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Melatonin, the RANKL/RANK/OPG system, and bone metabolism in girls with anorexia nervosa

Melatonina, system RANKL/RANK/OPG a metabolizm kostny u dziewcząt z jadłowstrętem psychicznym

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Abstract

Introduction: Young women and girls with anorexia nervosa (AN) suffer from abnormalities in melatonin (MEL) secretion, especially in the nocturnal phase. This is paralleled by a considerable bone mass loss and abnormalities of bone metabolism. As melatonin has been implicated in playing a role in inducing osteoporosis and that the effect could be mediated by the RANKL/RANK/OPG system, we decided to investigate the potential associations between MEL and bone status in girls with AN.
Aim: To evaluate the relationship between MEL, bone metabolism (as assessed by serum levels of bone turnover markers [OC and CTx]), and OPG and sRANKL in girls with AN.
Material and methods: A total of 57 girls with AN and 13 healthy girls, between 13 and 18 years of age, were enrolled in the study, and we evaluated BMI, fasting levels of OC, CTx, OPG and sRANKL, and levels of MEL (fasting levels and the levels at 2 a.m., at which time the secretion of the hormone peaks).
Results: We found a significantly increased mean serum level of MEL at 2 a.m. and an increased amplitude between nocturnal and morning levels of the hormone in girls with AN. We also showed a considerable suppression of the mean OC and CTx levels and an increase in serum OPG and RANKL levels paralleled by a significantly reduced OPG/sRANKL ratio. The changes in the MEL levels at 2 a.m. showed a statistically significant negative correlation with levels of the bone markers and a positive correlation with sRANKL. The changes in the amplitude between the nocturnal and morning levels of MEL showed a negative correlation with CTx levels and the OPG/ /sRANKL ratio.
Conclusions: Our results indicate that the abnormalities of bone metabolism in girls with AN are associated with changes in the nocturnal levels of MEL with RANKL appearing to play an important role in this mechanism. The increased amplitude between the nocturnal and morning levels of MEL may adversely affect the bone tissue in girls with AN with the effect most likely resulting from influences on the OPG/RANKL balance. **(Pol J Endocrinol 2010; 61 (1): 117–123)**

Key words: *melatonin, bone status, anorexia nervosa, girls*

Streszczenie

Wstęp: U młodych kobiet i dziewcząt z jadłowstrętem psychicznym (AN, *anorexia nervosa*) stwierdza się zaburzenia w wydzielaniu melatoniny (MEL, *melatonin*), zwłaszcza w fazie nocnej. Jednocześnie obserwuje się znaczny ubytek masy kostnej i zaburzenia w metabolizmie tkanki kostnej. Ponieważ istnieją sugestie, że MEL może mieć pewien udział w indukowaniu osteoporozy, i że efekt ten może być realizowany za pośrednictwem systemu RANKL/RANK/OPG, postanowiono zbadać ewentualne powiązania między MEL a stanem kośćca u dziewcząt z AN. Celem badań była ocena związku między MEL, metabolizmem tkanki kostnej (ocenianym na podstawie oznaczeń w surowicy krwi markerów obrotu kostnego (OC i CTx) a OPG i sRANKL u dziewcząt z AN.
Materiał i metody: Badaniami objęto 57 dziewcząt z AN i 13 zdrowych w wieku 13–18 lat, u których oceniono BMI oraz stężenia OC, CTx, OPG i sRANKL na czczo oraz MEL (2-krotnie w ciągu doby: na czczo i o godz. 2.00, odpowiadającej maksimum wydzielania hormonu).
Wyniki: U dziewcząt z AN stwierdzono istotne zwiększenie średniego stężenia MEL w surowicy o godzinie 2.00 oraz wzrost amplitudy pomiędzy nocnymi i porannymi wartościami hormonu. Wykazano także znaczną supresję średnich stężeń OC i CTx oraz wzrost OPG i sRANKL w surowicy przy istotnie obniżonej wartości wskaźnika OPG/sRANKL. Zmiany w stężeniach MEL o godzinie 2.00 korelowały ujemnie, znamiennie statystycznie ze stężeniami badanych markerów kostnych, a dodatnio z sRANKL. Zmiany w wartościach amplitudy pomiędzy nocnymi i porannymi stężeniami MEL korelowały ujemnie ze stężeniami CTx i wartościami wskaźnika OPG/sRANKL.
Wnioski: Uzyskane wyniki wskazują, że zaburzenia metabolizmu kostnego u dziewcząt z AN są związane ze zmianami stężeń MEL w godzinach nocnych, a istotną rolę w tym mechanizmie wydaje się odgrywać RANKL. Zwiększenie amplitudy pomiędzy nocnymi i porannymi stężeniami MEL może niekorzystnie wpływać na tkankę kostną u dziewcząt z AN; efekt ten jest najprawdopodobniej realizowany poprzez wpływ na równowagę OPG/RANKL. **(Endokrynol Pol 2010; 61 (1): 117–123)**

Słowa kluczowe: *melatonina, stan kośćca, jadłowstręt psychiczny, dziewczęta*

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Introduction

Osteoporosis is one of the common complications of anorexia nervosa (AN) [1–4]. Its aetiology is multifactorial, and the multiaxial disturbances of the formation and release of osteotropic factors, mainly hormones and possibly also melatonin (MEL), and cytokines may lead to reduced bone mineral density (BMD) or a lack of the expected BMD rise during puberty [1–3, 5, 6]. This results from excessive bone resorption and insufficient bone formation. In light of the most recent evidence, the effects of these factors, including MEL, on bone remodelling may be mediated by their direct influence on the receptor activator of nuclear factor-κB ligand/ /receptor activator of nuclear factor-κB/osteoprotegerin (RANKL/RANK/OPG) system [7–11]. It may also result from their direct inhibitory effect on the formation of pro-resorptive cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor α (TNF-α), and macrophage colony stimulating factor (M-CSF), and from their stimulatory effects on the formation of transforming growth factor β (TGF-β) and insulin-like growth factor 1 (IGF-1), which secondarily modulate the RANKL/RANK/OPG system [12–14].

RANKL, which is formed in the bone by osteoblasts and bone marrow stromal cells, is a type II transmembrane protein devoid of the signal peptide which stimulates the maturation, activity, and apoptosis of osteoclasts. It also affects the cells of the osteoclast cell line through RANK, which is a type I transmembrane protein of the TNF receptor superfamily that is only expressed by osteoclasts and its precursors. The stimulation of RANK triggers the differentiation of pluripotent osteoclast precursor cells to mature osteoclasts, reorganisation of the cytoskeleton, increased resorptive activity of mature osteoclasts, and the inhibition of apoptosis [7–11, 13, 14]. Similarly to RANKL, osteoprotegerin is a receptor protein of the TNF receptor superfamily that is mainly formed by osteoblasts and bone marrow stromal cells. It is a specific "trap receptor" for RANKL, as by binding with RANK it makes it prevents it from binding with RANK, which decreases the pool of active osteoclasts and limits bone resorption [7–11, 13, 14].

Results of the most recent studies indicate that MEL, which plays an important role in the regulation of synthesis and release of many osteotropic hormones and cytokines [15], may also affect the structural bone modelling and/or remodelling and bone mineralisation [16–18]. Koyama et al. [19] demonstrated that pharmacological doses of the hormone inhibit RANKL *in vitro* expression and increase OPG expression in MC3T3-E1 mouse osteoblasts, which suggests that MEL may affect bone status by means of OPG and/or RANKL. Studies on the experimental osteoporosis model [20–23] and the few studies in perimenopausal women [18, 24–26] indicate that in addition to the sex steroid deficiency that manifests with age and the changes in the levels of many well-studied local and systemic factors, MEL deficiency may also play a role in the development of osteoporosis. In light of the above evidence, it may be hypothesised that the abnormalities in MEL secretion seen in young women [27–31] and girls with AN [32] observed by some authors could be a contributing factor to bone loss and that the effect could be mediated by OPG and/ /or RANKL. Several authors have determined OPG in girls with AN and established, similarly to young women with AN [33], significantly elevated serum levels of this cytokine [34]. Only one study determined OPG and RANKL in adolescent girls with AN, showing a negatively correlating with RANKL and a positively correlating with BMD suppression of the OPG/RANK relation [35]. No studies have been conducted so far that would investigate the potential relationship between abnormalities in MEL secretion (especially in the nocturnal phase) in girls with AN and bone turnover, in terms of the role of OPG and/or RANKL.

The aim of our study was to evaluate the relationship between MEL, bone metabolism (as assessed by serum biochemical markers of bone formation, osteocalcin [OC], bone resorption, and C-terminal telopeptidea of type I collagen [CTx]) and OPG, and the soluble RANK ligand (sRANKL) in girls with AN.

Material and methods

The study enrolled 57 girls between 13 and 18 years of age, hospitalised at the Paediatric Endocrinology Ward of the Department of Paediatrics in Zabrze, Medical University of Silesia, Poland, diagnosed with AN following a paediatric examination and a psychiatrist consultation, in accordance with the ICD-10 and DSM-IV diagnostic criteria. We enrolled patients with primary or secondary amenorrhoea at the moment of diagnosis and a BMI equal to or lower than 17.5 kg/m^2. We qualified patients without serious somatic complications and with normal liver and kidney function in whom other psychiatric disorders had been ruled out. None of the patients was taking drugs that might affect nutritional status or bone metabolism at baseline. We excluded patients with serious somatic complications (gastrointestinal bleeding, chronic diarrhoea, dehydration, peptic ulcer disease, liver, or kidney dysfunction) and patients requiring medication.

The control group consisted of 13 healthy, regularly menstruating girls 13 to 18 years of age, with normal body mass, in whom endocrine and other disorders that might affect fat or bone metabolism had been ruled out.

All the girls were placed under the same nutritional conditions throughout the study (the meals were di-

spensed at 8 a.m., 1 p.m., and 6 p.m.), showed normal activity during the day, turned in at 10 p.m. (lights off) and slept until 6 a.m. (lights on).

We measured height and body mass, evaluated body proportions based on the physical growth percentile charts currently applicable in Poland, and calculated BMI.

Blood (5 ml) for determination of MEL, bone markers, OPG, and sRANKL was always collected under the same conditions, between 8 and 9 a.m. following 12-hour fasting (in the case of MEL an additional 3 ml sample was collected at 2 a.m. under red lighting conditions) and the centrifuged serum was frozen and stored at –74°C until analysis. Determinations of MEL were performed by RIA (DRG, USA), OC by ELISA (DLS Inc., USA), CTx by Serum CrossLaps ELISA (Nornic Bioscience Diagnostics A/D, Denmark), and OPG and RANKL by ELISA (Biomedica, Poland). The sensitivity, intra-assay error, and inter-assay error were 1 pg/ml, 7.5% and 9.5% for MEL, 0.05 nmol/ml, 5.8% and 7.3% for OC, 0.08 nmol/ml, 5.2% and 6.7% for CTx, 0.14 pmol/L, 7.0% and 7.5% for OPG, and 0.04 pmol/L, 5% and 7% for RANKL.

The results of the anthropometric measurements and evaluations were subjected to statistical analyses. In order to demonstrate significant differences between the groups of girls we used the *t*-Student test (for variables with a normal distribution) or the U Mann-Whitney test (for variables with a non-normal distribution). The relationship between BMI, levels of MEL, bone markers, OPG, sRANKL, and the OPG/sRANKL ratio in girls with AN were assessed by Pearson's correlation test (for variables with a normal distribution) or Spearman's correlation test (for variables with a non-normal distribution). The significance level was $p \leq 0.05$.

Table I. *Age, height, body mass, body mass index (BMI), melatonin (MEL), osteocalcin (OC), c-terminal telopeptide of type I collagen (CTx), osteoprotegerin (OPG), and receptor activator of nuclear-κB ligand (sRANKL) levels as well as values of OPG/sRANKL index in girls with anorexia nervosa and girls in the control group*

Tabela I. *Wiek, wzrost, masa ciała, wskaźnik masy ciała (BMI) oraz stężenia melatoniny (MEL), osteokalcyny (OC), c-końcowego usieciowanego telopeptydu łańcucha α1 kolagenu typu I (CTx), osteoprotegeryny (OPG), ligandu receptora aktywatora czynnika jądrowego-κB (sRANKL), a także wartość wskaźnika OPG/sRANKL u dziewcząt z jadłowstrętem psychicznym i w grupie kontrolnej*

Variables	Groups	
	Anorexia nervosa (n = 57)	Control (n = 13)
Age (years)	15.46 ± 1.59	15.85 ± 1.95
Height [m]	1.62 ± 0.07	1.63 ± 0.08
Weight [kg]	**39.50 ± 6.29***	52.58 ± 6.68
BMI [kg/m²]	**15.09 ± 1.96***	19.65 ± 1.53
MEL [pmol/L]		
na czczo	59.34 ± 50.26	66.26 ± 42.60
2.00 a.m.	**488.10 ± 76.19 ^**	417.45 ± 50.26
Δ [pmol/L]	**411.91 ± 59.13 ^**	351.19 ± 60.32
OC [nmol/ml]	**0.96 ± 0.66***	3.19 ± 1.59
CTx [nmol/L]	**6.90 ± 1.00 ^**	7.85 ± 0.90
OPG [pmol/L]	**3.97 ± 0.50#**	3.59 ± 0.70
sRANKL [pmol/L]	**0.499 ± 0.23***	0.275 ± 0.118
OPG/sRANKL	**10.24 ± 3.92#**	14.28 ± 3.99

#p ≤ 0.05; ^ p ≤ 0.01; *p ≤ 0.001 — significant difference compared to control group

Results

The mean body mass and BMI values were significantly lower in girls with AN versus controls (39.50 kg *v.* 52.58 kg and 15.09 kg/m² *v.* 19.65 kg/m², respectively).

Girls with AN showed a significantly higher mean MEL levels at 2 am compared to controls (448.10 pmol/L *v.* 417.45 pmol/L) and a significantly greater difference between the nocturnal and morning levels of the hormone (411.91 pmol/L *v.* 351.19 pmol/L). Patients with AN had a significant suppression of bone formation markers (OC, 0.96 nmol/ml *v.* 3.19 nmol/ml) and bone resorption markers (CTx, 6.90 nmol/L *v.* 7.85 nmol/L) (Figure 1). We also observed a considerable increase in OPG and sRANKL levels in patients with AN compared to controls (3.97 pmol/L *v.* 3.59 pmol/L and 0.499 pmol/L *v.* 0.275 pmol/L) paralleled by a significantly reduced OPG/sRANKL ratio (10.24 *v.* 14.28) (Table I).

Patients with AN showed a significantly positive correlation of BMI with the levels of OC and CTx and with the value of the OPG/sRANKL ratio, and a negative correlation with OPG and sRANKL. OPG levels showed a significant positive correlation with sRANKL and a negative correlation with the OPG/sRANKL ratio. sRANKL levels showed a significant negative correlation with OC, CTx, and OPG/sRANKL. We also found a positive significant correlation of OC with OPG/sRANKL. Girls with AN also showed a significant negative correlation of MEL at 2 a.m. with the bone markers and a positive correlation with sRANKL, and a negative significant correlation of amplitude between nocturnal and morning MEL levels with CTx and OPG/sRANKL ratio (Table II).

Discussion

Anorexia nervosa is a serious psychosomatic disorder with onset mainly during puberty, in which patients (generally girls and young women), by imposing upon

Table II. *Correlation between melatonin (MEL), osteocalcin (OC), c-terminal telopeptide of type I collagen (CTx), osteoprotegerin (OPG), receptor activator of nuclear-κB ligand (sRANKL) levels and body mass index (BMI) and OPG/sRANKL values in girls with anorexia nervosa (n = 57)*

Tabela II. *Korelacja między stężeniami melatoniny (MEL), osteokalcyny (OC), c-końcowego usieciowanego telopeptydu łańcucha α1 kolagenu typu I (CTx), osteoprotegeryny (OPG), ligandu receptora aktywatora czynnika jądrowego-κB (sRANKL) i wskaźnikami masy ciała (BMI) i OPG/sRANKL u dziewcząt z jadłowstrętem psychicznym (n = 57)*

Variables	OC [nmol/ml]	CTx [nmol/L]	OPG [pmol/L]	sRANKL [pmol/L]	OPG/sRANKL
MEL [pmol/L]					
na czczo	0.183	0.161	–0.122	–0.168	0.128
2.00 a.m.	**–0.364 ^**	**–0.659***	0.234	**0.790***	–0.219
Δ	–0.150	**–0.505***	0.211	0.235	**–0.294#**
BMI [kg/m²]	**0.254#**	**0.346 ^**	**–0.259#**	**–0.347 ^**	**0.267#**
OPG [pmol/L]	–0.110	–0.143	–	**0.258#**	**–0.402***
sRANKL [pmol/L]	**–0.298#**	**–0.380 ^**	**0.258***	–	**–0.741***
OPG/sRANKL	**0.323 ^**	0.228	**–0.402***	**–0.742***	–

#p ≤ 0.05; ^ p ≤ 0.01; *p ≤ 0.001 —— statistically significant values of correlation coefficients

themselves a restrictive diet and other activities (such as increased physical activity, vomiting, laxation), elicit weight loss [2, 4]. This leads to abnormalities in the formation and release of various hormones, mainly hormones of the pituitary-ovarian, pituitary-adrenal, pituitary-thyroid and somatotropin axes, calciotropic hormones, adipose tissue hormones [3, 5, 6, 34, 36–38], and MEL [27–32].

These abnormalities may lead to a BMD decrease or a loss of the expected BMD rise associated with the predominance of bone resorption over bone formation processes during physical growth [1, 3, 5, 36]. Patients with AN achieve lower values of peak bone mass and are therefore at risk of osteoporosis in adult life [3]. Determination of the bone formation and bone resorption markers in women with AN point to a reduced rate of bone formation (low levels of OC and the bone fraction of serum alkaline phosphatase) and increased bone resorption (elevated serum CTx and NTx and elevated urinary deoxypyridinoline) [39–42].

Such a bone metabolism status associated with increased bone resorption and reduced bone formation contributes to intensive bone loss. Maugars et al. [43] observed borderline high serum OC levels paralleled by elevated urinary hydroxyproline in patients with AN, which, in contrast to the previous studies, suggests a high bone turnover in this group of patients. Similarly, Owada et al. [33] showed elevated serum levels of OC and bone fraction of serum alkaline phosphatase and an increased urinary level of CTx in young women with AN which was paralleled by elevated OPG and unchanged RANKL.

In girls with AN, on the other hand, bone marker suppression generally correlating with BMI and BMD has been observed, which suggests reduced bone turnover [36, 44–48] and increased serum levels of OPG [34]. Although some authors have observed unchanged or increased levels of bone resorption markers [37, 38, 44, 49], OPG levels showed a negative correlation with BMI, body fat, and BMD [34]. Our study has shown a significant reduction in the mean serum levels of bone markers, both OC and CTx, more pronounced in the case of OC, paralleled by elevated OPG and sRANKL and significantly reduced OPG/sRANKL. We also found a significant positive correlation of BMI with OC, CTx, and OPG/sRANKL and a negative correlation with OPG and sRANKL. The confirmed increased OPG levels in girls with AN seems to be compensatory in nature relative to the increased bone resorption [34], as confirmed by the positive correlation between OPG and sRANKL and the negative correlation with OPG/ /sRANKL we observed. In addition, sRANKL levels showed a significant negative correlation with the bone markers we investigated and the OPG/sRANKL ratio. We further observed a significant positive correlation between OC and OPG/sRANKL. As we did in our study, Munoz-Calvo et al. [35] demonstrated a reduction in the OPG/sRANKL ratio in girls with AN that was significant and correlated with increased RANKL levels. Serum OPG levels were, on the other hand, only slightly elevated. A high positive correlation between the OPG/RANKL ratio and BMD was also found. The authors concluded that the reduction in the OPG/RANKL ratio in girls with AN partly explained the increases in bone loss observed in this group of patients. Other authors showed a significant increase in OPG levels and a significant negative correlation between OPG and BMD in girls with AN [34]. Others still observed eleva-

ted bone marker levels (OC and CTx) paralleled by increased OPG levels in young women with AN [33], while Khal et al. [50] found elevated OC and CTx in the serum paralleled by reduced OPG levels in young women with AN suffering from severe depression. These equivocal results may stem from the fact that the studies were not always conducted under similar conditions in terms of height, pubescence stage and bone modelling [35], and the duration of the disease in groups of adolescent girls with AN [47]. The differences in serum levels of OPG may also be related to the fact that OPG is not only formed by osteoblasts and bone marrow stromal cells, as OPG mRNA transcripts have also been found in lymphoid cells, kidneys, liver, thyroid gland, and many foetal tissues [51].

The bone turnover abnormalities that correlated with OPG and sRANKL and with the OPG/sRANKL ratio were accompanied by a significant increase in the MEL levels at 2 p.m., corresponding to the peak secretion of the hormone, and by a significant increase in the amplitude between the nocturnal and morning levels. These results suggest the possible involvement of MEL in the mechanism that leads to reduced BMD manifesting in girls with AN and resulting from bone turnover abnormalities. Studies conducted in recent years have shown that MEL, a hormone mainly synthesised by the pineal gland and playing an important role in the regulation of the synthesis and release of hormones [15], whose concentrations change significantly in patients with AN [3, 6], may affect the bone [16––18]. Lighting conditions, removal of the pineal gland, and long-term administration of MEL have been shown to modify circadian bone metabolism in rats [23, 52, 53]. Studies in ovariectomised rats [20–23] and the few studies in postmenopausal women [24–26] have shown that MEL deficiency may contribute to the induction of postmenopausal osteoporosis. On the other hand, administration of MEL to gelded female and male rats leads to increased BMD and suppression of bone markers, especially bone resorption markers [20, 21, 23, 54]. It has also been shown that excessive formation of the hormone during the day, as seen in obesity, may beneficially affect the bone after the menopause [17, 18]. Some authors, based on animal and human studies (*in vivo* and *in vitro*), suggest that MEL may directly and/or indirectly affect both the formation and resorption of bone [16–18]. Sex and adrenal steroids, calciotropic hormones, somatotropic axis hormones, thyroid axis hormones, and prostaglandin E_2 seem to play an important role in the indirect effect of MEL on bone cells [52, 53]. Other authors, based on *in vitro* and *in vivo* studies in mice and rabbits, have shown that MEL mainly affects bone resorption and does so indirectly rather than directly [19]. MEL at doses of 5–500 μM has been shown to reduce, in a dose-dependent manner, RANKL mRNA expression in the MC3T3-E1 osteoblast cell line of mice, and to increase OPG mRNA expression. Hence the hypothesis that MEL, by affecting OPG and/or RANKL expression in osteoblasts and bone marrow stromal cells, may regulate the size of the active osteoclast pool and therefore bone resorption [19]. The few human studies, mainly in obese postmenopausal women, seem to corroborate the above concept [17–18].

The studies conducted in patients with AN so far have mainly focused on evaluating the relationships between MEL and body mass composition and BMI, and have been conducted mostly in adult patients. However, results of these studies are equivocal. Tortosa et al. [29] found significant increases in the mean 24-hour and nocturnal levels of MEL in young women with AN without any changes in the acrophase. Similarly, other researchers [27, 28, 30, 31] observed a marked increase in MEL levels in the nocturnal phase without disturbances in the circadian rhythm, while Kennedy et al. [55, 56] found a significant reduction in nocturnal MEL levels, but only in women with AN suffering from depression. Other researchers [57–59], on the other hand, observed no significant changes in circadian levels of MEL in patients with AN. The discrepancies may be related to the fact that some of the AN patients were also suffering from depression, hence the reduced MEL values. Dalery et al. [32] showed higher MEL levels at time points corresponding to peak secretion in adolescents with AN than in depressed girls, although the studies by these authors did not include a group of healthy girls. In our study we found a significant increase in the mean serum levels of MEL at 2 a.m. and an increase in the amplitude between the nocturnal and morning levels of the hormone compared to healthy girls. We did not show any significant differences in the morning values of MEL between the girls with AN and the healthy girls. Because no studies investigating the relationship between MEL and bone metabolism, taking into account the involvement of the cytokines of the RANKL/RANK/OPG system, had been conducted at that time, we performed such a study. We showed that the changes in MEL levels at 2 a.m. in girls with AN showed a negative and statistically significant correlation with bone marker levels and a positive correlation with sRANKL, which suggests that MEL may modulate bone turnover through RANKL. This evidence suggests that the abnormalities in MEL secretion in the nocturnal phase could play a role in the mechanism leading to BMD reduction in girls with AN. This is supported by the fact that the amplitude values between the nocturnal and morning MEL levels showed a significant negative correlation with CTx and the OPG/sRANKL ratio.

Conclusions

Our results indicate that the abnormalities of bone metabolism in girls with AN are associated with changes in the nocturnal levels of MEL, with RANKL appearing to play an important role in this mechanism. The increased amplitude between the nocturnal and morning levels of MEL may adversely affect the bone tissue in girls with AN, with the effect most likely resulting from influences on the OPG/RANKL balance.

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