THE RELATIONSHIP BETWEEN BONE METABOLISM, MELATONIN AND OTHER HORMONES IN SHAM-OPERATED AND PINEALECTOMIZED RATS

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Objective. The influence of pinealectomy and long-term melatonin (MEL) administration on circadian oscillations of selected biochemical markers of bone metabolism [serum alkaline phosphatase (ALP) activity, carboxyterminal propeptide type I procollagen (PICP) and carboxyterminal telopeptide type I collagen (ICTP) concentrations as well as urinary excretion of hydroxyproline (HYP) and Ca] and possible involvement of circadian secretion of IGF-I, parathyroid, thyroid, adrenal cortex and gonads function in this mechanism was evaluated.

Methods. Studies were performed in 192 adult male Wistar rats weighing 145±9 g which were subjected to pinealectomy or sham operation. In half of the animals from each group MEL (Sigma, USA) in a dose of 50 mg/100 g b.w. was administered intraperitonealy (daily between 17.00 and 18.00 h for a 4-week period). Material for studies (blood and urine) was collected every 3 hours during a day. Hormones, PICP and ICTP concentrations were determined with the use of RIA methods, whereas ALP, HYP and Ca values - spectrophotometrically.

Results. The study has shown that pinealectomy had an inducing, while exogenous MEL a suppressing effect upon the level of investigated biochemical markers of bone metabolism. Furthermore, substantial changes in the values of amplitude and phase of their circadian oscillations were shown. Distinct, dependent on the time of day disturbances in circadian fluctuations of PICP, ICTP, HYP and Ca showing generally negative correlation with changes in endogenous MEL concentrations and positive with IGF-I and corticosterone (B) levels were found. In addition, changes in circadian oscillations of ALP and PICP correlated negatively with daily oscillations of calciotropic hormones and B. However, ICTP, HYP and Ca concentrations correlated positively with circadian fluctuations of B and FT3 (the latter only in sham operated rats receiving MEL).

Conclusions. This study showed that both pinealectomy and long-term MEL administration influence the circadian rhythm of bone metabolism and that an important role in the mechanism of this dependence is played by the changes of endogenous MEL levels. Secondary changes in circadian oscillations of calciotropic hormones, IGF-I and corticosterone concentrations, caused by pinealectomy and long-term MEL administration result in altered bone metabolism rhythm.

Key words*:* Pinealectomy - Melatonin - Bone metabolism - Hormones - Circadian rhythm - Male rats

The effect of pineal gland/melatonin (MEL) upon circadian secretion of hormones, especially those playing a crucial role in the regulation of bone metabolism (Badurski 1994; Galus 1995) appears significant. Early experimental studies have shown that light conditions, pinealectomy and also long-term MEL administration modify the synthesis and/or circadian release of growth hormone (GH), insulin-like

growth factor-I (IGF-I) as well as calciotropic, thyroid, adrenal cortex and testes hormones (Mess 1983; Kniazewski et al. 1990 a, b; Ostrowska et al. 1990, 1993, 2001 a, b, 2002; Vriend et al. 1990; Zwirska-Korczala et al. 1991; Vaughan et al. 1994; Karasek 1997). In our preliminary investigations in rats, we demonstrated that these hormones might play a role in the synchronization of the bone metabolism periodicity (Ostrowska et al. 2002). However, little is known about the effects of pineal gland/MEL on bone tissue metabolism. *In vitro* studies indicate, that MEL may play an essential role in regulating bone growth. It was shown that it stimulates osteoblast differentiation and mineralization of matrix in culture (Roth et al. 1999). MEL enhances also synthesis of collagenic and noncollagenic proteins of bone matrix (Nakade et al. 1999). In an experimental model of postmenopausal osteoporosis a successive lowering of nocturnal MEL concentrations was observed; these changes correlated negatively with the values of biochemical markers of bone metabolism (Ostrowska et al. 2001 a). It has also been demonstrated that MEL may be an important modulator of experimental osteoporosis induced in female rats by ovariectomy (Ladizesky et al. 2001; Ostrowska et al. 2002). Clinical studies suggest that characteristic changes of bone mass in postmenopausal osteoporosis may be related to MEL (Sandyk et al. 1992). Our own studies demonstrate that increased MEL secretion in obese women more than 20 % overweight has protective significance in loss of bone mass after menopause (Ostrowska et al. 2001 b).

The data mentioned above allow to postulate that the pineal gland/MEL most likely influence bone tissue metabolism and that a possible mechanism of this relationship might involve hormones playing a crucial role in the regulation of bone metabolism, such as IGF-I and also calciotropic, parathyroid, thyroid, adrenocortical and gonadal hormones.

The aim of this study in rats was to assess: 1. the influence of pinealectomy and long-term MEL administration on circadian oscillations of chosen biochemical markers of bone metabolism (serum alkaline phosphatase - ALP activity, carboxyterminal propeptide of type I procollagen - PICP and cross-linked carboxyterminal telopeptide of type I collagen - ICTP concentrations as well as urinary excretion of hydroxyproline - HYP and total calcium - Ca); 2. possible involvement of daily secretion of IGF-I and parathyroid, thyroid, adrenal cortex and gonads hormones in this mechanism.

Materials and Methods

Studies were performed in 192 adult male Wistar rats, weighing 145±9 g. Before the beginning of the experiment animals stayed for two weeks in a place with a temperature of 20-22 °C, air humidity of 80-85 % and regulated light/dark cycle (LD 12:12; light from 7 am to 7 pm). Rats were fed at the onset of the dark phase using a standard diet suitable for conducting bone metabolism research in experimental animals (Altromin Standard Diäten, Austria) and were given drinking water *"ad libitum"*. After two weeks of adaptation, half the animals (96 rats) were subjected to a pinealectomy according to Kuszak and Rodin (1977) and the remaining half sham operation. Two weeks after operation the rats were divided into four groups (48 animals in each): 1. SPx – control group after sham operation, which received intraperitonealy 5 % solution of ethyl alcohol in physiological saline; 2. SPx+MEL – after sham operation, which received intraperitonealy MEL (Sigma, USA) in the amount of 50 μg/100 g b.w. in solvent mentioned above; 3. Px – after pinealectomy, which received only solvent; 4. Px+MEL – after pinealectomy, which received 50 μg/100 g b.w. MEL in solvent mentioned above. Rats were administered with the MEL solution or the solvent daily between 17.00 and 18.00 h for a 4-week period. During the experiment, rats stayed in the same zoohygienical, alimentary and lighting conditions as during the adaptation period.

After the end of the experiment, rats were marked and placed separately in metabolic cages for 3 hours in order to collect urine aliquots (at 3-hour intervals within 24 hours) for HYP and Ca determination. The first urine samples were collected between 6.30 and 9.30 h. Six animals were sacrificed by decapitation at 3-hour intervals within 24 hours (starting at 8.00 h) on the following day. The blood was collected into test tubes: 1. with granulated mass (Sarstedt) in order to measure ALP, PICP, ICTP, MEL, IGF-I, free triiodothyronine (FT3), free thyroxine (FT4), corticosterone (B) and testoster-

one (T) concentrations; 2. with EDTA in order to measure parathormone (PTH) and calcitonin (CT) levels. The blood was centrifuged immediately (450 g, 10 min) and the obtained serum/plasma samples were stored frozen at -75 °C until determination. All activities in the dark phase were performed under red light.

The animals were adopted and accustomed to such individual metabolic cages, for instance by a repeated placing them to such cages before the experiment. They were also adopted to the presence of all personell participating at the procedure of sacrifice. The sacrificing has been performed in a room separated from that in which the metabolic cages were located. The study was conducted with the permission of the Ethics Committee of the Medical University of Silesia in Katowice.

Concentrations of hormones, PICP and ICTP were measured using RIA kits: MEL, PTH, CT (DRG, USA); IGF-I, B (DSL, USA), FT3, FT4, T, PICP, ICTP (FARMOS, Finland). Serum ALP activity and urinary excretion of Ca were determined using ALPHA DIAGNOSTICS kits (Poland). Urinary excretion of HYP was determined according to Drozdz et al. (1976). The sensitivity of assays was as follows: MEL, PTH, CT - 0.2 pg/tube, IGF-I - 2.73 nmol/l, B - 7 nmol/l, FT3 - 0.25 pmol/l, FT4 - 0.51 pmol/l, T - 0.3 nmol/l, PICP - 1.2 μg/l, ICTP - 0.34 μg/l, HYP - 6.6 μmol/l. The linearity for ALP and Ca methods were up to 1000 U and up to 16 mg/dl, respectively. The respective intra- and interassay coefficients of variations were: MEL - 11 and 12 %, PTH - 7.9 and 8 %, CT - 8.9 and 9.1 %, IGF-I - 3.7 and 6.1 %, B - 8.3 and 9.5 %, FT3 - 6.9 and 7.7 %, FT4 - 4.1 and 9.4 %, T - 5.5 and 6.2 %, PICP - 3.1 and 5.8 %, ICTP - 4.5 and 6 %, HYP - 5.5 and 7.2 %, ALP - 7.8 and 8.5 %, Ca - 4.7 and 6.8 %.

The results were evaluated statistically using variance analysis for parametric tests. After rejecting the variance uniformity hypothesis, further analysis of statistical significance was performed using Student t-test. The statistical analysis of circadian rhythms was carried out with the use of the cosinor method according to Halberg et al. (1967). The interrelation between the values of chosen hormones and biochemical markers of bone metabolism was assessed using the Pearson correlation test.

Results

Our results suggest that pinealectomy and long-term MEL administration considerably influence the circadian rhythm of both bone metabolism markers and hormones in rats. The effect is dependent on the time of day (Tables 1, 2 and 3).

Changes of bone metabolism markers and hormones in pinealectomized rats were generally the most pronounced at time points of significant MEL level decrease during the 24-hour period, i.e. from 20.00 until 11.00 h. An increase of bone formation marker values was observed in the dark phase while resorption markers rose during darkness and/or at the beginning of the light phase. An increase of PTH, CT and B concentrations was found from the early morning until afternoon, while that of thyroid hormones and T occurred at the turn of the light and dark phases and during the period of darkness. At the remaining time points of the 24-hour period the effect was usually suppressive. In pinealectomized rats IGF-I levels were substantially lowered at the majority of time points (Tables 1, 2 and 3).

The effect of long-term MEL administration on bone metabolism and endocrine function was rather inhibitory, while in the case of IGF-I it was stimulatory. MEL administration to pinealectomized rats resulted in the suppression of ALP, PICP and Ca values throughout the whole 24-hour period. However, ICTP and HYP concentrations were suppressed in the light phase. Concentration of PTH, CT, thyroid hormones, B and T was decreased while that of IGF-I was increased at the majority of time points, but mostly at the turn of light and dark phases and during the dark period, especially during its first half. MEL administration to rats with preserved pineal gland has resulted in changes of bone maker values, mainly at those time points where also an endogenous MEL increase was the most pronounced. A considerable decrease of bone formation markers was observed in the light phase, that of resorption markers in the light phase and at the beginning of the dark phase. Decreased values of PTH and CT were noted at the majority of time points; those of B and T at the turn of the light and dark phases and during darkness. IGF-I concentration increased from 2.00 until 11.00 h and at 20.00 h. At the remaining time points of the 24-hour period, inverse effects were

Table 1
Mean serum values of alkaline phosphatase (ALP), carboxyterminal propeptide of type I procollagen (PICP), cross-linked carboxyterminal telopeptide of type I collagen (ICTP) as well as urinary excretion of hydroxyproline (HYP) and total calcium (Ca) in sham operated (SPx) and pinealectomized (Px) rats after long-term MEL administration

	Bone formation markers				Bone resorption markers		
Groups	Hours	ALP (U/l)	PICP (μg/l)	ICTP (μg/l)	Hours	HYP (μmol/l)	Ca (mmol/l)
SPx	2.00	229.05 ± 8.14	11.71 ± 0.68	10.52 ± 1.19	0.30 - 3.30	10.80 ± 0.74	2.21 ± 0.26
	5.00	242.65 ±11.00	10.41 ± 1.47	11.02 ± 0.40	3.30 - 6.30	14.67 ± 0.48	1.86 ± 0.24
	8.00	292.50 ±10.15	12.62 ± 1.80	17.39 ± 0.75	6.30 - 9.30	14.09 ± 0.45	2.29 ± 0.27
	11.00	329.15 ±40.42	18.55 ± 1.77	17.34 ± 0.66	9.30 - 12.30	17.32 ± 0.78	3.26 ± 0.30
	14.00	344.80 ±30.81	20.17 ± 1.98	20.67 ± 0.62	12.30 - 15.30	17.26 ± 0.65	3.15 ± 0.27
	17.00	284.37 ±41.05	16.59 ± 1.26	18.20 ± 2.41	15.30 - 18.30	14.22 ± 0.32	2.73 ± 0.27
	20.00	263.50 ±10.04	9.07 ± 0.33	16.13 ± 1.17	18.30 - 21.30	14.04 ± 0.50	2.81 ± 0.39
	23.00	282.95 ±14.76	12.82 ± 1.42	12.05 ± 0.81	21.30 - 0.30	13.42 ± 1.05	2.49 ± 0.33
SPx + MEL	2.00	**176.02 ±14.00***	13.35 ± 1.49	**14.16 ± 0.69***	0.30 - 3.30	**12.98 ± 0.35***	2.63 ± 0.30
	5.00	**177.51 ±17.52***	13.98 ± 0.89	11.60 ± 0.31	3.30 - 6.30	12.77 ± 0.91	2.02 ± 0.30
	8.00	**180.80 ±15.98***	11.64 ± 1.87	**14.05 ± 0.49***	6.30 - 9.30	14.76 ± 0.71	2.67 ± 0.25
	11.00	**140.99 ±11.11***	**7.82 ± 1.09***	19.21 ± 1.13	9.30 - 12.30	16.02 ± 0.72	2.93 ± 0.31
	14.00	**221.19 ±12.49***	**8.01 ± 0.89***	**15.40 ± 0.91***	12.30 - 15.30	**12.19 ± 0.56***	**2.10 ± 0.13***
	17.00	220.79 ±13.46	13.93 ± 1.53	15.16 ± 1.46	15.30 - 18.30	13.01 ± 0.52	**1.83 ± 0.18***
	20.00	**182.70 ±11.06***	12.05 ± 1.35	12.60 ± 1.21	18.30 - 21.30	13.78 ± 0.88	2.29 ± 0.13
	23.00	**235.23 ± 7.66***	13.79 ± 1.36	**9.60 ± 0.88***	21.30 - 0.30	11.87 ± 0.79	2.46 ± 0.09
Px	2.00	**298.90 ±18.96***	**14.73 ± 0.82***	**15.57 ± 1.10***	0.30 - 3.30	**15.17 ± 0.25***	2.44 ± 0.23
	5.00	241.94 ±13.20	**15.08 ± 1.00***	**16.16 ± 1.17***	3.30 - 6.30	14.63 ± 1.20	2.28 ± 0.12
	8.00	**247.34 ±11.45***	12.84 ± 1.07	21.58 ± 1.82	6.30 - 9.30	**18.07 ± 0.77***	2.50 ± 0.28
	11.00	359.40 ±11.75	**11.01 ± 0.93***	19.19 ± 1.40	9.30 - 12.30	16.80 ± 0.40	3.35 ± 0.30
	14.00	328.60 ±27.57	**14.44 ± 1.63***	17.14 ± 1.58	12.30 - 15.30	15.71 ± 0.81	**2.46 ± 0.12***
	17.00	351.07 ±13.97	17.76 ± 1.18	16.30 ± 1.33	15.30 - 18.30	12.34 ± 0.45	2.60 ± 0.26
	20.00	**317.14 ±12.51***	12.11 ± 1.82	12.64 ± 1.10	18.30 - 21.30	11.60 ± 0.91	2.50 ± 0.27
	23.00	299.79 ±22.65	16.18 ± 1.99	**15.83 ± 1.01***	21.30 - 0.30	11.50 ± 1.17	2.90 ± 0.09
Px + MEL	2.00	**153.05 ± 9.78***	**9.08 ± 1.40***	17.64 ± 1.25	0.30 - 3.30	13.68 ± 0.76	2.21 ± 0.26
	5.00	**132.40 ±10.87***	**9.34 ± 1.40***	**19.61 ± 0.80***	3.30 - 6.30	15.60 ± 0.98	1.94 ± 0.38
	8.00	**172.10 ±13.56***	**6.69 ± 0.63***	**16.82 ± 1.59***	6.30 - 9.30	**16.11 ± 0.36***	2.16 ± 0.14
	11.00	**266.03 ±21.01***	8.80 ± 0.73	17.08 ± 1.60	9.30 - 12.30	**14.07 ± 0.69***	**2.15 ± 0.07***
	14.00	**175.87 ± 9.72***	**6.75 ± 0.83***	13.89 ± 1.02	12.30 - 15.30	**12.70 ± 0.21***	**1.98 ± 0.16***
	17.00	**208.50 ±10.91***	**8.96 ± 0.46***	**12.23 ± 0.67**	15.30 - 18.30	12.17 ± 1.18	2.36 ± 0.18
	20.00	**164.00 ± 9.04***	10.22 ± 0.53	14.07 ± 1.16	18.30 - 21.30	13.00 ± 0.44	**1.55 ± 0.15***
	23.00	**185.13 ± 5.01***	**10.27 ± 0.41***	16.97 ± 2.00	21.30 - 0.30	12.83 ± 0.31	**2.09 ± 0.15***

* $p \leq 0.05$ vs control group

generally observed concerning the studied markers of bone metabolism and hormones. Changes concerning the concentration of thyroid hormones were insignificant (Tables 1, 2 and 3).

As a consequence, circadian concentration changes of bone markers and hormones in pinealectomized and sham-operated rats receiving MEL resulted in disturbances of their rhythm chronoorganization (Tables 4 and 5). Considerably decreased mesor value and lack of MEL rhythm was accompanied by a markedly increased average circadian ALP, thyroid hormones and T concentration as well as by a decreased average circadian IGF-I concentration (Px group). A lowering of ICTP and IGF-I amplitude was observed, along with abolition of ALP, PICP, Ca, PTH, FT3 and B rhythms and/or peak shift for bone metabolism markers and PTH, CT and B from 27° up to 138°C. The rise of circadian MEL oscillation amplitude, and considerably increased average circadian concentration and lack of rhythm were ac-

Table 2
Mean serum concentrations of melatonin (MEL), parathormone (PTH), calcitonin (CT) and insulin-like growth factor (IGF-I) in sham operated (SPx) and pinealectomized (Px) rats after long-term MEL administration

Groups	Hours	MEL (pg/ml)	PTH (pg/ml)	CT (pmol/l)	IGF-I (nmol/l)
SPx	2.00	70.73 ± 4.11	120.58 ± 2.60	17.91 ± 1.41	113.39 ± 0.47
	5.00	49.54 ± 5.58	155.87 ± 2.47	22.23 ± 0.66	71.63 ± 7.30
	8.00	20.83 ± 1.56	135.85 ± 4.75	19.06 ± 1.19	89.15 ±10.20
	11.00	16.18 ± 1.99	91.98 ± 3.38	17.40 ± 0.88	149.82 ± 4.20
	14.00	8.72 ± 1.11	75.08 ± 2.93	12.56 ± 0.71	164.02 ± 3.67
	17.00	7.73 ± 1.22	91.98 ± 3.25	13.70 ± 0.89	144.25 ± 6.88
	20.00	12.30 ± 1.00	91.98 ± 0.78	16.27 ± 0.88	107.52 ± 7.05
	23.00	20.47 ± 1.84	98.73 ± 2.47	17.40 ± 0.81	112.02 ±10.86
SPx + MEL	2.00	74.28 ± 3.77	**63.57 ± 1.95***	**12.39 ± 1.62***	**160.32 ± 5.10***
	5.00	57.59 ± 1.80	**113.04 ± 5.07***	19.88 ± 1.97	**97.96 ± 4.42***
	8.00	**60.07 ± 4.41***	133.06 ± 3.77	19.51 ± 1.06	**136.44 ±13.56***
	11.00	**63.99 ± 3.81***	**69.62 ± 3.58***	**14.28 ± 0.98***	181.13 ±15.37
	14.00	**42.15 ± 3.43***	78.33 ± 10.14	11.56 ± 0.87	173.93 ±13.94
	17.00	**53.14 ± 4.79***	78.91 ± 7.48	12.11 ± 0.88	137.12 ± 7.35
	20.00	**49.26 ± 5.33***	**60.97 ±10.01***	**10.66 ± 0.74***	**138.84 ± 9.90***
	23.00	**51.09 ± 2.27***	**69.55 ±10.53***	**14.12 ± 0.66***	117.44 ± 7.88
Px	2.00	**8.99 ± 0.36***	**72.35 ± 5.46***	**14.25 ± 0.41***	**77.08 ± 3.59***
	5.00	**9.00 ± 0.47***	165.10 ±11.70	18.41 ± 0.72	61.32 ± 7.73
	8.00	**8.31 ± 0.12***	**189.93 ±10.99***	19.94 ± 2.43	102.06 ± 4.87
	11.00	**8.00 ± 0.16***	106.80 ± 8.19	**20.44 ± 0.78***	**92.94 ± 4.11***
	14.00	9.02 ± 0.10	**97.76 ± 5.40***	15.38 ± 1.14	**89.87 ± 8.65***
	17.00	8.89 ± 0.25	83.53 ± 3.90	12.74 ± 0.50	**105.82 ± 9.39***
	20.00	**9.12 ± 0.17***	81.90 ± 4.68	13.79 ± 1.45	**76.49 ± 3.47***
	23.00	**8.73 ± 0.22***	84.18 ± 4.81	**13.07 ± 0.57***	**70.34 ± 5.43***
Px + MEL	2.00	**37.87 ± 0.74***	**54.28 ± 3.45***	15.16 ± 1.07	**95.21 ± 3.44***
	5.00	**44.49 ± 0.92***	**119.93 ± 6.96***	**22.52 ± 0.56***	57.63 ± 4.80
	8.00	**39.00 ± 6.16***	156.98 ± 9.36	17.91 ± 1.60	93.35 ± 5.87
	11.00	**52.93 ± 2.71***	86.65 ± 9.36	19.06 ± 1.39	**112.33 ± 7.53***
	14.00	**71.51 ± 1.52***	**60.13 ± 8.65***	**7.98 ± 0.50***	**110.24 ± 8.65***
	17.00	**59.33 ± 1.80***	**60.58 ± 4.94***	13.51 ± 0.68	**135.64 ± 6.50***
	20.00	**58.37 ± 6.03***	95.23 ± 9.56	**9.23 ± 0.94***	78.36 ± 3.81
	23.00	**37.92 ± 1.88***	**122.72 ± 3.38***	**10.48 ± 0.82***	**104.83 ± 7.22***

* $p \leq 0.05$ vs control group

companied by decreased mesor values of certain bone metabolism markers, i.e. ALP, PICP, HYP, Ca as well as hormones (except B) and an increase of IGF-I average circadian concentration (Px+MEL group). Observed also were lower values of ALP, HYP, Ca, PTH and T, increased amplitudes of CT, IGF-I, FT3 and FT4 as well as a peak shift for bone turnover markers (except PICP) and PTH, IGF-I, B and T from 32° up to 108°C. The abolition of MEL rhythm and increased average circadian concentration have been accompanied by decreased mesor values for ALP, PICP, ICTP, PTH, CT, B and T and an increased value in the case of IGF-I (SPx+MEL group). Abolition of rhythm was observed for all markers of bone metabolism and PTH, along with a rise of FT4 circadian oscillations and/or maximum value shifts for ALP, PICP, HYP, Ca, PTH, FT3, B and T from 31° up to 195°C (Tables 4 and 5).

In pinealectomized rats and in both groups of animals receiving MEL a negative correlation was

Table 3
Mean serum values of free triiodothyronine (FT3), free thyroxine (FT4), corticosterone (B) and testosterone (T) in sham operated (SPx) and pinealectomized (Px) rats after long-term MEL administration

Groups	Hours	FT3 (pmol/l)	FT4 (pmol/l)	B (nmol/l)	T (nmol/l)
SPx	2.00	5.08 ± 0.70	21.70 ± 1.14	239.35 ± 24.32	0.89 ± 0.06
	5.00	4.78 ± 0.40	20.60 ± 2.64	195.74 ± 21.02	0.59 ± 0.09
	8.00	5.42 ± 0.24	26.00 ± 2.25	127.15 ± 12.61	0.72 ± 0.02
	11.00	7.33 ± 0.40	43.00 ± 3.73	204.29 ± 17.48	1.09 ± 0.09
	14.00	7.00 ± 0.56	33.60 ± 3.91	289.78 ± 11.09	1.82 ± 0.19
	17.00	5.53 ± 0.74	21.80 ± 1.85	693.69 ± 23.09	3.08 ± 0.20
	20.00	4.63 ± 0.31	26.40 ± 1.86	754.19 ± 14.48	1.92 ± 0.20
	23.00	4.83 ± 0.32	20.00 ± 1.51	270.15 ± 19.34	1.01 ± 0.16
SPx + MEL	2.00	5.92 ± 0.61	18.20 ± 1.96	**139.12 ± 5.34***	0.91 ± 0.02
	5.00	5.06 ± 0.61	22.00 ± 1.44	**266.56 ± 13.02***	**1.30 ± 0.09***
	8.00	6.13 ± 0.48	32.63 ± 2.56	**560.00 ± 59.01***	**1.81 ± 0.28***
	11.00	**6.73 ± 0.30***	35.90 ± 2.87	**488.00 ± 55.30***	1.21 ± 0.15
	14.00	6.47 ± 0.47	30.35 ± 2.28	338.70 ± 33.76	**0.40 ± 0.07***
	17.00	5.06 ± 0.61	26.00 ± 1.87	**225.16 ± 26.61***	**0.36 ± 0.04***
	20.00	4.48 ± 0.11	27.57 ± 2.52	**153.76 ± 16.54***	**0.70 ± 0.10***
	23.00	4.29 ± 0.32	18.50 ± 2.49	**123.03 ± 7.56***	**0.61 ± 0.03***
Px	2.00	5.14 ± 0.15	**28.28 ± 1.75***	207.50 ± 18.02	0.97 ± 0.15
	5.00	5.69 ± 0.21	26.66 ± 0.79	**529.34 ± 18.30***	**0.86 ± 0.08***
	8.00	**6.51 ± 0.04***	30.15 ± 1.43	**468.00 ± 37.30***	**0.99 ± 0.05***
	11.00	**8.62 ± 0.37***	44.22 ± 1.00	**577.25 ± 15.84***	0.88 ± 0.11
	14.00	**5.52 ± 0.06***	30.13 ± 0.85	345.09 ± 49.45	**2.41 ± 0.20***
	17.00	6.04 ± 0.17	**37.28 ± 1.34***	**306.68 ± 22.70***	2.70 ± 0.31
	20.00	**6.33 ± 0.44***	**33.06 ± 1.44***	**300.00 ± 11.08***	**3.08 ± 0.08***
	23.00	**5.95 ± 0.18***	**24.09 ± 0.64***	260.47 ± 5.21	1.35 ± 0.11
Px + MEL	2.00	4.64 ± 0.19	**13.86 ± 0.58***	**333.25 ± 23.43***	0.67 ± 0.06
	5.00	5.32 ± 0.22	23.44 ± 1.27	538.87 ± 71.13	0.70 ± 0.07
	8.00	**6.09 ± 0.06***	**20.41 ± 0.55***	438.72 ± 28.22	1.09 ± 0.05
	11.00	**6.56 ± 0.14***	**28.12 ± 1.30***	**457.40 ± 13.70***	**1.32 ± 0.12***
	14.00	**6.22 ± 0.23***	**37.47 ± 2.24***	251.75 ± 8.40	**0.98 ± 0.05***
	17.00	5.27 ± 0.29	**20.96 ± 0.54***	**214.64 ± 16.32***	**0.63 ± 0.07***
	20.00	**5.15 ± 0.18***	**21.36 ± 0.78***	307.60 ± 37.39	**0.55 ± 0.07***
	23.00	**4.71 ± 0.14***	**15.50 ± 1.07***	**185.14 ± 33.92***	**0.28 ± 0.05***

* $p \leq 0.05$ vs control group

found between circadian changes of PICP and ICTP concentration, or HYP and Ca level on the one hand, and changes of endogenous MEL concentration on the other (Table 6). Changes in ALP and MEL concentration showed a negative correlation only in the SPx+MEL group. In addition, changes of ALP and PICP circadian values in rats from SPx+MEL and Px groups showed a negative correlation with respect to PTH and CT. A directly proportional relationship was demonstrated between changes of PICP and IGF-I concentrations in those two groups. A positive correlation was also observed in all experimental groups between changes in circadian values of investigated bone formation markers and changes in B concentration. Changes in circadian concentration of bone resorption markers correlated positively with concentration changes of IGF-I (in SPx+MEL and Px groups), FT3 (in SPx+MEL group) and B (all experimental groups).

Discussion

Similarly to other authors (Ozaki and Lynch 1976; Lewy et al. 1980; Reiter 1990), we have shown that in pinealectomized rats the circadian MEL concentration is significantly decreased and that the nocturnal

Table 4
Changes in the chronobiologic parameters (M - mesor, A - aplitude, φ - acrophase) of the mean rhythms of chosen biochemical markers of bone metabolism (ALP, PICP and ICTP, all determined in serum as well as HYP and Ca, determined in urine) in sham operated (SPx) and pinealectomized (Px) rats after long-term MEL administration versus control group

Variables	Chronobiologic parameters	Groups (LD 12:12)			
		SPx	SPx+MEL	Px	Px+MEL
ALP	M (U/l)	283.62	**↓ 191.90**	**↑ 305.51**	**↓ 189.40**
	A (U/l)	45.20	**↓ 24.89**	49.27	**↓ 26.65**
	φ (h.min)	13.19	**→ 19.39**	**→ 15.23**	**12.02 ←**
	p	0.034*	**0.324**	**0.206**	**0.372**
PICP	M (μg/l)	13.87	**↓ 11.82**	14.27	**↓ 8.76**
	A (μg/l)	4.54	**↓ 2.68**	**↓ 1.31**	1.41
	φ (h.min)	13.17	**0.18 ←**	**→ 21.33**	22.41
	p	0.046*	**0.091**	**0.570**	**0.101**
ICTP	M (μg/l)	15.42	**↓ 13.97**	16.80	16.24
	A (μg/l)	4.74	**↓ 3.00**	**↓ 2.98**	3.00
	φ (h.min)	14.01	12.60	**9.19 ←**	**4.49 ←**
	p	0.001*	**0.085**	0.040*	0.005*
HYP	M (μmol/l)	14.58	13.42	14.48	13.77
	A (μmol/l)	2.43	**↓ 1.10**	**↑ 3.03**	**↓ 1.72**
	φ (h.min)	12.25	**10.07 ←**	**8.50 ←**	**6.34 ←**
	p	0.026*	**0.325**	0.005*	0.010*
Ca	M (mmol/l)	2.60	2.36	2.63	**↓ 2.05**
	A (mmol/l)	0.55	**↓ 0.23**	**↓ 0.14**	**↓ 0.10**
	φ (h.min)	15.04	**6.35 ←**	13.23	**8.31 ←**
	p	0.033*	**0.551**	**0.772**	**0.800**

↑ – relative increase of value by more than 10%
↓ – relative decrease of value by more than 10%
← – peak shift to earlier time points (more than 2 hours)
→ – peak shift to later time points (more than 2 hours)

***p** = less than 0.05 indicates statistically significant 24 h rhythm
– **significant differences, compared to control, are marked in bold**

secretion peak is suppressed. It appeared that even several weeks after the surgery MEL is still present in blood. This may result from the ongoing synthesis of this hormone outside of the pineal gland, i.e. in the retina, Harderian gland or digestive system (Ozaki and Lynch 1976; Hollaway et al. 1980; Yu et al. 1981; Huether 1993). Some authors have found that within several weeks after pinealectomy, MEL concentration in blood may even be slightly increased (Yu et al. 1981). The changes in circadian concentration of endogenous MEL following pinealectomy, demonstrated in our study were accompanied by distinct disturbances in circadian oscillations of investigated bone metabolism markers. This may point to the significance of this hormone in maintaining the physiological circadian rhythm of bone metabolism. The inversely proportional relationship between circadian changes of bone metabolism markers and changes in endogenous MEL concentration (significant for PICP, ICTP, HYP and Ca) seems to support the above-mentioned view. Following pinealectomy in rats, the increase of bone metabolism markers was generally dominating, most significantly at time points corresponding to a substantial decrease in the concentration of endogenous MEL, i.e. between 20.00 and 11.00 h. The rise of bone formation markers' values was observed in the dark phase; the rise of resorption markers' values was seen during the dark phase and/or at the beginning of the light phase. The values of average circadian concentrations of the studied bone metabolism markers did not reflect the circadian dependence of effect differentiation following pinealectomy in rats. Changes in values of two remaining chronobiological parameters, i.e. amplitude and acrophase of circadian oscillations seem to be better exponents. Lowering of amplitude was observed in pinealectomized rats for ICTP, along with the abolition of the rhythm for ALP, PICP and Ca as well as a shift of

Table 5
Changes in the chronobiologic parameters (M - mesor, A - aplitude, φ - acrophase) of the mean rhythms of chosen hormones in sham operated (SPx) and pinealectomized (Px) rats after long-term melatonin (MEL) administration versus control group

Variables	Chronobiologic parameters	Groups (LD 12:12)			
		SPx	SPx+MEL	Px	Px+MEL
MEL	M (pg/ml)	25.81	**↑ 56.45**	**↓ 8.76**	**↑ 50.18**
	A (pg/ml)	25.22	**↓ 8.72**	**↓ 0.34**	**↑ 14.51**
	φ (h.min)	3.21	4.46	**→ 21.37**	**15.18 ←**
	p	0.041*	**0.230**	**0.251**	**0.056**
PTH	M (pg/ml)	108.27	**↓ 83.33**	110.18	**↓ 94.58**
	A (pg/ml)	31.85	**↓ 24.18**	**↑ 45.63**	**↓ 24.90**
	φ (h.min)	4.46	**→ 7.38**	**→ 7.40**	**5.27 ←**
	p	0.021*	**0.176**	**0.082**	**0.082**
CT	M (pmol/l)	17.07	**↓ 14.32**	16.00	**↓ 14.48**
	A (pmol/l)	3.60	3.94	3.89	**↑ 5.61**
	φ (h.min)	4.52	6.27	**→ 8.35**	6.42
	p	0.015*	0.046*	0.005*	**0.058**
IGF - I	M (nmol/l)	118.98	**↑ 142.90**	**↓ 84.49**	**↑ 98.45**
	A (nmol/l)	33.93	**↓ 21.91**	**↓ 15.27**	**↑ 20.79**
	φ (h.min)	15.14	13.21	13.22	**→ 15.39**
	p	0.067	0.341	0.142	0.227
FT3	M (pmol/l)	5.58	5.52	**↑ 6.23**	**↓ 5.50**
	A (pmol/l)	1.17	**↓ 1.02**	**↓ 0.76**	**↑ 0.91**
	φ (h.min)	12.17	**10.16 ←**	11.35	11.25
	p	0.037*	0.049*	**0.400**	0.001*
FT4	M (pmol/l)	26.63	26.39	**↑ 31.74**	**↓ 22.64**
	A (pmol/l)	5.17	**↑ 7.73**	**↑ 5.97**	**↑ 8.07**
	φ (h.min)	12.11	12.01	13.23	12.50
	p	0.101	**0.015***	0.182	0.061
B	M (nmol/l)	346.79	**↓286.79**	374.29	340.91
	A (nmol/l)	271.43	**↓ 203.80**	**↓ 146.54**	141.35
	φ (h.min)	18.43	**10.06 ←**	**9.21 ←**	**7.12 ←**
	p	0.034*	0.004*	**0.077**	0.043*
T	M (nmol/l)	1.39	**↓ 0.91**	**↑ 1.66**	**↓ 0.78**
	A (nmol/l)	1.00	**↓ 0.58**	**↑ 1.11**	**↓ 0.41**
	φ (h.min)	17.06	**6.44 ←**	17.48	**10.28 ←**
	p	0.015*	0.025*	0.012*	0.004*

↑ – relative increase of value by more than 10%
↓ – relative decrease of value by more than 10%
← – peak shift to earlier time points (more than 2 hours)
→ – peak shift to later time points (more than 2 hours)

***p** = less than 0.05 indicates statistically significant 24 h rhythm
– **significant differences, compared to control, are marked in bold**

maximum values of investigated bone turnover markers from 27° up to 122°C. Our results also demonstrate that the mechanism of endogenous MEL circadian concentration dependence on investigated markers of bone metabolism may be influenced by pinealectomy-induced changes in the excretion of studied hormones, especially of PTH, CT, B and IGF-I. It is known that following pineal gland removal in animals increased secretion is generally observed for the majority of hormones (MESS 1983; KNIAZEWSKI et al. 1990 a, b; OSTROWSKA et al. 1990, ZWIRSKA-KORCZALA et al. 1991; WEIDENFELD et al. 1993; VAUGHAN et al. 1994; KARASEK 1997). The data reported herein demonstrates that the changes in concentration of calciotropic and thyroid hormones, B, T and IGF-I are not equally remarkable during the 24-hour period which causes considerable irregularities in their chronoorganization such as the changes of amplitude and rhythm phase. These changes may, in turn, substantially influence the circadian metabolism of bone tissue (BADURSKI 1994; GALUS 1995). It has been shown that after the removal of pineal gland in rats there is a negative cor-

Table 6
The correlation between the values of melatonin (MEL), insulin-like growth factor (IGF-I), calciotropic hormones (PTH, CT), thyroid hormone (FT3), corticosterone (B), testosterone (T) and chosen biochemical markers of bone metabolism (ALP, PICP and ICTP, all determined in serum as well as HYP and Ca, determined in urine) in sham operated (SPx) and pinealectomized (Px) rats after long-term melatonin (MEL) administration. Table includes only these variables that show significant correlation in at least one study group

Dependent variable	Independent variables	Groups (LD 12 : 12)		
		SPx + MEL	Px	Px + MEL
ALP (U/l)	MEL (pg/ml)	**-0.289***	NS	NS
	PTH (pg/ml)	**-0.316***	**-0.900*****	NS
	CT (pmol/l)	**-0.287***	**-0.545***	NS
	B (nmol/l)	**-0.666*****	**-0.754*****	**-0.470*****
PICP (µg/l)	MEL (pg/ml)	**-0.570*****	**-0.393****	**-0.427***
	PTH (pg/ml)	**-0.666*****	**-0.542*****	NS
	CT (pmol/l)	**-0.442****	**-0.932*****	NS
	IGF-I (nmol/l)	**0.297***	**0.289***	NS
	B (nmol/l)	**-0.366****	**-0.286***	**-0.295***
ICTP (µg/l)	MEL (pg/ml)	**-0.574*****	**-0.905*****	**-0.529*****
	IGF-I (nmol/l)	**0.292***	**0.311***	NS
	FT3 (pmol/l)	**0.544*****	NS	NS
	B (nmol/l)	**0.286***	**0.412****	**0.560*****
HYP (µmol/l)	MEL (pg/ml)	**-0.444****	**-0.629*****	**-0.384****
	IGF-I (nmol/l)	**0.608*****	**0.472*****	NS
	FT3 (pmol/l)	**0.700*****	NS	NS
	B (nmol/l)	NS	**0.394****	NS
Ca (mmol/l)	MEL (pg/ml)	**-0.884*****	**-0.832*****	**-0.297***
	IGF-I (nmol/l)	**0.775*****	**0.416****	NS
	FT3 (pmol/l)	**0.799*****	NS	NS
	B (nmol/l)	**0.410****	**0.436****	**0.306***

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ statistically significant value of correlation coefficient

relation between the changes in circadian values of bone formation markers and changes in the concentration of calciotropic hormones and B. Induction of circadian PICP rhythm disturbances was additionally mediated by the changes in concentration of IGF-I. However, differences in circadian concentration of investigated bone resorption markers showed a significant positive correlation with the changes of IGF-I and B concentration. The results obtained show that pineal gland removal induces not only disturbances in the chronoorganization of bone turnover markers and examined hormones, but also the changes in their mutual circadian relationships. It appears that, in rats, not only pineal gland removal but also the functions of the GH/IGF-I axis, parathyroids and adrenal cortex, disturbed under this condition, may participate in causing circadian rhythm anomalies of bone metabolism markers.

MEL administration at the end of the light phase, i.e. at the time of strongest MEL receptors' sensitiv-

ity (GAUER et al. 1993, 1994 a, b) to rats that were either pinealectomized or had the gland preserved, causes a considerable increase of endogenous MEL circadian concentration, a decrease of circadian oscillations' amplitude and a marked extension of night time peak duration. These changes were accompanied by disturbances in the chronoorganization of bone metabolism markers, calciotropic and thyroid hormones, B, T and IGF-I as well as by disturbances of mutual circadian relationships, which may suggest that the latter ones are not accidental. Our earlier studies (KNIAZEWSKI et al. 1990 a, b; ZWIRSKA-KORCZALA et al. 1991; OSTROWSKA et al. 1993), other reports (JOHN et al. 1990; PERSENGIEV and KANCHEV 1991; JETTON et al. 1994; VAUGHAN et al. 1994; KARASEK 1997) and the results presented in this paper point to the substantial effect of MEL in the regulation of circadian activity of the GH/IGF-I axis and parathyroids, thyroid, adrenal cortex and testes functions. These reports also demonstrate that MEL administration to pinealectomized rats only partially prevents changes in the circadian oscillations of calciotropic and thyroid hormones, B, T and IGF-I. This may point to the participation of other pineal gland-derived substances, apart from MEL, in the mechanism of the above relationship. The present study corroborates also the earlier formulated concept (ZWIRSKA-KORCZALA et al. 1991; OSTROWSKA et al. 1993) that MEL participation in circadian profile disturbance induction of investigated hormones is greater in rats with pineal glands preserved than in pinealectomized animals, which is confirmed by much higher values of correlation coefficients.

Since there have been no experiments reported so far assessing the impact of MEL administration and endocrine function changes induced by this upon the circadian metabolism of bone tissue in rats, we undertook this problem in our study. We show that MEL administration to rats with pineal glands removed causes a suppressive round-the-clock effect with respect to ALP, PICP, Ca and mainly a light-phase effect with respect to ICTP and HYP; at the remaining time points the effect was contrary. As a result, lower mesor values of ALP, PICP and Ca are seen along with disturbances such as decreased amplitude of ALP, HYP and Ca circadian oscillation. There may also be a small shift of PICP concentration peak by 16°C and a considerable shift for the remaining bone metabolism markers, from 32°C up to 103°C. The distinct influence of long-term MEL administration to pinealectomized rats upon amplitude and/or rhythm phase of investigated bone markers suggests that this hormone may be important for modulation of their circadian oscillations. The fact that circadian changes in endogenous MEL concentration correlated negatively with changes of investigated bone markers (significantly in case of PICP, ICTP, HYP and Ca) corroborates the above hypothesis. As in the case of hormones, MEL administration did not normalize but only partially leveled off pinealectomy-related changes in the chronoorganization of the investigated bone markers. This may point to pineal gland-derived substances other than MEL that takes part in generating the observed disturbances of bone metabolism. It has been established that there are compounds other than MEL, mainly indoles that are formed in the pineal gland (KARASEK 1997; REITER 1989, 1990). Correlation analysis has also shown that circadian changes in B concentration, following MEL administration to pinealectomized rats are of substantial importance in inducing disturbances in concentration of bone metabolism markers, especially ALP, PICP, ICTP and Ca. However, concentration changes of other hormonal factors are most probably insignificant.

MEL administration to rats with their pineal gland preserved had usually a slightly less intense effect upon inhibition of bone tissue metabolism and the effect was generally limited to the 24-hour period during which the increase of endogenous MEL was at its maximum. Lowering of the bone formation markers' values was observed in the light phase and that of resorption markers in the light phase and at the beginning of the dark phase. During the remaining hours no effect could be shown or the effect was a stimulating one. As a consequence of these irregularities, a lowering of mesor values of ALP, PICP, ICTP was noted along with disturbances in their circadian oscillation patterns, occurring as rhythm abolition of all investigated markers and a shift of maximum ALP, PICP, HYP and Ca values from 33°C up to 195°C. Participation of MEL in the induction of bone metabolism circadian changes in rats with preserved pineal glands was considerably higher than in pinealectomized animals. This is stressed by the negative correlation between circadian changes of

MEL concentration and all investigated markers of bone metabolism as well as higher values of correlation coefficients. What is more, MEL-induced changes in the secretory activity of parathyroids, thyroid (especially triiodothyronine), adrenal cortex and IGF-I concentration may be of importance for the mechanism of the above relationship. It is attested by the negative correlation between circadian changes of bone formation markers' values and changes in PTH, CT and B concentration, as well as by the positive correlation between PICP and IGF-I. Also, concentration changes of the bone resorption markers showed a significant positive correlation with changes of IGF-I, triiodothyronine and B.

The presented results clearly show that the effect of exogenous MEL upon bone tissue metabolism and the endocrine function of rats depends on the pineal gland, although the mechanism has not been unambiguously elucidated. Some studies showed that exogenous MEL may directly change biochemical and morphological parameters of the pineal gland (Karasek 1997). Being a target organ for this hormone, the pineal gland itself could modulate the effects of its own activity by the agency of MEL-specific receptors. Their presence in the pineal gland was indeed established (Morgan et al. 1994). Increase in NAT and MEL content in the pineal gland (Miguez et al. 1996) seen after exogenous MEL administration seems to confirm this concept, which is also corroborated by the effect of exogenous MEL administration, i.e. the extension of peak nocturnal hormone expression and causes morphological changes in the pineal gland akin to those induced by a short light cycle. It has been established that the density of MEL receptors is inversely proportional to the blood concentration of this hormone (Gauer et al. 1993, 1994 a, b) hence the maximum MEL effect occurs at the end of the light phase. Other studies suggest the possibility of an indirect effect of exogenous MEL on the pineal gland that would be mediated by the changes in concentration of hormones (Niles et al. 1979; Mess 1983; Armstrong et al. 1986; John et al. 1990; Reiter 1991; Karasek 1997) which may be fed back to the pineal gland (Champney et al. 1985; Catala et al. 1988; Bondarenko 1991; Rom-Bugoslavoskaya et al. 1991; Zhao and Touitou 1993). It turns out, however, that the results assessing effects of various hormones upon the pineal gland are not always unequivocal. In rats with the pituitary removed the nighttime NAT and MEL increase was lower, which might suggest a substantial participation of pituitary tropic hormones in modulating the biosynthesis and release of this hormone from the pineal gland (Sabry and Reiter 1988). However, PRL and GH administration (either together or separately) to rats that underwent hypophysectomy did not cause any high NAT and MEL values to return at nighttime, which suggests that these two hormones do not influence particularly the MEL production *in vivo* (Sabry and Reiter 1988). However, *in vitro* studies (Vacas et al. 1987) showed that PRL in a dose of 1 ng/ml stimulates MEL synthesis and release from the pineal gland whereas at 100 ng/ml these processes are inhibited. However, FSH at 100 ng/ml inhibits MEL production whereas LH, TSH and GH stimulate it and ACTH has no effect. Bauer et al. (1989) investigations were unable to demonstrate the effect of pituitary-adrenal or -thyroid axes upon synthesis and release of MEL from the pineal gland. However, Zhao and Touitou (1993) concluded that the effect of glucocorticosteroids (GC) on MEL production is dose-dependent; high doses of B and dexamethazone bring about a decrease of MEL synthesis while low concentrations of both steroids do not exert any effect. It has also been concluded that GC inhibit β-agonist induced increases of NAT activity in the pineal gland. However, no significant GC effect upon HIOMT activity was evident (Yuwiler 1989). Some other studies (Champney et al. 1985) show that MEL synthesis and secretion from the pineal gland, especially at night, depend on thyroid function. It has been demonstrated that under *in vitro* conditions, triiodothyronine stimulates MEL synthesis and secretion during the day, and inhibits during the dark period (Catala et al. 1988). An increase of the nighttime MEL secretion peak has been observed in rats affected by hypothyroidism but following thyroxine administration, MEL secretion decreased (Bondarenko 1991). It has been also shown that thyroxine stimulates serotonin O-methylation in the pineal gland whereas calcitonin inhibits it (Rom-Bugoslavskaya et al. 1991).

The mechanism of MEL action on the circadian metabolism of bone tissue is difficult to explain, based on results obtained *in vivo*. It is not only the question of independent effects of its action but also of the in-

teractions with other endogenous factors, not necessarily of hormonal. One may merely postulate the possibility of direct and/or indirect MEL interaction with the elements of bone tissue. *In vitro* studies indicate that MEL stimulates proliferation and type I collagen synthesis in human bone cells (NAKADE et al. 1999; ROTH et al. 1999). Besides acting directly as suggested, MEL might also affect the circadian rhythm of bone formation and resorption by acting upon endogenous hormonal and growth factors playing a fundamental role in bone metabolism regulation (BADURSKI et al. 1994; GALUS 1995, OSTROWSKA et al. 2002). Our studies indicate that the mechanisms of this dependence involve calciotropic hormones (PTH and CT), IGF-I, B and FT3 concentrations (the latter only in sham operated rats receiving MEL).

In conclusion, our results suggest that pinealectomy in rats and long-term MEL administration significantly influence circadian metabolism of bone tissue and an important role in the mechanism of this dependence seems to be played by the changes of endogenous MEL concentration. A possible mechanism of this relationship might involve calciotropic hormones, IGF-I, corticosterone and FT3 concentrations (the latter only in sham operated rats receiving MEL).

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