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Assessment of the relationship between melatonin, hormones of the pituitary-ovarian, -thyroid and -adrenocortical axes, and osteoprotegerin and its ligand sRANKL in girls with anorexia nervosa

Ocena zależności między melatoniną, hormonami osi przysadkowo-jajnikowej, -tarczycowej i -korowo-nadnerczowej a osteoprotegeryną i jej ligandem sRANKL u dziewcząt z jadłowstrętem psychicznym

Zofia Ostrowska[1,A,C,D,E,F], Katarzyna Ziora[2,D,F], Joanna Oświęcimska[2,D,F], Kinga Wołkowska-Pokrywa[1,B], Bożena Szapska[1,B]

[1] Clinical Biochemistry Division, Department of Biochemistry, Medical University of Silesia, Zabrze, Poland
[2] Department of Pediatrics, Medical University of Silesia, Zabrze, Poland

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Background: It has been suggested that disturbances in melatonin (MEL) secretion might play a role in osteoporosis development in females with anorexia nervosa (AN). It might be hypothesized that changes in the levels of hormones of the pituitary-ovarian, -thyroid and -adrenocortical axes might mediate the potential relationship between MEL and bone tissue.

Aim: We investigated whether a relationship existed between MEL and LH, FSH-E2, TSH-FT3, FT4 and ACTH-cortisol axes in girls with AN. We also aimed to establish whether such a relationship might adversely affect the balance of the OPG/sRANKL system.

Material/Methods: Eighty-six girls with AN and 21 healthy subjects aged 12.6 to 18.2 years participated in the study. The serum levels of hormones as well as OPG and sRANKL were determined by radio-immunoassay (RIA), immunoradiometric assay (IRMA) or enzyme-linked immunosorbent assay (ELISA) methods.

Discussion: Our study participants with AN showed a significant reduction in body mass and body mass index (BMI), a decrease in LH, E2 and FT3 concentrations, increased MEL concentration at 02.00 hours and increased amplitude between its nocturnal and morning levels (Δ MEL2.00/9.00) as well as an increase in cortisol concentration. These changes were associated with a significant increase of OPG and sRANKL levels and a decrease in the OPG/sRANKL ratio. BMI values correlated positively with LH, FSH, E2, FT3 and the OPG/sRANKL ratio while the correlation between BMI and cortisol was negative. Δ MEL2.00/9.00 correlated positively with cortisol and negatively with LH, FSH, E2, FT3 concentrations and the OPG/sRANKL ratio. A positive correlation was observed between LH, E2 and the OPG/sRANKL ratio as well as between cortisol and sRANKL while the correlation between LH and OPG as well as between cortisol and the OPG/sRANKL ratio was negative. E2 and LH were shown to be significant and independent predictors of Δ MEL2.00/9.00. LH turned out to be a significant and independent predictor of OPG, cortisol and FT3 were significant and independent predictors of sRANKL, while LH, E2, Δ MEL2.00/9.00 and FT3 were significant predictors of the OPG/sRANKL ratio.

Conclusions: Alterations in OPG and sRANKL levels observed in girls with AN are associated with changes in nocturnal MEL secretion, the circadian rhythm of MEL, and LH, E2, FT3 and cortisol levels. Dysregulatiotn of the relationships between MEL and LH, E2, FT3 and cortisol found in girls with AN might affect the balance of the OPG/sRANKL system. Low values of the OPG/sRANKL ratio associated with high OPG and sRANKL levels suggest some defect in the mechanism compensating for bone remodeling disturbances.

Keywords: **anorexia nervosa · female adolescents · melatonin · LH, FSH-E2 axis · ACTH-cortisol axis · TSH-FT3, FT4 axis · OPG · sRANKL**

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Author address: Prof. Zofia Ostrowska, Clinical Biochemistry Division, Department of Biochemistry Medical University of Silesia, ul. Jordana 19, 41-808 Zabrze, Poland; e-mail: ozdrasiek@wp.pl

Introduction

Females with anorexia nervosa (AN) show disturbances in the production of multiple osteotropic factors, mainly hormones, including those of the pituitary-ovarian, -thyroid and -adrenocortical axes [10,12,14,23,26,28,29,30,32, 33,35,47,64,66,73,75] all controlled by the main neurohormone secreted by the pineal gland, i.e., melatonin (MEL) [4,67]. The disturbances might affect the skeletal system in adult [12,14,27,32,48,74] and adolescent females with AN [3,12,17,31,33,34,36,37,41,42,43,45,46,61], the effect occurring directly and/or indirectly through the regulation of osteoprotegerin (OPG) and/or receptor activator of nuclear factor-kB ligand (RANKL) [15,72]. It has also been documented that MEL might affect bone remodeling [8,58]. Koyama et al. [22] demonstrated that pharmacological doses of MEL inhibited in vitro expression of RANKL and increased OPG expression in mouse MC3T3-E1 osteoblastic cells, suggesting that the hormone participated in OPG and/or RANKL-mediated skeletal effects. Models of experimental postmenopausal osteoporosis [24,25,53,56] and investigations carried out in peri- and postmenopausal women [18,50,51,54,58] have revealed that, apart from age-related estrogen deficiency and altered concentrations of several well-recognized local and systemic factors, a decrease in MEL levels might also play a role in the development of osteoporosis, possibly via the OPG/sRANKL system. Based on the above, we hypothesized that MEL secretion dysregulation observed by several authors in young females [2,6,11,16,71] and girls [9,59] with AN might induce bone loss acting through OPG and/or RANKL. Some authors investigated OPG concentrations in girls with AN [38,43,59,60] and found a significant elevation of serum levels of this cytokine similar to the increase seen in young females with AN [48]. Munoz-Calvo et al. [46] and Ostrowska et al. [59,60] investigated serum OPG and sRANKL in adolescents with AN, and demonstrated suppression of the OPG/sRANKL ratio with normal [46] or significantly increased OPG concentrations [59,60] and a significant increase in sRANKL levels [46,59,60]. Our previous studies regarding girls with AN [59] showed a significant increase in nocturnal MEL concentrations and an increase in the amplitude between nocturnal and morning levels of the hormone (Δ MEL2.00/9.00) with marked suppression of bone markers (osteocalcin [OC] and carboxy-terminal collagen cross-links [CTx]), increase of the RANKL/RANK/OPG system cytokines (especially sRANKL) and a decline of the OPG/sRANKL ratio. Changes in nocturnal MEL levels correlated negatively with the concentrations of bone markers under investigation, and positively with sRANKL. Changes in Δ MEL2.00/9.00 correlated negatively with CTx levels and the OPG/sRANKL ratio [59]. Thus, we formulated a hypothesis that bone metabolism disturbances observed in girls with AN might be related to alterations in nocturnal synthesis of MEL possibly mediated by RANKL. The relationship between MEL secretion and the OPG/RANKL system in patients with AN has not been investigated so far; a potential role of hormones of the pituitary-ovarian (LH, FSH-E2), -thyroid (TSH-FT3, FT4) and -adrenocortical axes (ACTH-cortisol) has not been investigated either.

We investigated whether a relationship existed between MEL and LH, FSH-E2, TSH-FT3, FT4 and ACTH-cortisol axes in girls with AN. We also aimed to establish whether such a relationship might adversely affect the balance of the OPG/sRANKL system.

Material and methods

The study involved 86 girls aged 12.6 to 18.2 years, hospitalized at the Pediatric Endocrinology Ward of the Pediatric Department in Zabrze (Medical University of Silesia in Katowice, Poland), who, following pediatric examination and psychiatric consultation, were diagnosed with AN according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [1]. The average disease duration was 12.1 months (range 3-36 months). All examined girls were at Tanner puberty stages IV and V. They had normal liver and kidney functions; no severe somatic complications or psychiatric disorders were observed. On recruitment, no patients were taking medications known to affect the nutritional and bone status, including calcium or vitamin D supplements. Exclusion criteria included severe somatic complications, i.e., gastrointestinal bleeding, chronic diarrhea, dehydration, peptic ulcer disease, liver, or kidney dysfunction, and pharmacotherapy, e.g., anti-anxiety or psychotropic drugs. During hospitalization, patients were placed at bed rest, which is the standard care. The control group comprised 21 healthy, regularly menstruating adolescent females between 13 and 17 years of age, with normal body mass and no endocrine or other disorders that could possibly influence adipose and bone tissue metabolism. During the three-month period before the study, the control participants did not take calcium or vitamin D3 supplements.

The height (stadiometer), body mass (electronic scale), and body mass index (BMI) of each female subject were measured and documented. Blood samples (8 ml) for determination of MEL, hormones of the pituitary-ovarian (luteinizing hormone – LH, follicle-stimulating hormone – FSH and 17b-estradiol – E2), -thyroid (thyroid-stimulating hormone – TSH, free triiodothyronine – FT3 and free thyroxin – FT4), and -adrenocortical (adrenocorticotropin – ACTH and cortisol – C) axes, OPG and its soluble ligand sRANKL were always collected under the same conditions, at 09.00 hours following a 12-hour fast (in the case of MEL an additional 3 ml sample was collected at 02.00 hours under red lighting conditions). Centrifuged serum was frozen and stored at -75°C until assay. Determinations of MEL were performed by RIA (DRG, USA), ACTH, LH, FSH, TSH by IRMA (Immunotech, France), E2 by ELISA (IBL International GmbH, USA), FT3, FT4, C by RIA (Immunotech, France), and OPG and sRANKL by ELISA (Biomedica, Austria). The sensitivity, intra-assay error, and inter-assay error were 1 pg/ml, 7.5% and 9.5% for MEL, 1.2 pg/ml, 7.6% and 8.2% for ACTH, 0.2 U/l, 5.9% and 6.7% for LH, 0.2 U/l, 2.9% and 5.8% for FSH, 0.015 mU/l, 4.9 and 5.8% for TSH, 9.71 pg/ml, 4.7% and 7.8% for E2, 0.33 pg/ml, 5.4% and 6% for FT3, 0.31 ng/dl, 2.9% and 4% for FT4, 0.35 µg/dl, 5.6% and 8.7% for C, 0.14 pmol/l, 7% and 7.5% for OPG, and 0.04 pmol/l, 5% and 7% for sRANKL.

The database was prepared using Excel 2000 (Microsoft Corporation). Statistical analysis was carried out with Statistica 5.5 for Windows (StatSoft Inc., USA). The t-test was used to determine the significance of intergroup differences (normal distribution of variables). In the case of non-normal distribution, the significance was tested using the Mann-Whitney U test. We used univariate and multiple regression analyses to determine predictors of OPG and sRANKL levels, the OPG/sRANKL ratio, and Δ MEL2.00/9.00 value.

The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice (KNW/0022/KB1/105/09). Written informed consent was obtained from all examined participants and their parents or legal guardians before participation.

Results

Compared to the controls, our study participants with AN showed a significant reduction of the mean body mass and BMI, and a significant increase in the mean MEL concentration at 02.00 hours, and of Δ MEL2.00/9.00. These changes were associated with a marked decrease in the mean LH, E2 and FT3 concentrations, and a significant increase in the mean cortisol (C) levels when compared to the controls. A significant increase in the mean OPG and sRANKL levels, and a decrease in the OPG/sRANKL ratio were also found (Table 1).

BMI of girls with AN correlated positively and significantly with LH, FSH, E2, FT3 levels and the OPG/sRANKL ratio while its correlation with cortisol concentration was negative. Δ MEL2.00/9.00 correlated positively and significantly with cortisol and negatively with LH, FSH, E2, FT3 concentrations and the OPG/sRANKL ratio. A significant and positive correlation was also revealed between LH, E2 and the OPG/sRANKL ratio as well as between cortisol and sRANKL concentrations while the correlation between LH and OPG and between cortisol and the OPG/sRANKL ratio was negative (Tables 2 and 3).

Using a mixed-model regression analysis, we examined the relationship between LH, FSH, E2, TSH, FT3, FT4, ACTH, cortisol, and Δ MEL2.00/9.00 as well as between Δ MEL2.00/9.00, LH, FSH, E2, TSH, FT3, FT4, ACTH, cortisol, and OPG, sRANKL and OPG/sRANKL ratio (Table 3). In patients with AN, E2 and LH were shown to be significant and independent predictors of Δ MEL2.00/9.00 ($R2 = 0.0688$; $p = 0.0056$). However, LH was a significant and independent predictor of OPG ($R2 = 0.0661$; $p = 0.0090$); cortisol and FT3 were significant and independent predictors of sRANKL ($R2 = 0.0874$; $p = 0.0005$); LH, E2, Δ MEL2.00/9.00 and FT3 were significant and independent predictors of OPG/sRANKL ratio ($R2 = 0.5184$; $p < 0.0001$).

Discussion

The majority of untreated females with AN demonstrate suppression of gonadoliberin (GnRH), FSH and/or LH, E2 and progesterone secretion [13,73]. There are also disturbances in positive feedback effects of E2 on pituitary LH secretion, and, most probably, in E2 actions on GnRH production in the hypothalamus [75]. Individuals with AN are

Table 1. Mean values of age, height, body mass, body mass index (BMI), chosen hormonal parameters, osteoprotegerin (OPG), soluble receptor activator of nuclear factor-κB ligand (sRANKL) and OPG/sRANKL ratio in girls with anorexia nervosa and the control group

Variables		Groups	
		Anorexia nervosa (n-86)	Control group (n-21)
Age [years]		15.48 ± 1.58	15.86 ± 1.94
Height [m]		1.63 ± 0.06	1.64 ± 0.07
Body mass [kg]		39.51 ± 6.28[b]	52.59 ± 6.67
BMI [kg/m^2]		14.29 ± 1.95[b]	19.67 ± 1.51
MEL [pmol/l]	9.00	72.54 ± 12.34	67.22 ± 19.31
	2.00	798.54 ± 154.76[b]	583.09 ± 134.17
	Δ2.00/9.00	714.52 ± 118.32[b]	554.93 ± 101.18
Lutropin (LH) [U/l]		1.18 ± 2.52[c]	5.69 ± 4.76
Folitropin (FSH) [U/l]		4.09 ± 3.68	4.87 ± 2.02
17β-estradiol (E2) [pg/ml]		32.24 ± 19.4[c]	71.26 ± 35.90
Tyreotropin (TSH) [mU/l]		2.09 ± 1.01	2.39 ± 0.91
Free triiodothyronin (FT3) [pg/ml]		2.05 ± 0.71[b]	3.62 ± 0.69
Free thyroxin (FT4) [ng/dl]		1.47 ± 0.24	1.14 ± 0.26
Adrenocorticotropin (ACTH) [pg/ml]		24.27 ± 20.80	29.92 ± 15.03
Cortisol (C) [μg/dl]		24.17 ±11.91[b]	13.45 ± 4.67
OPG [pmol/l]		4.05 ± 0.87[a]	3.48 ± 0.71
sRANKL [pmol/l]		0.421 ± 0.143[b]	0.276 ± 0.117
OPG/sRANKL ratio		9.61 ± 0.35[a]	12.65 ± 4.88

[a]$p \leq 0.05$, [b]$p \leq 0.01$, [c]$p \leq 0.001$ vs control group

also characterized by E2 metabolism alterations, which, as some authors believe, are secondary to low T3 syndrome [75]. Similar to other investigations [12,33,36,41,42], and compared to the control group, our patients with AN also had a significant decrease in LH and E2 levels, additionally associated with a decrease in FT3 levels; this seems to confirm the above concept. It has been well established that the hypothalamic-pituitary-gonadal axis suppression in AN is not only related to weight loss, body fat decrease, malnutrition and an increase in physical activity [75]. Pituitary-adrenocortical axis (p-ac) hyperactivity has also been mentioned as a potential suppressor of the hypothalamic-pituitary-gonadal axis. Secretion of sex hormones can also be dysregulated by overactivity of corticotropin-releasing hormone (CRH) secreting neurons [30]. Some contribution of decreased peripheral androgen-to-estrogen conversion, resulting from adipose tissue loss, cannot be ruled out [70]. Factors involved in GnRH release disturbances also include the loss of pulsatile leptin secretion, suppression of gonadotropic effects of insulin and IGF-I, dysregulation of the dopamine system, and increased levels of endogenous opioids [45,70]. The above-mentioned p-ac hyperactivity is suggestive of primary CRH hypersecretion or peripheral syndrome of resistance to glucocorticosteroids [29]. Hypercortisolism (also found in our girls with AN) without any disruption of the normal circadian rhythm of cortisol secretion as well as less suppression of cortisol after dexamethasone ingestion are indicators of p-ac axis hyperactivity [29,33,36,45]. Although normal ACTH concentrations are sometimes observed (which was also the case in our investigations), the response to ACTH administration is usually reduced [45]. The most frequent thyroid abnormality shown by females with AN includes decreases in serum total and free triiodothyronine (T3 and FT3), commonly referred to as the low T3 syndrome [47,64,66]. TSH concentrations tend to be normal; measurements of total and free thyroxine (T4 and FT4) are within the normal reference range but may also be low [45,70]. TSH levels of our patients with AN were comparable to the control values, FT3 was decreased while FT4 was within the normal range. Similar results were demonstrated in female adolescents examined by other authors [12,33,36,64]. Low T3 levels seem to result from compromised peripheral conversion of T4 to T3 due to preference conversion of T3 to hormonally inactive reverse T3 [28]. Decreased TSH seen in a few females with AN might be related to hyperexpression of dopaminergic receptors [28].

Since the secretion of hormones of the pituitary-ovarian, -thyroid and -adrenocortical axes is regulated by MEL [4,67], it might be suspected that abnormal concentra-

Table 2. Correlation between body mass index (BMI), chosen hormonal parameters, osteoprotegerin (OPG), soluble receptor activator of nuclear factor-κB ligand (sRANKL) and OPG/sRANKL ratio values in girls with anorexia nervosa (AN; n-86)

Variables		Values of correlation coefficients – R
BMI [kg/m^2]	Melatonin (MEL) [pmol/l] Δ2.00/9.00	NS
	Lutropin (LH) [U/l]	0.457^c
	Folitropin (FSH) [U/l]	0.457^c
	17β-estradiol (E2) [pg/ml]	0.228^a
	Thyreotropin (TSH) [mU/l]	NS
	Free triiodothyronin (FT3) [pg/ml]	0.293^b
	Free thyroxin (FT4) [ng/dl]	NS
	Adrenocorticotropin (ACTH) [pg/ml]	NS
	Cortisol (C) [μg/dl]	-0.278^b
	OPG [pmol/l]	NS
	sRANKL [pmol/l]	NS
	OPG/sRANKL ratio	0.208^a

$^a p \leq 0.05$, $^b p \leq 0.01$, $^c p \leq 0.001$ – statistically significant values of correlation coefficients

Table 3. Correlation[1] between chosen hormonal parameters, osteoprotegerin (OPG), soluble receptor activator of nuclear factor-κB ligand (sRANKL) and OPG/sRANKL ratio values in girls with anorexia nervosa (AN; n-86)

Variables	ΔMEL [pmol/l] 2.00/9.00	OPG [pmol/l]	sRANKL [pmol/l]	OPG/ sRANKL ratio
Melatonin (MEL) [pmol/l] Δ2.00/9.00	–	NS	NS	-0.262^b
Lutropin (LH) [U/l]	-0.667^c	-0.250^b	NS	0.629^c
Folitropin (FSH) [U/l]	-0.344^c	NS	NS	NS
17β-estradiol (E2) [pg/ml]	-0.262^b	NS	NS	0.587^c
Tyreotropin (TSH) [mU/l]	NS	NS	NS	NS
Free triiodothyronin (FT3) [pg/ml]	-0.511^c	NS	NS	NS
Free thyroxin (FT4) [ng/dl]	NS	NS	NS	NS
Adrenocorticotropin (ACTH) [pg/ml]	NS	NS	NS	NS
Cortisol (C) [μg/dl]	0.402^c	NS	0.236^a	-0.402^c
Stepwise regression model[2]	E2, LH	LH	C, FT3	LH, E2, Δ MEL, FT3
R^2	0.0688	0.0661	0.0874	0.5184
p	0.0056	0.0090	0.0005	< 0.0001

NS – non significant values of correlation coefficients ($p > 0.05$)

$^a p \leq 0.05$, $^b p \leq 0.01$, $^c p \leq 0.001$ – statistically significant values of correlation coefficients

[1] Displayed value represents the model variance explained by these parameters

[2] Hormones entering the stepwise regression model

tions of these hormones in adult and adolescent females with AN might be induced by some abnormalities in MEL production. Women with AN generally demonstrate significant alterations in nocturnal MEL concentrations, which, however, are not associated with disturbances in the acrophase of circadian rhythm. Tortosa et al. [71] observed a significant increase in the mean diurnal and nocturnal plasma MEL concentrations in young females with AN but without changes in the MEL circadian profile. Other investigators [2,6,11,16] found a significant increase in nocturnal MEL concentrations without disturbances in acrophase values. Still others [5,44,62] did not observe any significant alterations in circadian MEL concentrations of AN patients. Kennedy et al. [19,20,21], on the other hand, demonstrated significantly lower nocturnal MEL values in AN females suffering from major depression. Very few authors have investigated MEL concentrations in adolescent girls with AN [9,59]. Dalery et al. [9] noted significantly higher nocturnal MEL levels in adolescent girls with AN and depression compared to age-matched

controls with depression. However, the authors did not compare their results to those of healthy girls. In our previous [59] and present investigations regarding adolescent girls with AN, we demonstrated a significant increase in nocturnal MEL concentrations compared to the controls. Δ MEL2.00/9.00 was also increased, suggesting dysregulation of the circadian rhythm of this hormone. The negative correlation between Δ MEL2.00/9.00 and LH, FSH, E2, FT3 concentrations, and the positive correlation of this parameter with cortisol levels observed in the present investigations seem to confirm the concept that alterations in nocturnal MEL secretion observed in girls with AN might induce some disturbances in gonadotropins, E2, FT3 and cortisol secretions. Stepwise regression analysis revealed, that, in girls with AN, up to 6.88% of the Δ MEL2.00/9.00 variance was due to E2 and LH. This might indicate that, in adolescent girls with AN, some interaction exists between E2, LH, and Δ MEL2.00/9.00.

It has recently been suggested that alterations in the concentrations of MEL, some hormones of the pituitary-ovarian, -thyroid, and -adrenocortical axes, hormones of the somatotropin axis, and adipose tissue hormones might affect the skeleton in girls with AN [3,12,17,31,33,34,36,37,41,42,43,45,46,59,61], and that these alterations might be significantly affected by the OPG/RANKL system [46,59,60]. As no multidirectional investigations into potential changes in the relationship between MEL and hormones of the pituitary-ovarian, -thyroid, and adrenocortical axes and the effect thereof on the balance of the OPG/sRANKL system have been carried out so far, we decided to try to shed some light on this issue. It should be emphasized that previous investigations regarding girls with AN mainly concerned the relationships between E2, cortisol, leptin, growth hormone (GH), IGF-I and bone markers and/or bone mineral density (BMD) [3,12,17,31,33,34,36,37,41,42,43,45,46,61]. These research projects generally revealed suppression of bone formation and resorption markers which correlated with BMI and/or BMD, indicating decreased bone turnover [7,33,37,39,43,45,61,65,68,69]. It has also been shown that E2 [12,39,40], GH [12,38,39,40], IGF-I [38,68], cortisol [12,38,39,40] and/or leptin [39] were predictors of bone turnover and/or BMD. However, the concentrations of the RANKL/RANK/OPG system cytokines in relation to MEL, E2, cortisol, leptin, GH or IGF-I have only rarely been investigated [38,43,46,59]. An increase in serum OPG of AN girls found by Misra et al. [38,43] correlated negatively with BMI, leptin and BMD; no correlations were observed between serum OPG and E2, serum IGF-I, and urinary free cortisol. Misra et al. [43] believe that the decrease in markers of bone resorption observed in this population may reflect the suppressive effects of higher OPG levels on osteoclast differentiation and activity. However, the authors did not investigate RANKL concentrations or the OPG/sRANKL ratio. Our previous investigations of girls with AN [59,60] revealed suppression of bone formation and resorption markers (OC and CTx) as well as a significant increase in serum OPG and sRANKL levels associated with a significant decrease in the OPG/sRANKL ratio. We also found a significant positive correlation between BMI and CTx, BMI and the OPG/sRANKL ratio, and OC and the OPG/sRANKL ratio, while OC concentrations correlated negatively with CTx and sRANKL. Some alterations in the relationship between OPG and sRANKL and bone markers [59,60], and low values of the OPG/sRANKL ratio associated with high OPG and sRANKL concentrations found in the present study seem to suggest disturbances in the mechanism that controls the bone remodeling process or, alternatively, in the mechanism compensating for enhanced bone mass loss. Munoz-Calvo et al. [46] observed a significant decrease in the OPG/RANKL ratio in girls with AN, which correlated with an increase in serum RANKL. They also found a high positive correlation between OPG/RANKL and BMD, but did not observe a significant increase in serum OPG or a relationship between OPG and RANKL, leptin or BMD. According to Munoz-Calvo et al. [46], the decrease in the OPG/RANKL ratio in girls with AN might only partly account for the increased bone mass loss observed in this population. E2 has been considered the main determinant of bone turnover, BMD and/or OPG in females and adolescent girls with AN. It should be remembered though that cytokines of the RANKL/RANK/OPG system, and OPG in particular, are not exclusively regulated by E2; they are also under the influence of other cytokines, hormones and mesenchymal transcription factors, which can change their concentrations in the direction opposite to that of E2 [15,72]. The ultimate result of these interactions is difficult to predict.

The results of our investigations show that changes in the secretion of several hormones of the pituitary-ovarian, -thyroid, and -adrenocortical axes observed in girls with AN correlated with OPG, sRANKL and/or the OPG/sRANKL ratio, and were also associated with a significant increase of MEL secretion at 02.00 hours (consistent with peak MEL secretion). Δ MEL2.00/9.00 was also significantly increased, correlating positively with cortisol, and negatively with LH, FSH, E2 and FT3 levels and the OPG/sRANKL ratio. A positive correlation was also found between LH, E2 and the OPG/sRANKL ratio as well as between cortisol and sRANKL, while the correlation between LH and OPG as well as between cortisol and OPG/sRANKL ratio was negative. Stepwise regression analysis revealed that, in girls with AN, up to 6.61% of the OPG variance was due to LH; up to 8.74% of the sRANKL variance was due to cortisol and FT3; and up to 51.84% of the OPG/sRANKL ratio variance was due to LH, E2, Δ MEL2.00/9.00 and FT3. These results suggest a potential role of MEL in the mechanism leading to BMD decrease in girls with AN, which occurs as a consequence of bone turnover disturbances. Stepwise regression analysis also suggests the involvement of LH, E2, FT3 and cortisol in the mechanism.

Several experimental and clinical studies have demonstrated a potential effect of MEL on bone tissue [8,58]. It has been found that lighting conditions, pinealectomy and prolonged MEL administration modify the circa-

dian bone tissue metabolism in rats [52,53,55]. Research into ovariectomized rats [24,25,53,56] and the few investigations conducted so far in postmenopausal women [18,50,51,54] have indicated that MEL deficiency might, among other factors, induce postmenopausal osteoporosis. However, MEL administration to neutered female and male rats causes BMD increase and suppression of bone markers, and especially bone resorption markers [24,25,53,55,57]. It has also been demonstrated that hypersecretion of MEL found in obese individuals might exert beneficial effects on postmenopausal bone tissue [49]. Based on animal experiments and human studies, some authors suggest that MEL can directly and/or indirectly affect bone formation and resorption processes [8,58]. The latter might be under considerable influence of sex and adrenal steroids, thyroid hormones and IGF-I [52,55]. Other authors believe that MEL has an indirect mediatory effect on the inhibition of bone resorption [22], which seems to be confirmed by sparse human studies, mainly in obese postmenopausal women [57,58] and female patients with postmenopausal osteoporosis [50]. Our correlation analysis revealed that alterations in gonadotropic hormones, E2, FT3 and cortisol seen in girls with AN may not only modify the circadian rhythm of MEL secretion (as assessed based on Δ MEL 2.00/9.00), but also (especially LH and E2) mediate the effects of MEL on the OPG/RANKL system. Thus, it can be suspected that the above hormones might influence the OPG/RANKL system not only directly [15,72] but also via MEL mediation. The investigations of Pawlikowska et al. [63] also indicate that the dysfunction of the hypothalamic-pituitary-gonadal axis observed in AN patients might play a role in the dysregulation of MEL secretion.

Conclusions

• Alterations in OPG and sRANKL levels observed in girls with AN are associated with changes in nocturnal MEL secretion, the circadian rhythm of melatonin, and LH, E2, FT3 and cortisol levels.

• Dysregulation of the relationships between MEL and LH, E2, FT3 and cortisol found in girls with AN might affect the balance of the OPG/sRANKL system.

• Low values of the OPG/sRANKL ratio associated with high OPG and sRANKL levels seen in girls with AN suggest some defect in the mechanism compensating for bone remodeling disturbances.

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