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Effects of oestrogen deficiency on bone mineralisation in girls during "adolescent crisis"

Wpływ niedoboru estrogenów na mineralizację kości u dziewcząt w okresie „kryzysu adolescencji"

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Abstract

Puberty is a critical bone mineralisation period, and peak bone mass attained by adolescent girls is one of the most significant predictive factors for postmenopausal osteoporosis. Adolescent girls' peak bone mass depends on genetic factors as well as on general condition, nutritional status and body mass; lifestyle is also important, along with physical exercise and the use of prescription drugs. Additionally, hormones, including oestrogens, play an important role during pubertal accumulation of bone mass. Therefore, oestrogen deficiency during puberty has serious consequences for bone mineralisation. During puberty, particularly during the initial years after menarche, hypothalamic dysfunction can develop due to psycho-emotional burden, excessive physical exercise or increasing number of responsibilities; psychologists refer to this period as the "crisis of adolescence". Its symptoms include behavioural disorders and juvenile depression, both affecting the hypothalamic neurosecretion to an extent that can be reflected by secondary hypo-oestrogenism and amenorrhea. The administration of oestroprogestagens in the treatment of low bone mineral density and hypo-oestrogenism-associated menstrual disorders results in resumed regular menstrual bleedings and maintains, or even improves, bone mineral density. This observation seems to be important not only in terms of short term clinical applications, but also in the context of the long term prevention of osteoporosis. Consequently, hormonal therapy has to be accompanied by a thorough education of patients and their parents, particularly in terms of proper nutrition and modification of levels of physical activity. Puberty is the optimal time period for modifying environmental factors that are associated with bone mass gain. **(Pol J Endocrinol 2011; 62 (6): 538–546)**

Key words: *amenorrhea, bone mineralisation, densitometry, oestrogens, hypo-oestrogenism*

Streszczenie

Dojrzewanie jest krytycznym okresem dla mineralizacji kości, a szczytowa masa kostna osiągana przez nastolatki podczas tego okresu jest jednym z najbardziej istotnych czynników predykcyjnych osteoporozy pomenopauzalnej. Szczytowa masa kostna u nastolatek zależy od uwarunkowań genetycznych, a ponadto od ogólnego stanu zdrowia, odżywiania, masy ciała; nie bez znaczenia jest również styl życia, aktywność fizyczna i przyjmowanie leków. Istotne znaczenie dla akumulacji masy kostnej w okresie pokwitania mają także hormony, w tym estrogeny. Dlatego niedobór estrogenów w okresie rozwojowym ma istotny wpływ na mineralizację kości. W okresie dojrzewania, szczególnie w pierwszych latach po menarche, u nastolatek może dochodzić do dysfunkcji podwzgórza na tle nadmiernego obciążenia psychoemocjonalnego i wysiłku fizycznego lub z powodu nadmiaru obowiązków; psycholodzy nazywają ten okres „kryzysem adolescencji". Mogą mu towarzyszyć zaburzenia zachowania i depresja młodzieńcza. Wpływając na neurosekrecję podwzgórza, stany te mogą prowadzić do wtórnego niedoboru estrogenów i braku miesiączki. Leczenie estroprogestagenami niskiej gęstości mineralnej kości i zaburzeń miesiączkowania związanych z hipoestrogenizmem skutkuje przywróceniem regularnych cykli miesiączkowych oraz ma wpływ na utrzymanie lub poprawę gęstości mineralnej kości. Jest to istotne w doraźnym postępowaniu klinicznym oraz długoterminowej profilaktyce osteoporozy. Ze względu na wieloczynnikową etiologię zaburzeń miesiączkowania i obniżonej gęstości mineralnej kości terapia hormonalna powinna być uzupełniona o odpowiednią edukację pacjentek i ich opiekunów w zakresie właściwego postępowania dietetycznego i aktywności fizycznej. Dojrzewanie jest bowiem odpowiednim okresem modyfikacji czynników środowiskowych związanych z nabywaniem masy kostnej. **(Endokrynol Pol 2011; 62 (6): 538–546)**

Słowa kluczowe: *brak miesiączki, mineralizacja kości, densytometria, estrogeny, hipoestrogenizm*

Introduction

Puberty is a critical bone mineralisation period as approximately half of the peak bone mass is reached during its early stages [1]. Peak bone mass (PBM) attained by adolescent girls during puberty is one of the most significant predictive factors for postmenopausal osteoporosis [2]. Bone mass accumulation can continue up to the third decade of life, but approximately 26% of bone mineral mass is formed during the peripubertal

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growth spurt, and nearly 60% during the peripubertal period [3, 4].

The effects of peak bone mass, menopause and age-related loss of the mass on the risk of osteoporosis has been determined with the aid of a mathematical model [5]. According to this model, a 10% increase in the peak bone mass will delay the onset of osteoporosis by 13 years, compared to only two years of delay as a consequence of a 10% increase in mineralisation taking place during the menopausal or postmenopausal period [5]. Therefore, this analysis has confirmed the vital role puberty plays in the process of bone mineralisation.

Adolescent girls' peak bone mass depends on genetic factors as well as on general condition, nutritional status (ingestion of vitamin D and calcium) and body mass; lifestyle is also important, along with physical exercise and the use of prescription drugs. Additionally, hormones, including oestrogens, play an important role during pubertal accumulation of bone mass.

The most frequent and important causes of bone demineralisation observed during puberty are set out in Table I [6–8]. The clinical course of these disorders and lifestyle modifications is nearly always associated with hypoestrogenism along with primary or secondary amenorrhea.

Menstrual disorders associated with puberty most frequently occur during the initial years after menarche, and their prevalence decreases with time from the first menstrual bleeding. Previous studies examining the peripubertal period have found that in girls (including a group of Polish girls) the average number of normal menses observed three years after menarche approached 85–97% [9, 10]. According to previous research, menstrual disorders observed in the second year after menarche were natural consequences of incomplete neuroendocrine development of the brain. However, clinical observations suggest that this group also includes a significant proportion of girls whose menstrual disorders are predictors of future, more severe, neuroendocrine pathologies. Therefore, the medical history of each adolescent patient with menstrual disorder (using, for example, a questionnaire survey) should be analysed for risk factors of peak bone mass deficiency (Table II) [11, 12]. According to several authors, even abnormal menstrual cycles observed during the initial years after menarche require endocrine

Table I. *The most frequent causes of bone demineralisation during puberty [6]*
Tabela I. *Najczęstsze przyczyny demineralizacji kości w okresie dojrzewania [6]*

Specific endocrinopathies	Chronic medical conditions (multifactorial aetiologies)	Medications (medium- to long-term use)	Deleterious behaviors
Cushing syndrome	Prolonged immobilisation	Anticonvulsants	Female athlete triad*:
Growth hormone deficiency	Anorexia nervosa	Glucorticoids	— among disordered eating
Hyperthyroidism	Asthma	Cyclosporine A	— amenorrhea
Hyperparathyroidism	Celiac disease	Gonadotropin-releasing hormone agonists	— osteoporosis
Hyperprolactinaemia	Cerebral palsy and other neuromuscular conditions	Heparin	Excessive alcohol consumption
Hypopituitarism	Chronic renal failure	Lithium	Tobacco use
Hypothyroidism	Cranial irradiation (particularly pituitary)	Depot medroxyprogesterone acetate	
Sex hormone deficiency (hypogonadism acquired or genetic)	Cystic fibrosis*	Methotrexate	
	Diabetes mellitus	Other chemotherapeutic agents	
	Epilepsy		
	Human immunodeficiency virus infection		
	Inflammatory bowel disease		
	Malignancies		
	Organ transplantation (solid and bone marrow)		
	Rheumatologic diseases		
	Sickle cell disease		
	Thalassemia		
	Turner syndrome		

*Indicates that an official position statement has been published (cystic fibrosis [7]; female athlete triad [8])

Table II. *Questionnaire for the risk assessment of osteoporosis in pubescent girls*
Tabela II. *Kwestionariusz oceny ryzyka osteoporozy u dziewcząt w okresie dojrzewania*

I. Menstrual cycle	
Age of patient (years and months, e.g. 16 6/12)	.....
Age at menarche (years and months, e.g. 12 5/12)	.....
Did regular menstrual bleedings occur during the 1st year after menarche?	Yes ☐ No ☐
Did regular menstrual bleedings occur during 2 years after menarche?	Yes ☐ No ☐
Did regular menstrual bleedings occur during 3 years after menarche?	Yes ☐ No ☐
Was any recurrent irregular menstrual bleeding noted after menarche?	Yes ☐ No ☐
Did any amenorrhea occur?	Yes ☐ No ☐
Number of menstrual bleedings during the first year after menarche	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Usual duration (in months) of previous amenorrheas, if any	up to 3 ☐ up to 6 ☐ > 6 ☐
Average duration of menstrual cycles in days (months)	
Character of menstrual bleedings: 1. Heavy 2. Normal 3. Weak	1 ☐ 2 ☐ 3 ☐
Character of menstrual bleedings: 1. Painful 2. Non-painful	1 ☐ 2 ☐
Has the patient ever used oral contraceptives?	Yes ☐ No ☐
Has the patient ever undergone EP treatment?	Yes ☐ No ☐
II. Physical activity	
Does the patient participate in any sports?	Yes ☐ No ☐
If yes, what discipline?	
How many hours a day does she participate in this sport?	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
How many hours a week does she participate in this sport?	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Does she practice any recreational gymnastics?	Yes ☐ No ☐
If yes, how many hours a day does she practice it?	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Has she ever practiced gymnastics in order to reduce body weight?	Yes ☐ No ☐
Has she ever practiced gymnastics in order to improve her physical condition?	Yes ☐ No ☐
III. Nutrition	
Patient's appetite 1. Good 2. Poor	1 ☐ 2 ☐
Does she eat her meals willingly and often?	Yes ☐ No ☐
Is proper nutrition important to the patient's family?	Yes ☐ No ☐
Can the patient's attitude towards nutrition be summarised as: "eat anything so as to not be hungry"?	Yes ☐ No ☐
Is the number of meals consumed by the patient supervised by her parents?	Yes ☐ No ☐
Does she always have breakfast before school?	Yes ☐ No ☐
Does she always have second breakfast at school?	Yes ☐ No ☐
In school, she eats meals that are prepared: 1. At home 2. In the canteen	1 ☐ 2 ☐
Does her everyday diet contain a glass of milk or yoghurt?	Yes ☐ No ☐
Does her everyday diet contain cheese or cottage cheese?	Yes ☐ No ☐
Does the patient supplement with calcium preparations on a daily basis?	Yes ☐ No ☐
Does the patient supplement with vitamin preparations on a daily basis?	Yes ☐ No ☐
Has she ever been on an elimination, i.e. vegetarian, diet?	Yes ☐ No ☐
Has she ever been on an elimination, i.e. vegan, diet?	Yes ☐ No ☐

diagnostics and puberty monitoring because they may constitute a discrete clinical manifestation of conditions presented in Table I. Consequently, one should consider regular menstrual cycles in adolescent girls as a clinical marker of health and normal somatosexual development.

Psychogenic type amenorrhea during puberty

In the case of pubertal girls, irregular menses observed during the first year after menarche can result from an unstable secretory function of the hypothalamus; however, they can also predict several disorders, including puberty-specific psychogenic type disorders.

Puberty can be considered as an amplification of previously existing hormonal mechanisms rather than the onset of a new secretion process. In the hypothalamus, puberty starts with the activation of GnRH neurons, which are stimulated by *Kiss1* neurohormone and its GPR54 receptor. Secretion of gonadoliberin (GnRH) is modulated by the coordinated action of the neuronal systems of *Kiss1* neurons, proopiomelanocortin (POMC), neuropeptide Y (NPY), and adipocyte-released leptin [13, 14].

Leptin (cytokine-like peptide hormone) indirectly modulates GnRH secretion, influencing the metabolism of *Kiss1* neurons [14]. Leptin concentrations are proportional to the mass of the adipose tissue. Therefore, as proposed by Frisch and Revelle [15], reaching a sufficient body mass during puberty is of crucial importance, and, as further suggested by these authors, a "critical body mass" is required for the onset of menstruation.

Initially, tonic, and then pulsatile release of GnRH by hypothalamic neurons is vital for the onset of puberty and the stabilisation of menses. This release is also dependent on the synchronised action of other neurons secreting various neuropeptides and neurotransmitters that stimulate or inhibit the secretion of GnRH. The most important compounds are: noradrenaline, dopamine, β-endorphin, NPY, and GABA, as well as neuroactive amino acids — glutamate, aspartate and others. Additionally, via feedback systems, oestradiol exerts positive and negative regulatory effects on the synthesis and secretion of GnRH. The functional regulation of GnRH neurons by oestrogens is mediated by their receptors, ER-α and ER-β. As currently suggested, selective activation of ER-β is reflected by an increase in GnRH secretion, while the activation of ER-α has the opposite effect. This dual effect of oestrogens on the reactivity of GnRH neurons depends both on the density of oestrogen receptors and oestrogen concentration [16]. Pulsatile release of GnRH induces hypothalamic gonadotropins release, and thus indirectly stimulates the ovaries to secrete oestrogen [17]. This complex regulatory mechanism of the hypothalamic-pituitary-ovarian axis is responsible for the regular menstrual cycle, usually 28 ± 3–5 days long, along with ovulation, and is usually observed shortly after menarche in healthy girls with normal body mass [18].

Disorders of hypothalamic neurohormonal secretion, along with secondary disorders of pituitary secretion, lead to abnormal gonadal function which is manifested by delayed menarche, irregular menses and anovulatory cycles; hypoestrogenism can also secondarily impair the regulation of GnRH release.

During puberty, particularly during the initial years after menarche, hypothalamic dysfunction can also develop due to psycho-emotional burden, excessive physical exercise or increasing number of responsibilities (e.g. school). Psychologists refer to this period as the "crisis of adolescence"; it can be accompanied by a set of transient, puberty-related mental symptoms. These symptoms include behavioural disorders and juvenile depression, both affecting hypothalamic neurosecretion to an extent that can be reflected by secondary amenorrhea [12].

Recently, a so-called "deprivation syndrome" has been reported with increasing frequency in adolescent girls, as a result of an inability to act or to fulfill strong needs of physiological (e.g. hunger), social (isolation and loneliness), emotional (lack of connection with peers or parents) and mental nature due to failures and disappointments. Deprivation syndrome can lead to aggressive behaviours, somatic disorders and even suicide attempts [19].

Long-term exposure to psychogenic factors (e.g. in the course of deprivation syndrome) can be reflected by functional hypothalamic amenorrhea (FHA). The prevalence of this disorder is estimated at 2.6–8.5% [20, 21], but the proportion can increase to 100% in cases of persistent stress, for example, in athletes who practice several sport disciplines [10].

The mechanism by which psychogenic factors affect hypothalamic function during puberty is not fully understood, but the results of many recently published studies suggest the involvement of neuropeptides, such as CRF and β-endorphin [22, 23].

Stress has been shown to increase CRF concentration, which further stimulates β-endorphin secretion and inhibits pulsatile release of GnRH. B-endorphin is known to modulate behaviour, emotions, psychological drive and learning processes, and can directly influence functions of ovarian follicle and corpus luteum, leading to secondary menstrual disorders [24]. Both psychogenic and somatic stress is reflected by elevated hypothalamic concentration of this neuropeptide, impairing the pulsatile release of GnRH, and inhibiting dopamine secretion, and thus increasing

prolactin concentrations. Functional hyperprolactinaemia, detected during dynamic challenges with MCP and TRH, exerts central effects and impairs both the pulsatile release and the amplitude of LH pulses. This results in impaired proliferation of granulosa cells and lowered synthesis of oestradiol with consecutive lack of the LH peak, and inhibition of ovarian follicle maturation and ovulation [25]. Furthermore, stress can lead to secondary increase of ACTH, catecholamine and cortisol concentrations [26, 27]. Children with elevated levels of aggression and stress are characterised by high concentrations of dopamine along with lower serotonin levels and higher levels of DHEA-S and testosterone; all these abnormalities can be reflected by abnormal menstrual cycles [28].

The intensity of clinical symptoms depends on the extent of hormonal abnormalities underlying the impaired GnRH secretion. Potential reasons for infrequent menstruation include anovulatory cycles, corpus luteum insufficiency and low concentrations of progesterone. Initially infrequent, then infrequent and scanty periods, and finally secondary amenorrhea, are a result of the functional impairment of the hypothalamic-pituitary-ovarian axis and the inhibition of oestrogen secretion.

As a result, in girls undergoing intense somatosexual development, the sequential developmental stages can be delayed or prolonged which can manifest in the form of pubertal clinical signs that are not specific for a given stage, such as OHA type menses (oligo/hypo/amenorrhea) or secondary amenorrhea. Other than propelling sexual development, oestrogens play a vital role among hormonal factors that specifically modulate bone mineralisation during puberty.

Involvement of oestrogens in bone mineralisation

The biological effects of oestrogens are mediated by the oestrogen receptor. Two isoforms of this receptor, ER-α and ER-β, have been identified thus far. Previous studies have revealed that binding ER-α with its ligand induces transcription and that ER-β modulates this reaction. Consequently, ER-α plays a crucial role in the regulation of the oestrogen-mediated stimulation of osteoblast function [29, 30]. Additionally, ER-α receptors have also been detected in osteoclasts; however, it was found that oestrogens influence osteoclasts mainly indirectly by stimulating expression of regulatory factors synthesised by osteoblasts. According to published research, oestrogens can exert various effects on the skeletal cell activity acting via ER-α and ER-β receptors; oestrogen influences depend on ER-α to ER-β ratio, as well as on the receptor density within a cell [31].

Both α and β oestrogen receptors have been detected on the chondrocytes of human growth plate, as well as on the osteoblasts located on the surface of bone trabecules. These findings suggest that oestrogens are directly involved in skeletal growth and bone mineralisation [32]. Furthermore, the effects of biomechanical forces appear to be mediated by the oestrogen receptor α. According to growing evidence, osteocyte and osteoblast longevity is a crucial determinant of bone durability, and the activity of these cells is modulated by mechanical forces in addition to hormones and local factors. Moreover, it has been revealed that a lack of mechanical stimulation is reflected by an increased prevalence of osteocyte apoptosis and lower durability of the bone [33].

During puberty, oestrogens stimulate secretion of the growth hormone (GH) from the pituitary gland. This increase in GH synthesis exerts direct and indirect effects (via increased IGF-I) on bone growth, and skeletal modelling and remodelling rates. Furthermore, oestrogens increase the amplitude of the pulsatile GH release, thus further potentiating their effects [34]. Growth hormone significantly influences bone growth, mainly due to local generation of IGF-I. This stimulates the growth plate, with resulting enhanced bone growth. An increase in IGF-I concentration begins in childhood, but the peak secretion of this molecule is observed during puberty [35].

Oestrogens, however, have also been shown to antagonise the effects of GH and IGF-I at the metaphyseal growth cartilage, inducing mineralisation and physeal closure with simultaneous inhibition of bone growth in length [36]. Additionally, IGF-I has been observed to reduce the levels of sex hormone binding globulin (SHBG), with resulting increase in the pool of biologically active oestradiol inhibiting the skeletal effects of GH and IGF-I [37].

Oestrogens influence the expression of genes involved in the synthesis of cytokines and growth factors by osteoblasts [38]. Oestradiol has been observed to stimulate osteoblasts to synthesise and secrete TGF-β, IGF-I and IGF II, while inhibiting the synthesis and secretion of several other cytokines (IL-1, IL-6 and TNF-α), granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor (M-CSF) and prostaglandin E2 (PGE-2), all being involved in bone resorption [38]. TGF-β inhibits the recruitment of osteoclast precursors as well as the activity of already existing osteoclasts, therefore preventing bone resorption. This factor potentiates the effects of oestrogens in osteoblasts, inhibiting proliferation of the latter; additionally, TGF-β increases the activity of alkaline phosphatase [39]. The intensity of bone resorption is determined by the number of osteoclasts and their ac-

tivity. Osteoclast differentiation and function, in turn, are markedly modulated by interleukins and tumour necrosis factor alpha (TNF-α) [40].

Cytokines IL-1, IL-6 and IL-11, by increasing the proliferation of osteoclast precursors and subsequent stimulation of their differentiation, indirectly induce bone resorption [38]. Oestrogens attenuate the effect of IL-6, inhibiting both the ligand itself and its receptor [41]. As a result, both osteoclast differentiation and bone resorption are inhibited. Furthermore, osteoclast recruitment and differentiation can be modulated by M-CSF; concentration of this latter protein decreases in response to oestrogen administration [42]. Additionally, exposure to oestrogens modulates the location and flow of lysosomal enzymes. A decrease in cathepsin L, β-glucuronidase and lysozyme concentrations have been observed after oestrogen administration, along with an increase in cathepsin B and tartrate-resistant acid phosphatase (TRAP) levels. These findings suggest that oestrogens modulate the resorptive activity of osteoclasts, regulating the synthesis and secretion of lysosomal enzymes by these cells [43].

Oestrogen involvement in the programmed cell death of osteoclasts has been demonstrated alongside the participation of TGF-β in this process. Moreover, common metabolic pathways of apoptosis have been postulated for osteoblasts and osteoclasts [43, 44]. The results of recently published studies suggest that the OPG/RANKL/RANK signalling cascade is the principal signalling pathway involved in bone remodelling [45]. Osteoprotegerin (OPG) and RANK (receptor activator of nuclear factor *k*B) belong to the superfamily of TNF receptors (tumour necrotic factors receptor, TNFR), and RANKL (receptor activator of nuclear factor *k*B ligand) is the ligand of RANK. Binding the RANKL to RANK receptor induces the cascade of intracellular processes. Through the activation of transcription factors, these processes lead to osteoclast activation and differentiation, while the apoptosis of osteoclasts is inhibited [45, 46].

In contrast to other TNF superfamily receptors, osteoprotegerin is a cell-released glycoprotein (without cytoplasmic and transmembrane domains). It forms the so-called decoy receptor for RANKL ligand. OPG blocks binding RANKL to the RANK receptor, thus preventing activation of the latter on osteoclast surface. Oestrogen-induced synthesis and activation of OPG is reflected by an inhibition of the terminal phase of osteoclast genesis, attenuated activation of mature osteoclasts and induction of their apoptosis. Consequently, osteoprotegerin reduces the number of active osteoclasts and prevents bone resorption [47, 48].

The predominance of RANKL effects over those of OPG leads to an increasing pool of active osteoclasts and enhanced bone resorption, while the predominance of OPG exerts the opposite effect. Oestrogens, along with IL-α, IL-1β, TNF-α, TNF-β, TGF-β and calcium, stimulate the synthesis of OPG by osteoblasts, while the synthesis of this molecule is inhibited by $1,25(OH)_2D_3$, PTH, glucocorticoids and PGE-2. In turn, the osteoblast synthesis of RANKL is induced by parathyroid hormone, active metabolite of vitamin D_3 — $1,25(OH)_2D_3$, glucocorticoids, IL-1, IL-11 and PGE-2, and is inhibited by TGF-β [49].

Via their receptors (ER-α), oestrogens stimulate the expression of genes involved in the osteoblast synthesis of OPG. Furthermore, a significant relationship has been demonstrated between OPG level and oestradiol concentration during exposure to oestrogens. Specifically, prolonged exposure and higher oestrogen concentrations correlated positively with OPG levels [49]. Additionally, oestradiol inhibits the synthesis of pro-resorptive cytokines: IL-1, IL-6, TNF-α, M-CSF and PGE-2, and stimulates the synthesis of TGF-β and IGF-1 [49].

Oestrogen deficiency during puberty has serious consequences for bone mineralisation. Therapeutic modification of the RANKL/RANK/OPG signalling pathway during pubertal development is associated with the involvement of 17-β oestradiol in:

— suppression of the endogenous secretion of RANKL (additionally enhanced by mechanical stimulation);
— suppression of further stages of the RANKL/RANK signalling pathway;
— an increase in the endogenous synthesis of OPG.

Consequently, oestrogens can be considered to be the regulators of skeletal homeostasis, and their deficiency can be reflected by elevated bone turnover parameters, leading to lowered peak bone mass and decreased mechanical resistance, both being risk factors for future fractures [38, 50, 51].

Effects of oestrogen therapy on BMD in adolescent girls with hypothalamic functional amenorrhea

Hypoestrogenism-associated menstrual disorders of adolescent girls require the implementation of oestrogen therapy in order to prevent bone demineralisation. Various protocols of hormonal therapies are currently used in the clinical practice of menstrual disorder management, but evidence regarding their effects on bone mineral density is sparse.

Previous studies of these protocols have analysed the effects of oestrogen preparations on BMD in patients suffering FHA due to anorexia nervosa or intense gymnastic or athletic training. The administration of conjugated oestrogens (Premarin, 0.3–0.625 mg) and

medroxyprogesterone acetate (Provera, 5–10 mg) to FHA patients over a period of 1.5–4.3 years did not improve their BMD compared to the control group [52–56]. One study analysed the effects of a protocol consisting of: 1) oestriol (1 mg) and oestradiol (2 mg) administered for 12 days; 2) oestriol (1 mg), oestradiol (2 mg) and norethisterone acetate (1 mg) given for ten days; and 3) oestriol (0.5 mg) and oestradiol (1 mg) given for another six days; additionally 1,000 mg/d of calcium carbonate was included. The treatment was continued for nine months; compared to baseline values, it was not reflected by significant changes in BMD of the lumbar spine and femur [53]. However, a 19.3% increase in BMD was observed in a subgroup of FHA patients who resumed menses spontaneously.

Therefore, one can conclude that FHA-associated bone demineralisation has a complex aetiology, and besides hypo-oestrogenism, metabolic abnormalities are also involved in this process. Accordingly, oestrogen deficiency seems to be one potential consequence of this disorder rather that its reason. Furthermore, therapeutic outcomes have been shown to depend on the pubertal stage and menarcheal age, i.e. were associated both with the duration of previous exposure to natural oestrogens, and the duration of hypoestrogenism and secondary amenorrhea.

No long-term studies of bone mineralisation in girls subjected to oestroprogestagen therapy due to psychogenic-type secondary amenorrhea developed in response to stress have been performed thus far. The only exception is a four-year prospective observation of postmenarcheal girls receiving oestroprogestagen treatment due to scanty and irregular menses associated with hypo-oestrogenism. Triphasic preparation was included in this study, comprising 17-β oestradiol (1 mg) administered for 21 days (e.g. from the 5th to the 25th day of the menstrual cycle) and gestagen, medroxyprogesterone acetate (5 mg) or didrogesterone (10 mg), given for ten days (from the 16th to the 25th day of the cycle). The resumption of natural, spontaneous secretion of oestrogens was controlled every 3–6 months. The goal of the treatment was to induce regular menstrual bleeding using a minimal dose of 17-β oestradiol (reducing an initial substitutive 2 mg dose down to 0.5–1 mg). Lack of menstrual bleeding after treatment with a substitutive or lower dose of the preparation, or low blood oestradiol concentration (negative progesterone challenge) was taken as an indication for returning to an increased therapeutic dosage. Such therapy (mimicking physiological concentrations of hormones throughout the cycle) was reflected by linear increase in bone mineral density, the most intense during the second year of the therapy [12]. After 4–5 years of the therapy, spontaneous menses resumed in all patients, but a further increase in BMD was not observed during the ten-year follow-up (not published). This finding is consistent with the literature, since growth potential in girls is the highest between one and two years before menarche, co-existing with peak accumulation of bone mass [3, 35]. These findings suggest that hormonal disorders leading to hypoestrogenism are not the only factor predisposing to impaired bone mineralisation in pubertal girls. This is consistent with the literature, according to which lowered BMD of growing girls can also be a direct consequence of incorrect diet, disturbed metabolism and genetic predispositions [57, 58].

Evidence in the literature of the effects of hormonal contraceptives on BMD in FHA girls is inconclusive [59]. Administered at low (0.03 mg of ethinyloestradiol) or ultra-low doses (0.02 mg ethinyloestradiol), oral contraceptives caused only a 2.4–2.5% increase in the lumbar spine BMD, while BMD in the controls decreased by 1.2% [60]. This observation supports the hypothesis according to which oral contraceptives prevent bone demineralisation but do not improve BMD. This phenomenon results from the fact that the administration of a contraceptive is reflected by a slower rate of bone turnover (as suggested by decreased values of both bone resorption and bone mineralisation markers). Furthermore, blocking bone resorption by exogenous oestrogens can secondarily reduce osteoblast activity; as a result, osteoblasts will not be able to fill post-resorptive defects made by osteoclasts [61, 62].

Another preparation, depot medroxyprogesterone acetate (Depo-Provera, DMPA) inhibits ovulation in most women due to its high progesterone content. This preparation blocks hormonal function of the ovary, leading to ultralow concentrations of oestradiol. Resulting oestrogen deficiency can be reflected by a decreased bone mineral density. However, this method of contraception is still preferred in adolescent girls in some clinical circumstances (e.g. in cases of mental disability) due to only four intramuscular injections being required every year. In view of this advantage, the WHO does not limit DMPA application in spite of its potentially unfavourable effects on bone density [63]. Nevertheless, all potential advantages and risks should be scored prior to prescribing DMPA to an adolescent girl. In view of the potential risk of peak bone mass deficiency, the patient should be supplemented with calcium and vitamin D preparations, and regular physical activity should be recommended. Additionally, oestrogens at low doses can be administered in girls with osteopenia or other risk factors of BMD deficiency [64].

In summary, the evidence from previous studies of the effects of hormonal contraceptives on bone mineralisation is still insufficient to conclude that these preparations can modulate fracture risk in women [65].

The administration of oestroprogestagens in the treatment of low bone mineral density and hypoestrogenism-associated menstrual disorders results in resumed regular menstrual bleedings and maintains, or even improves, bone mineral density. This observation seems to be important, not only in terms of short term clinical applications, but also in the context of the long term prevention of osteoporosis. Consequently, hormonal therapy has to be accompanied by a thorough education of patients and their parents, particularly in terms of proper nutrition and modification of levels of physical activity. Puberty is still the optimal time period for modifying environmental factors that are associated with bone mass gain.

References

1. Adams Hillard PJ, Nelson LM. Adolescent girls, the menstrual cycle, and bone health. J Pediatr Endocrinol Metab 2003; 16 (suppl 3): 673–681.
2. Seeman E. Reduced bone density in women with fractures: contribution of low peak bone density and rapid bone loss. Osteoporos Int 1994; 4 (suppl 1): 15–25.
3. Bonjour JP, Theintz G, Buchs B et al. Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. J Clin Endocrinol Metab 1991; 73: 555–563.
4. Theintz G, Buchs B, Rizzoli R et al. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab 1992; 75: 1060–1065.
5. Hernandez CJ, Beaupre GS, Carter DR. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. Osteoporos Int 2003; 14: 843–847.
6. Loud KJ, Gordon CM. Adolescent bone health. Arch Pediatr Adolesc Med 2006; 160: 1026–1032.
7. Aris RM, Merkel PA, Bachrach LK et al. Guide to bone health and disease in cystic fibrosis. J Clin Endocrinol Metab 2005; 90: 1888–1896.
8. Otis CL, Drinkwater B, Johnson M et al. American College of Sports Medicine position stand. The Female Athlete Triad. Med Sci Sports Exerc 1997; 29: i–ix.
9. Sowinska E. Verification of indications for the treatment of menstrual disorders in girls on the basis of clinical analysis, including the role of prolactin and beta-endorphin. Szczecin 1990. Doctoral dissertation.
10. Gordon CM, Nelson LM. Amenorrhea and bone health in adolescents and young women. Curr Opin Obstet Gynecol 2003; 15: 377–384.
11. Deligeoroglou E, Tsimaris P, Deliveliotou A et al. Menstrual disorders during adolescence. Pediatr Endocrinol Rev 2006; 3 (suppl 1): 150–159.
12. Sowinska-Przepiera E, Chelstowski K, Friebe Z et al. Bone mineral density in girls with functional hypothalamic amenorrhea subjected to estroprogestagen treatment — a 4-year prospective study. Gynecol Endocrinol 2011 [Epub].
13. Donato J, Jr., Cravo RM, Frazao R et al. Hypothalamic sites of leptin action linking metabolism and reproduction. Neuroendocrinology 2011; 93: 9–18.
14. Backholer K, Smith JT, Rao A et al. Kisspeptin cells in the ewe brain respond to leptin and communicate with neuropeptide Y and proopiomelanocortin cells. Endocrinology 2010; 151: 2233–2243.
15. Frisch RE, Revelle R. Height and weight at menarche and a hypothesis of menarche. Arch Dis Child 1971; 46: 695–701.
16. Hu L, Gustofson RL, Feng H et al. Converse regulatory functions of estrogen receptor-alpha and -beta subtypes expressed in hypothalamic gonadotropin-releasing hormone neurons. Mol Endocrinol 2008; 22: 2250–2259.
17. Sizonenko PC. Endocrinology in preadolescents and adolescents. I. Hormonal changes during normal puberty. Am J Dis Child 1978; 132: 704–712.
18. Zhang K, Pollack S, Ghods A et al. Onset of ovulation after menarche in girls: a longitudinal study. J Clin Endocrinol Metab 2008; 93: 1186–1194.
19. Romanowska-Łakomy H. On genesis of a young person`s personal crisis. In: Grochmal-Bach B, Knobloch-Gala A, eds. Crises developmental age children and youth: a comprehensive textbook. Impuls, Kraków 2005; 15–29.
20. Liu JH, Bill AH. Stress-associated or functional hypothalamic amenorrhea in the adolescent. Ann NY Acad Sci 2008; 1135: 179–184.
21. Bomba M, Gambera A, Bonini L et al. Endocrine profiles and neuropsychologic correlates of functional hypothalamic amenorrhea in adolescents. Fertil Steril 2007; 87: 876–885.
22. Vitoratos N, Papatheodorou DC, Kalantaridou SN et al. "Reproductive" corticotropin-releasing hormone. Ann NY Acad Sci 2006; 1092: 310–318.
23. Drolet G, Dumont EC, Gosselin I et al. Role of endogenous opioid system in the regulation of the stress response. Prog Neuropsychopharmacol Biol Psychiatry 2001; 25: 729–741.
24. Katz N, Mazer NA. The impact of opioids on the endocrine system. Clin J Pain 2009; 25: 170–175.
25. Deligeoroglou E, Athanasopoulos N, Tsimaris P et al. Evaluation and management of adolescent amenorrhea. Ann NY Acad Sci 2010; 1205: 23–32.
26. Kiapekou E, Zapanti E, Mastorakos G et al. Update on the role of ovarian corticotropin-releasing hormone. Ann NY Acad Sci 2010; 1205: 225–229.
27. Kalantaridou SN, Zoumakis E, Makrigiannakis A et al. Corticotropin-releasing hormone, stress and human reproduction: an update. J Reprod Immunol 2010; 85: 33–39.
28. Barzman DH, Patel A, Sonnier L et al. Neuroendocrine aspects of pediatric aggression: can hormone measures be clinically useful? Neuropsychiatr Dis Treat 2010; 6: 691–697.
29. Bord S, Horner A, Beavan S et al. Estrogen receptors alpha and beta are differentially expressed in developing human bone. J Clin Endocrinol Metab 2001; 86: 2309–2314.
30. Hall JM, McDonnell DP. The estrogen receptor beta-isoform (ERbeta) of the human estrogen receptor modulates ERalpha transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. Endocrinology 1999; 140: 5566–5578.
31. Monroe DG, Getz BJ, Johnsen SA et al. Estrogen receptor isoform-specific regulation of endogenous gene expression in human osteoblastic cell lines expressing either ERalpha or ERbeta. J Cell Biochem 2003; 90: 315–326.
32. Kusec V, Virdi AS, Prince R et al. Localization of estrogen receptor-alpha in human and rabbit skeletal tissues. J Clin Endocrinol Metab 1998; 83: 2421–2428.
33. Lanyon L, Armstrong V, Ong D et al. Is estrogen receptor alpha key to controlling bones' resistance to fracture? J Endocrinol 2004; 182: 183–191.
34. Veldhuis JD, Anderson SM, Patrie JT et al. Estradiol supplementation in postmenopausal women doubles rebound-like release of growth hormone (GH) triggered by sequential infusion and withdrawal of somatostatin: evidence that estrogen facilitates endogenous GH-releasing hormone drive. J Clin Endocrinol Metab 2004; 89: 121–127.
35. Sabatier JP, Guaydier-Souquieres G, Laroche D et al. Bone mineral acquisition during adolescence and early adulthood: a study in 574 healthy females 10–24 years of age. Osteoporos Int 1996; 6: 141–148.
36. Matkovic V. Skeletal development and bone turnover revisited. J Clin Endocrinol Metab 1996; 81: 2013–2016.
37. Pfeilschifter J, Scheidt-Nave C, Leidig-Bruckner G et al. Relationship between circulating insulin-like growth factor components and sex hormones in a population-based sample of 50– to 80-year-old men and women. J Clin Endocrinol Metab 1996; 81: 2534–2540.
38. Riggs BL. The mechanisms of estrogen regulation of bone resorption. J Clin Invest 2000; 106: 1203–1204.
39. Oursler MJ, Cortese C, Keeting P et al. Modulation of transforming growth factor-beta production in normal human osteoblast-like cells by 17 beta-estradiol and parathyroid hormone. Endocrinology 1991; 129: 3313–3320.
40. Hofbauer LC, Khosla S, Dunstan CR et al. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. J Bone Miner Res 2000; 15: 2–12.
41. Franchimont N, Wertz S, Malaise M. Interleukin-6: an osteotropic factor influencing bone formation? Bone 2005; 37: 601–606.
42. Kimble RB, Srivastava S, Ross FP et al. Estrogen deficiency increases the ability of stromal cells to support murine osteoclastogenesis via an interleukin-1and tumor necrosis factor-mediated stimulation of macrophage colony-stimulating factor production. J Biol Chem 1996; 271: 28890–28897.
43. Rickard DJ, Subramaniam M, Spelsberg TC. Molecular and cellular mechanisms of estrogen action on the skeleton. J Cell Biochem 1999; Suppl 32–33: 123–132.
44. Vaananen HK, Zhao H, Mulari M et al. The cell biology of osteoclast function. J Cell Sci 2000; 113: 377–381.
45. Khosla S. Minireview: the OPG/RANKL/RANK system. Endocrinology 2001; 142: 5050–5055.
46. Al-Dujaili SA, Lau E, Al-Dujaili H et al. Apoptotic osteocytes regulate osteoclast precursor recruitment and differentiation in vitro. J Cell Biochem 2011; 112: 2412–2423.
47. Shevde NK, Bendixen AC, Dienger KM et al. Estrogens suppress RANK ligand-induced osteoclast differentiation via a stromal cell independent mechanism involving c-Jun repression. Proc Natl Acad Sci USA 2000; 97: 7829–7834.

48. Hofbauer LC, Khosla S, Dunstan CR et al. Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. Endocrinology 1999; 140: 4367–4370.
49. Hadjidakis DJ and Androulakis, II. Bone remodeling. Ann NY Acad Sci 2006; 1092: 385–396.
50. Valentino R, Savastano S, Tommaselli AP et al. The influence of intense ballet training on trabecular bone mass, hormone status, and gonadotropin structure in young women. J Clin Endocrinol Metab 2001; 86: 4674–4678.
51. Joy EA and Campbell D. Stress fractures in the female athlete. Curr Sports Med Rep 2005; 4: 323–328.
52. Karlsson MK, Weigall SJ, Duan Y et al. Bone size and volumetric density in women with anorexia nervosa receiving estrogen replacement therapy and in women recovered from anorexia nervosa. J Clin Endocrinol Metab 2000; 85: 3177–3182.
53. Gibson JH, Mitchell A, Reeve J et al. Treatment of reduced bone mineral density in athletic amenorrhea: a pilot study. Osteoporos Int 1999; 10: 284–289.
54. Warren MP, Brooks-Gunn J, Fox RP et al. Persistent osteopenia in ballet dancers with amenorrhea and delayed menarche despite hormone therapy: a longitudinal study. Fertil Steril 2003; 80: 398–404.
55. Munoz MT, Morande G, Garcia-Centenera JA et al. The effects of estrogen administration on bone mineral density in adolescents with anorexia nervosa. Eur J Endocrinol 2002; 146: 45–50.
56. Klibanski A, Biller BM, Schoenfeld DA et al. The effects of estrogen administration on trabecular bone loss in young women with anorexia nervosa. J Clin Endocrinol Metab 1995; 80: 898–904.
57. Ferrari SL. Osteoporosis: a complex disorder of aging with multiple genetic and environmental determinants. World Rev Nutr Diet 2005; 95: 35–51.
58. Havill LM, Mahaney MC, Binkley T et al. Effects of genes, sex, age, and activity on BMC, bone size, and areal and volumetric BMD. J Bone Miner Res 2007; 22: 737–746.
59. Liu SL, Lebrun CM. Effect of oral contraceptives and hormone replacement therapy on bone mineral density in premenopausal and perimenopausal women: a systematic review. Br J Sports Med 2006; 40: 11–24.
60. Castelo-Branco C, Vicente JJ, Pons F et al. Bone mineral density in young, hypothalamic oligoamenorrheic women treated with oral contraceptives. J Reprod Med 2001; 46: 875–879.
61. Garnero P, Sornay-Rendu E, Delmas PD. Decreased bone turnover in oral contraceptive users. Bone 1995; 16: 499–503.
62. Grinspoon SK, Friedman AJ, Miller KK et al. Effects of a triphasic combination oral contraceptive containing norgestimate/ethinyl estradiol on biochemical markers of bone metabolism in young women with osteopenia secondary to hypothalamic amenorrhea. J Clin Endocrinol Metab 2003; 88: 3651–3656.
63. WHO Statement on hormonal contraception and bone health. Available on: http://www.who.int/reproductivehealth/publications/family_planning/hc_bone_he lth/en/index.html. Accessed September 6, 2011.
64. Cromer BA, Scholes D, Berenson A et al. Depot medroxyprogesterone acetate and bone mineral density in adolescents — the Black Box Warning: a Position Paper of the Society for Adolescent Medicine. J Adolesc Health 2006; 39: 296–301.
65. Lopez LM, Grimes DA, Schulz KF et al. Steroidal contraceptives: effect on bone fractures in women. Cochrane Database Syst Rev 2006; CD006033.