in increased fragility and significantly increased fracture risk [10]. Studies have shown that a 10% increase in PBM can delay the onset of osteoporosis by 13 years [11].

3. Peak bone mass: factors influencing gain, including genetic determinants, physical activity, and nutrition

Several factors have been found to influence PBM, which can be categorized into genetic and non-genetic factors. The latter is primarily related to lifestyle choices and is estimated to impact adult PBM in approximately 20-40% [8]. PBM has shown a high heritability in familial and twin studies.

As PBM is primarily acquired during childhood and adolescence, genes that affect bone density during these periods play a crucial role in its development. Studies have identified the LRP5 and ESR1 genes to be associated with bone mineral density (BMD) [12,13]. Furthermore, the Avon Longitudinal Study of Parents and Children (ALSPAC) has linked the Sp7 transcription factor, receptor activator of nuclear factor-kappa B (RANK), and osteoprotegerin (OPG) to (BMD) in children [14].

Physical activity is a key non-pharmacological approach for optimizing and maintaining BMD. It plays a vital role in preserving PBM and strength. The beneficial effect of exercise on PBM depends on the type of physical activity performed. Research indicates that individuals engaged in high-impact or odd-impact sports tend to have higher BMD. Similarly, impact exercise, strength training, and brief high-impact jump training interventions have been shown to increase BMD in premenopausal women [15]. Regular physical activity, particularly during childhood and adolescence, has the potential to enhance PBM and reduce the risk fractures in adulthood [16].

Dietary habits are an important contributor to PBM in childhood and adolescence. Nutrients such as calcium, phosphorus, and protein play a crucial role in bone mass accumulation [17]. Vitamin D plays a vital role in regulating calcium metabolism in the body and maintaining stable calcium concentration in the extracellular fluid. It is thus essential for bone mass accumulation, contributing to bone homeostasis [18]. Adequate phosphate supply is necessary for the mineralization of cartilage and osteoid tissue [19]. Food rich in protein, such as dairy products, meat, or nuts, are excellent dietary sources of phosphorus. Moreover, a higher protein intake during growth has been positively associated with improved bone size, bone mass, and estimated bone strength [17].

While certain dietary habits can enhance PBM, there are also nutritional factors that can negatively impact its development. Substances such as tobacco and alcohol have been identified as impairing factors. Tobacco increases blood acidity and promotes bone dissolution [14]. Similarly, alcohol inhibits the growth of marrow mesenchymal stem cells and their transformation into osteoblasts [20]. Research has found that smoking during adolescence for an average of four years is significantly associated with decreased BMD [21].

4. The role of estrogen and androgen receptors in bone health

Bone growth and remodeling involves two types of cells: osteoblasts, responsible for depositing new bone matrix and its mineralization, and osteoclasts, which reabsorb mineralized bone tissue. Osteoblasts derive from the mesenchymal cell

differentiation pathway, while osteoclasts develop from hematopoietic precursor cells [22].

Sex hormones play a vital role in achieving PBM, growth accrual, and final height in both genders [23]. Androgens and estrogens stimulate endochondral bone development, but the epiphyseal growth plate closure is majorly mediated by estrogen receptors (ERs) at the end of puberty through aromatization of androgen to estrogen [24]. Estrogens and androgens exert their effects on osteoclasts, osteoblasts, and osteocytes by binding to the ERs α and β (also known as NR3A1 and NR3A2, respectively) and the androgen receptor (AR) (also known as NR3C4) [22].

The ER and AR consist of an amino-terminal domain (NTD), a DNA-binding domain (DBD), and a carboxy-terminal ligand-binding domain (LBD) [25]. Estrogens and androgens inhibit bone resorption in trabecular and endocortical bone surfaces by reducing the number of osteoclasts. This inhibition occurs by impeding the differentiation process of osteoclasts and promoting apoptotic mechanisms in these cells. In osteoclasts, the ER-α regulates the proapoptotic effect of estrogens, while estrogens also exhibit an antiapoptotic effect on osteoblasts, promoting bone tissue buildup [22]. Binding of estrogens or androgens to their corresponding receptors promote the transcription of target genes. This can occur through direct interactions of the receptor proteins with DNA or interactions with other transcription factors [26].

Androgens are known to stimulate longitudinal bone growth as well as radial bone growth, thereby increasing the cortical bone size. Testosterone shows an anabolic effect on the skeletal system via binding to the AR, and the conversion to 17-beta estradiol, which then binds to ERs. The activation of the AR and ER-α, but not ER-β, is associated with maintenance of the trabecular bone. The effects of ER-α activation preserved the thickness and number of trabeculae, while the AR preserved the number of trabeculae [24].

Estradiol (E2) is the dominant hormone that regulates bone homeostasis in women. It is essential for development of adolescent peak BMD and physiological levels prevent the rapid bone resorption, and thus preventing BMD loss usually observed in most adults. However, decreasing E2 levels trigger bone resorption. Progesterone (P4), the main progestin involved in bone formation and maintenance acts in accord with E2 during the menstrual cycle. In bone tissue, P4 is responsible for the increased P4-receptor-mediated, slow osteoblastic bone formation. When menstrual cycles are of normal length and ovulatory, E2 and P4 are balanced and BMD is stable [27].

Several conditions involving hypoestrogenism are linked to poor bone health, which supports the link between the balance of sex hormones and bone homeostasis. A prime example of hypoestrogenism reflecting poorly on bones is functional hypothalamic amenorrhea (FHA). Causes of FHA include eating disorders, overtraining, and psychological or physical stress. Women with exercise-related FHA have low BMD. When FHA manifests at a young age, it irreversibly impairs bone mass accrual. Hypoestrogenism appears to impact mainly on trabecular (e.g. spine) bone [28].

In exercise-induced FHA, a set of symptoms called The Female Athlete Triad can be observed. It is a condition in physically active young women that includes low energy availability, menstrual dysfunction and low BMD [29]. Amenorrheic athletes have impaired microarchitecture and bone strength, and a higher risk of stress fractures compared to eumenorrheic athletes and nonathletes [28].

5. Interplay between bone and pituitary hormones: TSH, FSH, GH

In recent decades, it has become evident that pituitary hormones have broader effects beyond their traditionally understood targets. Research has shown that receptors for several pituitary hormones, including GH, follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), prolactin, oxytocin, and vasopressin are expressed in bone tissue. This discovery highlights the direct influence of pituitary hormones on skeletal actions, leading to a new understanding of bone physiology and the identification of potential novel therapeutic targets.

The decline in bone mass that occurs during the transition to menopause, despite largely unaltered estrogen levels in this group, has prompted further investigation into other potential factors contributing to this observed decline. Multiple animal and human studies in the past decade have implicated FSH as a crucial hormone involved in bone mass decrement. Sowers *et al*. [30] found that the loss of BMD during the perimenopause was strongly associated with initial FSH levels and longitudinal FSH changes, rather than estrogen or androgen levels.

Subsequently, Sun *et al*. [31] conducted an experimental animal study showing that neither FSH-β nor FSH receptor null mice experienced bone loss despite severe hypogonadism. Furthermore, they demonstrated that the elimination of FSH-β prevented bone loss and even increased bone mass [31]. They also demonstrated that FSH stimulates osteoclastogenesis and acts directly on bone, independent of estrogen. Elevated FSH levels are a characteristic feature of POI. Lana *et al*. [32] investigated a group of 40 women diagnosed with POI, concluding that high FSH concentrations, but not estradiol, are positively associated with bone mass loss. Given the significant role of FSH in regulating bone metabolism and the increased FSH levels in patients with POI, anti-FSH agents represent a promising therapeutic option in preventing osteoporosis in this group of patients.

For many years, TSH has been known for its indirect influence on bone metabolism through thyroid hormones. However, recent groundbreaking studies by Abe *et al*. [33] revealed the presence of TSH receptors on osteoblast and osteoclast precursors, leading to subsequent clinical investigations that documented the direct effects of TSH on bone. Abe *et al*. [33] confirmed these independent mechanisms in an animal study, demonstrating that reduced expression of the TSH receptor led to the development of osteoporosis in mice. Further research elucidated the mechanism of TSH action, showing increased osteoclastogenesis in TSH receptor deficient mice mediated by tumor necrosis factor α, followed by the inhibition of osteoblast differentiation and type 1 collagen expression [34]. Epidemiological human studies reported a relationship between TSH levels, bone loss parameters, and fracture risk. A prospective study by Murphy *et al*. [35], involving over 2,300 postmenopausal women, found that higher

TSH levels were associated with a protective effect against bone loss, with a reduction in nonvertebral fracture up to 35%. Several studies have confirmed the association between low-normal TSH levels and an increased risk of fracture [36,37], while some regression models demonstrated that TSH was associated with osteoprotection. Promising outcomes were observed in interventions using recombinant human thyrotropin, where postmenopausal women showed a significant decline in bone resorption after subcutaneous injection of recombinant human TSH, suggesting its potential as a new therapeutic agent in osteoporosis treatment [38].

Originally, GH was simply considered the primary driver of linear growth during childhood and adolescence. However, our current understanding of its role in bone metabolism reveals a far more complex picture. GH and IGF-1 form an axis that exerts pleiotropic effects on the skeleton. GH/IGF actions peak during pubertal growth and decline with age. GH directly stimulates osteoblastogenesis and bone formation, as demonstrated in a study by Kassem *et al*. [39], which showed GH's direct anabolic effects on human osteoblasts. Boot *et al*. [40] conducted a human study in which they observed that germline mutations leading to GH deficiency result in growth retardation, delayed sexual maturation, and subsequently, reduced BMD. Activation of the GH/IGF-1 axis leads to increased bone formation, accompanied by elevated bone resorption and consequently the augmentation of bone turnover. In general, the GH/IGF-1 axis shifts the balance toward bone formation; however, GH excess as observed in patients with acromegaly, induces heightened bone turnover and decrease BMD [41]. The potential use of GH therapy in osteoporotic women remains a topic of deliberation since GH may not improve bone density but could be beneficial in reducing fracture risk, enhancing bone metabolism, and improving bone geometry [42]. An experimental study of GH treatment for POI in a mouse model focused solely on its effects on promoting ovarian tissue repair and did not investigate its impact on bone [43].

6. Bone and incretin and hunger hormones: GLP-1, GIP, ghrelin, leptin

Gut hormones play a significant role not only in modulating metabolic processes, such as glucose homeostasis and food intake, but more recently, their involvement in orchestrating the process of bone remodeling has been demonstrated. This important connection has been emphasized by the introduction of the term "gut-bone axis". Leptin, known for many years as a major regulator of food intake and energy homeostasis, has been found to have broader effects in the human body, including its influence on the immune system, reproduction, blood pressure, and bone mass.

Leptin plays a role in bone metabolism through both direct and indirect mechanisms. An animal study conducted in the early 2000s demonstrated a connection between leptin and bones. The study investigated leptin-deficient and leptin receptor-deficient, hypogonadal, obese mice and found that both types of mutant mice exhibited increased bone formation, leading to high bone mass despite hypogonadism [44]. This groundbreaking study was the first to identify leptin as a potent inhibitor of bone formation, leading to further research establishing the pathways by which leptin affects bone metabolism. However, prospective studies on the bone phenotype of leptin-deficient ob/ob mice have yielded conflicting results, as some studies have demonstrated low bone mass in leptin-deficient mice [45]. Nevertheless, it has been confirmed that leptin can directly act on bones, as leptin receptors have been found in adult primary osteoblasts and chondrocytes [46].

Leptin also impacts osteocalcin, a hormone that regulates bone metabolism. Studies in mice have revealed that leptin is one of the most important negative hormonal regulators of osteocalcin activity [47]. Additionally, leptin may exert indirect effects through a central relay in the brain, where leptin receptor activation suppresses the production of serotonin and subsequently activates the sympathetic nervous system. The sympathetic nervous system inhibits bone mass accrual by preventing bone formation and favoring bone resorption [48]. However, contrary to this, long-term metreleptin administration in women with hypothalamic amenorrhea and hypoleptinemia increases lumbar spine BMD and alters bone remodeling to favor bone formation [49]. Furthermore, leptin treatment for mice with bone loss after ovariectomy reduces trabecular bone loss and changes in trabecular structure, suggesting that leptin could participate in bone reconstruction in patients with POI [50].

Ghrelin, a gut-derived peptide hormone involved in the regulation of energy homeostasis, has also garnered attention in relation to bone metabolism. It is produced by enteroendocrine cells of the gastrointestinal tract, primarily the stomach, and was first described as a GH releasing peptide. As the interaction between hunger hormones and bone metabolism grew as a subject of interest, several studies have aimed to establish the role of ghrelin in skeletal integrity. Ghrelin has shown to modulate osteoblast differentiation and function, both directly and indirectly. A direct mechanism of ghrelin action on bone metabolism, independent of GH, was confirmed in an animal study where administration of ghrelin in GH-deficient rats increased BMD without affecting body weight or food intake [51]. Moreover, the same study indicated that ghrelin significantly increased the number of osteoblast-like cells, DNA synthesis, and the expression of osteoblast differentiation markers, alkaline phosphatase activity, and calcium accumulation in the matrix. These effects were dose-dependent [51]. Indirect effects on bones are mediated through the GH/IGF-1 axis. Ghrelin stimulates GH release, and this effect depends on the GH secretagogue receptor (GHSR). GH subsequently increases the liver's production of IGF-1, which exerts mitogenic effects on bone cells, leading to increased collagen production and matrix apposition [52]. In a cohort study on healthy Italian women, serum ghrelin levels were positively correlated with trabecular BMD [53]. A recent study by Erener *et al*. [54] investigated the effects of ghrelin on fracture healing, and demonstrated that ghrelin directly contributes not only to fracture healing, but also improves BMD, breaking strength, and stiffness thus holding promise for potential pharmacological applications [54].

With the increasing research on the enteroendocrine-bone axis, glucose-dependent insulinotropic polypeptide (GIP) has emerged as another hormone suspected of having an impact on bone health. GIP is secreted from the gut and acts as a weak inhibitor of gastric

acid secretion but its primary role is to enhance insulin secretion in a glucose-dependent manner. Recent findings have highlighted the importance of GIP in bone remodeling, as its receptor, glucose-dependent insulinotropic polypeptide receptor (GIPR), has been found to be expressed in bones [55]. GIP is believed to have a positive effect on bone strength and quality.

Mieczkowska *et al*. [55] provided evidence that genetic ablation of the GIP receptor in mice led to significant alterations in bone microarchitecture, including a marked reduction in ultimate load, stiffness, total absorbed, and post-yield energies. Furthermore, bone resorption was significantly increased in animals deficient in GIPR, while bone formation remained unchanged. Moreover, the deficiency in GIPRs has also been associated in mice with a dramatic decrease in bone quality and a subsequent increase of fracture risk [56]. These results were subsequently validated in human studies, as loss-of-function mutations in the GIPR in humans were associated with low BMD and a significant increase in fracture risk [57]. Human studies have also demonstrated that GIP decreases levels of the bone resorption marker carboxy-terminal type 1 collagen crosslink, confirming its role in suppressing bone resorption [58,59]. Additionally, bone formation in humans, assessed by procollagen type 1 amino-terminal propeptide (P1NP), was increased by 10–15% during GIP infusion [60].

The association between type 2 diabetes mellitus (DM2) and osteoporosis has led to numerous studies investigating the impact over bone metabolism of glucagon-like peptide-1 (GLP-1) and GLP-1 receptor agonists, both commonly used in DM2 treatment [61]. GLP-1 has been found to increase the number of osteoblasts, enhance gene expression related to bone formation, and elevate serum levels of bone formation markers, while simultaneously reducing the number of osteoclasts and serum levels of bone resorption markers [62]. Additionally, GLP-1 influences the differentiation of mesenchymal stem cells, diverting them from adipocytes toward osteoblasts [62]. Another mechanism by which GLP-1 promotes bone formation is through the regulation of glucose metabolism. Research has demonstrated a reverse relationship between hyperglycemia and lumbar BMD, with hyperglycemia being a factor that reduces BMD [63]. Animal studies in which GLP-1 and its related peptide, exendin 1-39 amide (Ex-4), were administered to rats showed decreased osteocalcin gene expression, reduced OPG/RANK ligand ratio, and reversal of bone alterations. [64] Similarly, in a study on ovariectomy-induced osteoporotic rats, exendin-4, a glucagon-like peptide-1 receptor agonist, was found to enhance bone strength and prevent further deterioration in trabecular microarchitecture. These results suggest that GLP-1 receptor agonists hold promise as potential treatment options for postmenopausal osteoporosis in aging individuals [65]. However, the relationship between GLP-1 and the risk of bone fractures has not been fully elucidated [62].

7. BMD in estrogen-deficient women: The effects of POI on BMD and the evaluation of BMD in POI patients

Estrogens play a crucial role in determining BMD, making estrogen deficiency a key factor in the development of osteoporosis. A deficiency of estrogen leads to an accelerated rate of bone remodeling, increasing the risk of low bone density in affected women [66]. Estrogen receptors are highly expressed on the surface of osteoblasts, osteoclasts, and osteocytes, providing protective effects in bone health. However, in estrogen deficiency, there is an upregulation of osteoclast formation and bone resorptive activity, coupled with decreased osteogenesis [67]. Additionally, a slower mineralization process has been observed in new bone formation among women with POI, resulting in lower mineralization compared to older bone [68]. Estrogens also indirectly influence calcium metabolism, and their deficiency reduces both intestinal and renal calcium absorption [69]. Moreover, recent studies have reported that hypoestrogenism exacerbates bone loss by diminishing the cells' resistance to oxidative stress [70]. These findings highlight the multifaceted effects of estrogen deficiency on bone health, affecting bone remodeling, mineralization, calcium metabolism, and cellular responses to oxidative stress.

Hypoestrogenism in young women can be attributed to various factors such as nutritional deficiency, X chromosomal haploinsufficiency, associated malignancy, chemotherapy, excessive exercise, or spontaneous primary ovarian insufficiency. FHA is, alongside other causes, a common cause of secondary amenorrhea in young premenopausal women and results in severe hypoestrogenism. Since estrogen deficiency during adolescence determines low PBM, the age at which hypoestrogenism occurs affects severity of bone damage. In comparison to POI in FHA not only hypoestrogenism but also the alteration of other hormones can affect bone health. Hypothalamic amenorrhea also results in hypothyroidism, elevated cortisol level, nutritionally acquired resistance to GH and imbalance in vitamin D levels. Contrary to hypoestrogenism which impacts mainly on trabecular bone, other factors presented in FHA patients, like low body mass index, decreased lean mass and mechanical load appear to act on cortical bone [28].

Estrogen deficiency, a characteristic manifestation of POI, significantly increases the risk of bone impairment. Studies have shown that women with POI exhibit reduced bone density compared to those with regular menstruation [66]. Bachelot *et al*. [71] reported that up to 60% of women with POI had alterations in BMD, with a prevalence of osteoporosis ranging from 8% to 15% [72]. A meta-analysis of 10 studies concluded that significant decreases in BMD of the femur neck and forearm were associated with POI in affected women [73]. Delay in diagnosis further increases the risk of lower bone density [66]. Several collateral risk factors for osteoporosis in this group have been established, including age of onset of menstrual irregularity before age 20, low vitamin D levels, low body weight, lack of regular exercise, poor calcium intake, and noncompliance with hormone replacement therapy (HRT) [66].

Not only is bone density affected in women with POI, but there is also an imbalance in bone microarchitecture. Decreased trabecular bone score (TBS) in POI women indicates an accelerated loss of microarchitectural integrity due to prolonged hypoestrogenism [72]. TBS can provide additional information and may serve as an independent predictor of fracture risk in the future [72]. The increased fracture risk observed in women with early menopause highlights the significance of addressing bone health throughout their lifespan. Dual-Energy X-ray Absorptiometry (DXA) is

the primary diagnostic and management tool for assessing bone health in women with POI and is considered the gold standard for measuring BMD [6]. According to recommendations from the ESHRE, if a diagnosis of osteoporosis is made and estrogen replacement or other therapies are initiated, BMD measurement should be repeated within 5 years [6].

8. Impact of hormone therapy (HT) on the bone density in women with POI: regimens and routes of administration, as well as a comparison between HT and combined oral contraceptives (COC)

Systemic HT has proven to be an effective treatment for hypoestrogenism in women with POI. It not only improves bone health but also helps prevent cardiovascular disease and urogenital atrophy. It is recommended that women with POI initiate HT at the time of POI onset and continue estrogen treatment until at least the average age of menopause (51–52 years) to prevent bone loss [6]. A study by Benetti-Pinto *et al*. [74] revealed that women with POI who received estrogen and progestogen therapy maintained stable bone mass throughout an 8-year follow-up period. Measurements of BMD at 2, 4, 6, and 8 years did not differ significantly from baseline. However, it should be noted that the treatment did not decrease the number of women who experienced some degree of reduction in bone density, situation that has raised concerns. Furthermore, standard dose HT with 1 mg estradiol was found to be insufficient in reducing the incidence of osteoporosis/osteopenia in POI patients [75]. Therefore, it was suggested that higher doses of estrogen might be necessary to achieve a positive effect on bone. Podfigurna *et al*. [76] conducted a study involving 132 patients diagnosed with spontaneous POI who were treated daily with oral 2 mg 17-β-estradiol and 10 mg dydrogesterone. After three years of follow-up, they observed a significant increase in BMD, indicating that higher doses of estradiol can have a positive influence on bone mass.

Most authors favor 2 mg estradiol for oral use or 100 μg for transdermal use as the initial dose. Recently Fruzzetti *et al*. [77] have proposed adjusting doses of estradiol according to the patient's age, needs, and preferences to create tailored hormonal approaches. Authors recommended starting hormonal therapy in young girls at age 10–11 years, preferably with low-dose estradiol administered by systemic route. This dose should be gradually increased over a period of 4 or more years, based on the stages of puberty. After puberty, a full adult dose was defined as 100 or 200 μg transdermal estradiol, 2–4 mg micronized oral estradiol or 20 μg oral ethinyl estradiol. With age exacerbation of estrogen deficiency symptoms can occur which may be an indication to reconsider HT. After the age of natural menopause, the dose should be adjusted according to the minimal effective dose for the control of symptoms.

In an interesting observational study, the use of COC was compared with low-dose HT and high-dose HT in women with POI. Continuous oral contraceptives were associated with increased BMD compared to low-dose HT, and both the COC and high-dose HT groups showed similar improvements [78]. However, Cartwright *et al*. [79] demonstrated that after 12 and 24 months of treatment, the HT group had significantly increased bone density compared to the COC group. According to recommendations from the ESHRE, while COCs may be appropriate for some women, their effects on BMD are less favorable compared to HT [6]. Additionally, there was no statistical difference observed between the transdermal and oral routes of administration in terms of femoral neck or total hip BMD [80]. Transdermal preparations may be preferred over oral estrogens for women with risk factors for venous thromboembolism or stroke [81].

Another pros of transdermal route is that compared to oral administration transdermal route can achieve higher plasma levels of circulating estradiol with a lower treatment dose and therefore fewer circulating estrogen metabolites. Additionally, transdermal route of administration produces a physiologic E2:E1 ratio, presumably because it avoids first-pass metabolism, unlike oral estrogen therapy [82]. Women with POI for whom HT is contraindicated, such as those with breast cancer, should be referred to specialist care for consideration of alternative antiresorptive therapies, such as bisphosphonates or denosumab [81].

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