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The relationship between the daily profile of chosen biochemical markers of bone metabolism and melatonin and other hormone secretion in rats under physiological conditions

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Abstract

BACKGROUND: The mechanism of generating and synchronizing daily rhythms of bone and mineral metabolism markers has not been entirely explained. Most studies indicate that LD cycle and/or feeding schedules provide important synchronizers of these rhythms. It seems that endogenous factors, including systemic and local hormones can be important in the mechanism of dependence of bone and mineral metabolism parameters rhythms on LD cycle and feeding schedule.

OBJECTIVE: To assess the relationship between the daily profile of chosen biochemical parameters of bone and mineral metabolism (serum ALP, PICP, ICTP and iP and urinary excretion of HYP and total calcium) and daily secretion of MEL, GH/IGF-I axis activity and parathyroid, thyroid, adrenal cortex and gonads function in 48 adult male rats.

METHODS: Material for studies was collected every 3 hours within a day. Hormones, PICP and ICTP concentrations were determined with the use of RIA method and ALP, HYP, total calcium and iP values – spectrophotometrically.

RESULTS: Existence of a negative correlation between daily oscillations of studied markers of osteogenesis (ALP and PICP) and daily profile of MEL and PTH secretion and positive – with daily fluctuations of GH and IGF-I was shown. Moreover, ALP values correlated negatively with daily oscillations of CT. Concentrations of bone resorption markers (ICTP, HYP and total calcium) correlated negatively with daily fluctuations of MEL and – positively with GH, IGF-I and thyroid hormones.

CONCLUSION: The present results suggest that physiological daily rhythmicity of PTH, MEL, GH, IGF-I and thyroid hormones most probably play an important role in regulating the daily rhythm of biochemical markers of bone metabolism.

ORIGINAL ARTICLE

Abbreviations

ALP alkaline phosphatase
B corticosterone
CT calcitonin
FT4 free thyroxine
FT3 free triiodothyronine
GH growth hormone
HYP hydroxyproline
ICTP cross-linked carboxyterminal telopeptide of type I collagen
IGF-I insulin-like growth factor-I
MEL melatonin
iP inorganic phosphorus
PICP carboxyterminal propeptide of type I procollagen
PTH parathormone
T testosterone
T4 total thyroxine
T3 total triiodothyronine

Introduction

Biochemical parameters of bone and mineral metabolism show a daily rhythm in experimental animals and humans [1–11], but the physiological mechanisms underlying these rhythms have not been entirely explained. In animal studies, the predominant mechanism underlying the daily rhythms of bone and mineral metabolism parameters seems to be exogenous and depends on intake of calcium and phosphorus [12, 13]. Numerous investigators have reported the influence of feeding schedules (meal timing) on the circadian changes in biochemical parameters of bone and mineral metabolism [1, 6, 14–16]. Most of the data support the conception that the feeding schedule is a powerful Zeitgeber that can override the influence of the light:dark (LD) cycle. Nevertheless, the LD cycle remained the predominant synchronizer for the bone matrix formation rhythm [3]. There is some evidence that endogenous mechanisms could also be important. Several published observations suggest that parathormone (PTH) and glucocorticoids might play a role in the synchronization of the bone metabolism periodicity [4, 10, 17–20]. However, an effect of other hormones, including melatonin (MEL), cannot be excluded.

The aim of the study was to investigate the influence of the endogenous daily fluctuations of MEL and other hormones such as growth hormone (GH), insulin-like growth factor-I (IGF-I), total and free triiodothyronine (T3, FT3), total and free thyroxine (T4, FT4), corticosterone (B), testosterone (T) in serum and PTH and calcitonin (CT) in plasma on the daily rhythmicity of chosen biochemical parameters of bone and mineral metabolism (alkaline phosphatase – ALP, carboxyterminal propeptide of type I procollagen – PICP, cross-linked carboxyterminal telopeptide of type I collagen – ICTP, inorganic phosphorus – iP levels in serum and urinary excretion of hydroxyproline – HYP and total calcium) in rats under physiological conditions.

Material and methods

The experiment was carried out from 2 to 18 March 1999 on 48 adult male Wistar rats weighing 175±18 g. During the experiment the animals were housed under conditions of uniform temperature (20–22 °C), air humidity (80–85%) and natural LD cycle (light on ~ 6.25 am: light off ~ 5.35 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 2 weeks adjustment, the rats were divided into eight equal subgroups of 6 animals in each. At the appropriate time, i.e. at 3-hour intervals within 24 hours, the animals were marked and placed separately in metabolic cages for 3 hours in order to collect urine aliquots for HYP and total calcium determination. The first urine samples were collected between 6.30 and 9.30 am. The rats were decapitated at 3-hour intervals within 24 hours (starting at 8 am) on the following day. The blood was collected into test tubes: a) with granulated mass (Sarstedt) in order to measure ALP, PICP, ICTP, iP, MEL, GH, IGF-I, T3, FT3, T4, FT4, B and T concentrations; b) with EDTA in order to measure PTH and CT levels. The blood was centrifuged immediately (450 g, 10 min) and the obtained serum or plasma samples were stored frozen at –75°C until determination. All activities in the dark phase were performed under red light. The study was conducted with the permission of the Ethics Committee at the Silesian Academy of Medicine in Katowice.

Concentrations of hormones, PICP and ICTP were measured using rat radioimmunological (RIA) kits: MEL, PTH, CT (DRG, USA); GH, IGF-I, B (DSL, USA), T3, FT3, T4, FT4, T, PICP, ICTP (FARMOS, Finland). Serum ALP activity, iP levels and urinary excretion of total calcium were determined using ALPHA DIAGNOSTICS kits (Poland). Urinary excretion of HYP was determined using a colorimetric micromethod [21]. The sensitivity of assays was as follows: MEL, PTH, CT – 0.2 pg/tube, GH – 1 ng/ml, IGF-I – 2.73 nmol/l, B – 7 nmol/l, T3 – 0.2 nmol/l, FT3 – 0.25 pmol/l, T4 – 5 nmol/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, iP and total calcium methods were: up to 1000 U, up to 15 mg/dl and up to 16 mg/dl, respectively. The respective intraassay and interassay coefficients of variations were: MEL – 11 and 12%, PTH – 7.9 and 8%, CT – 8.9 and 9.1%, GH – 6.2 and 8.1%, IGF-I – 3.7 and 6.1%, B – 8.3 and 9.5%, T3 – 3.3 and 7.5%, FT3 – 6.9 and 7.7%, T4 – 6.8 and 8%, 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%, iP – 8.7 and 9.9%, total calcium – 4.7 and 6.8%.

The results were analyzed 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 [22]. The interrelation between the values of chosen hormones and biochemical markers of bone metabolism was assessed using the Pearson correlation test.

Results

In all studied rats serum ALP activities and PICP and ICTP levels were subjected to significant daily oscillations with acrophase at 2.49 pm, 1.39 pm and 1.26 pm, respectively. The respective mesor and amplitude values were: ALP – 265.32 and 50.01 U/l, PICP – 15.02 and 4.49 µg/l, ICTP – 15.69 and 4.18 µg/l. Also HYP excretion was subjected to daily rhythmic fluctuation with acrophase at 12.38 pm, mesor value of 14.59 µmol/l and amplitude of 2.00 µmol/l (Tables 1 and 2, Fig. 1).

Daily rhythm of plasma calcium-regulated hormones (PTH and CT) in rats with acrophase respectively at 4.48 am and 4.39 am, mesor values of 116.82 pg/ml and 18.41 pmol/l and amplitude of 34.96 pg/ml and 4.02 pmol/l was also shown. Urinary excretion of total calcium was subjected to daily fluctuation with acrophase at 2.39 pm, mesor value of 2.64 mmol/l and amplitude of 0.54 mmol/l. Daily rhythm of serum iP concentrations with peak at 12.31 pm, mesor value of 2.70 mmol/l and amplitude – of 0.20 mmol/l was also shown (Tables 1 and 2, Fig. 2).

Table 1. The chronobiologic parameters (mesor, amplitude, acrophase) of the mean rhythms of biochemical markers of bone metabolism (alkaline phosphatase - ALP, carboxyterminal propeptide of type I procollagen - PICP, cross-linked carboxyterminal propeptide of type I collagen - ICTP in serum and hydroxyproline - HYP in urine), calciotropic hormones (PTH, CT) in plasma, total calcium in urine and inorganic phosphorus (iP) in serum and other hormones (melatonin - MEL, growth hormone - GH, insulin-like growth factor-I - IGF-I, thyroid hormones - T3, FT3, T4, FT4, corticosterone - B and testosterone - T in serum) in rats under physiological conditions

The chronobiologic parameters					
Variables	Mesor	Amplitude	Acrophase h.min	PR %	p
ALP (U/l)	265.32	50.01	14.49	74.92	0.031*
PICP (µg/l)	15.02	4.49	13.39	84.88	0.008*
ICTP (µg/l)	15.69	4.18	13.26	87.80	0.004*
HYP (µmol/l)	14.59	2.00	12.38	97.12	0.001*
PTH (pg/ml)	116.82	34.96	4.48	85.89	0.007*
CT (pmol/l)	18.41	4.02	4.39	86.85	0.009*
total calcium (mmol/l)	2.64	0.54	14.39	74.66	0.031*
iP (mmol/l)	2.70	0.20	12.31	75.30	0.029*
MEL (pg/ml)	26.01	25.76	3.00	75.63	0.030*
GH (ng/ml)	23.47	7.89	6.22	80.90	0.019*
IGF-I (nmol/l)	117.99	32.17	15.23	48.59	0.189
T3 (nmol/l)	0.99	0.32	11.12	82.01	0.015*
FT3 (pmol/l)	5.72	1.50	11.49	75.60	0.028*
T4 (nmol/l)	36.92	6.43	11.46	58.99	0.109
FT4 (pmol/l)	28.47	4.77	12.29	12.80	0.742
B (nmol/l)	350. 99	252.21	18.52	71.00	0.048*
T (nmol/l)	1.26	0.82	17.29	72.43	0.041*

* p = less than 0.05 indicates statistically significant 24 h rhythm

Table 2. The correlation coefficients between the values of melatonin (MEL), growth hormone (GH), insulin-like growth factor (IGF-I), calciotropic hormones (PTH, CT), thyroid hormones (T3, FT3, T4, FT4), corticosterone (B), testosterone (T) and chosen biochemical markers of bone metabolism (ALP, PICP, ICTP, iP, all determined in serum and HYP and Ca, determined in urine) in rats under physiological conditions

Dependent variables	Independent variables ALP (U/l)	PICP (µg/l)	ICTP (µg/l)	HYP (µmol/l)	Ca (mmol/l)	iP (mmol/l)	MEL (pg/ml)
PTH (pg/ml)	–0.517***	–0.346*	NS	NS	NS		–0.387**
CT (pmol/l)	–0.400**	NS	NS	NS	NS		–0.326**
MEL (pg/ml)	–0.538***	–0.318*	–0.585***	–0.382**	–0.451**		–
GH (ng/ml)	0.462**	0.512***	0.287*	0.381**	0.341*		0.496**
IGF-I (nmol/l)	0.510***	0.543***	0.305*	0.365*	0.465***		0.516***
T3 (nmol/l)	NS	NS	0.383**	0.483***	0.405**	NS	–0.289*
FT3 (pmol/l)	NS	NS	0.340*	0.482***	0.332*		–0.551*
T4 (nmol/l)	NS	NS	0.285*	0.362*	0.330*		–0.287*
FT4 (pmol/l)	NS	NS	0.293*	0.474***	0.338*		–0.299*
B (nmol/l)	NS	NS	NS	NS	NS		–0.436***
T (nmol/l)	NS	NS	NS	NS	NS		–0.595***

* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001 – statistically significant value of correlation coefficient

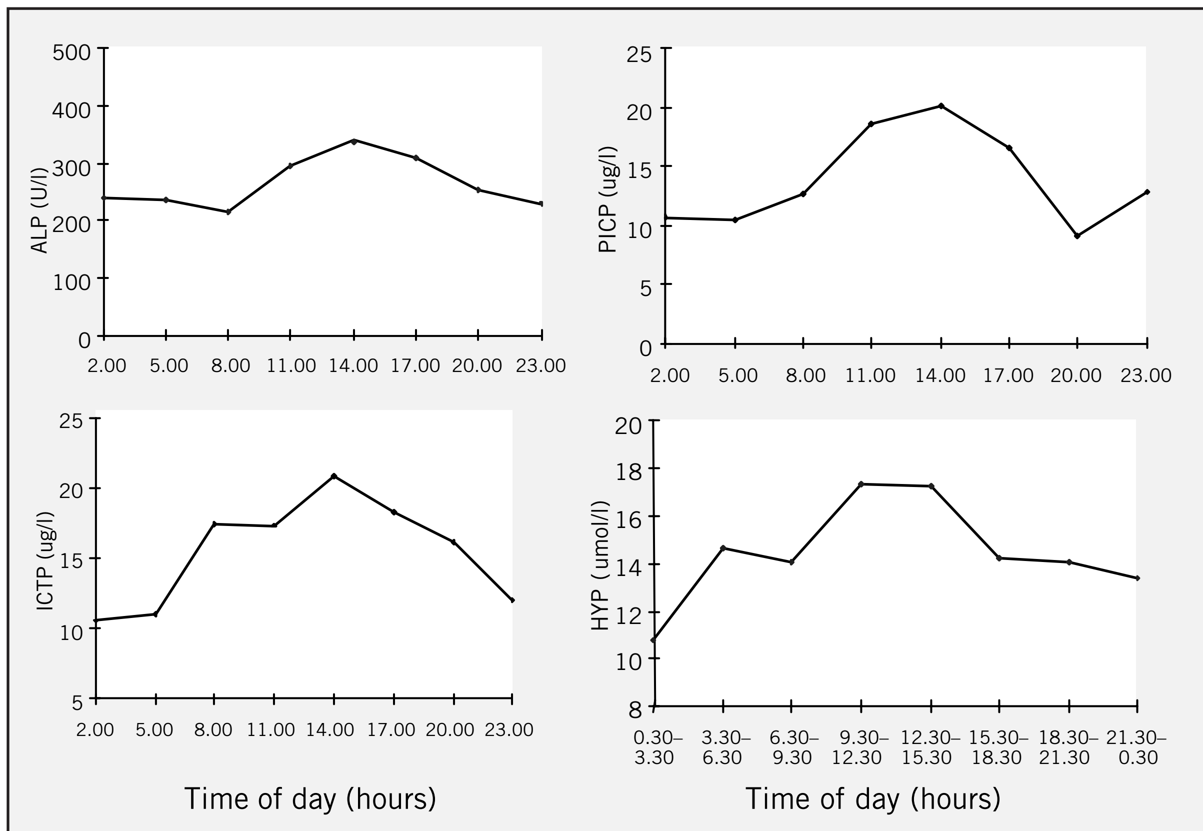

Fig. 1. Daily oscillations of bone metabolites (alkaline phosphatase - ALP activity and carboxyterminal propeptide of type I procollagen - PICP, cross-linked carboxyterminal telopeptide of type I collagen - ICTP concentrations in serum and urinary excretion of hydroksyproline - HYP) in rats under physiological conditions

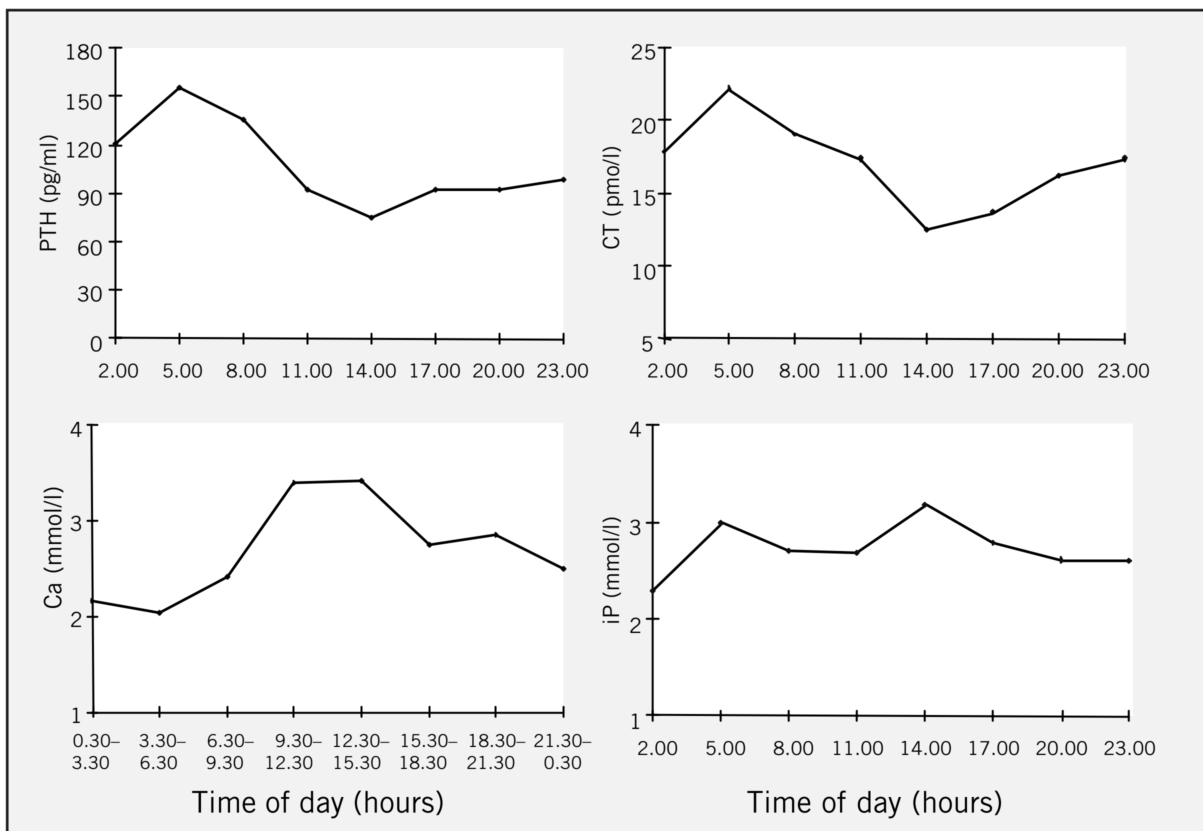

Fig. 2. Daily oscillations of plasma calciotropic hormones (parathormone - PTH, calcitonin - CT), urinary excretion of total calcium (Ca) and serum levels of inorganic phosphorus (iP) in rats under physiological conditions

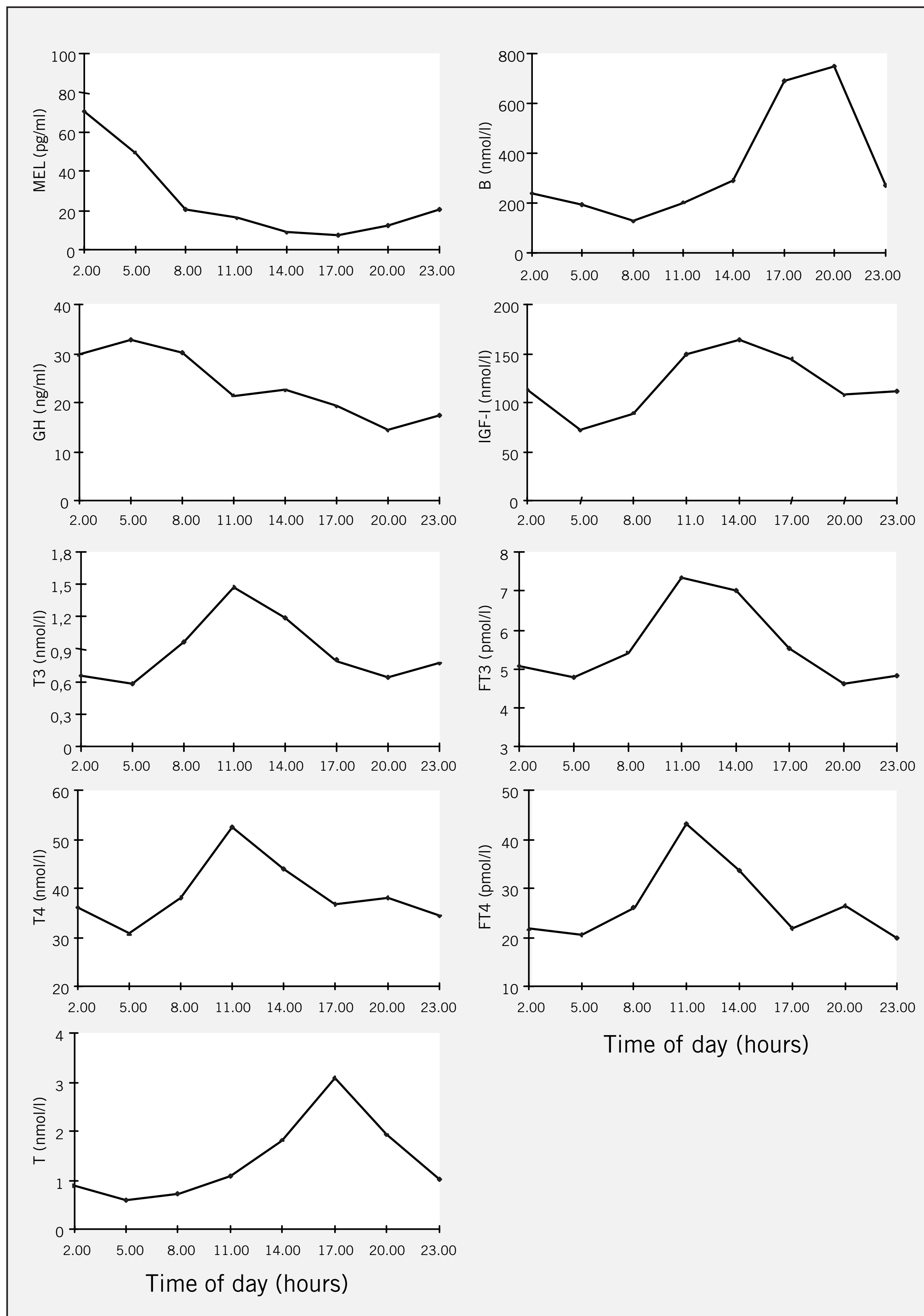

Fig. 3. Daily oscillations of other hormones (serum melatonin - MEL, corticosterone - B, growth hormone - GH, insulin-like growth hormone-I - IGF-I, total and free triiodothyronine - T3, FT3, total and free thyroxine - T4, FT4, testosterone - T concentrations) in rats under physiological conditions

Most hormones in rats were subjected to daily rhythm with acrophase for MEL at 3.00 am, for GH at 6.22 am, for T3 at 11.12 am, for FT3 at 11.49 am, for T at 5.29 pm and for B at 6.52 pm. The respective mesor and amplitude values were: MEL – 26.01 and 25.76 pg/ml, GH – 23.47 and 7.89 ng/ml, T3 – 0.99 and 0.32 nmol/l, FT3 – 5.72 and 1.50 pmol/l, T – 1.26 and 0.82 nmol/l and B – 350.99 nmol/l and 252.21 nmol/l. Serum concentrations of IGF-I, T4 and FT4 in rats were subjected to slight daily oscillations with maximum at 3.23 pm, 11.46 am and 12.29 pm. The respective mesor and amplitude values were: IGF-I – 117.99 and 32.17 nmol/l, T4 – 36.92 and 6.43 nmol/l, FT4 – 28.47 and 4.77 pmol/l (Tables 1 and 2, Fig. 3).

Existence of a negative correlation between daily oscillations of studied markers of osteogenesis (ALP and PICP) and daily profiles of MEL and PTH, and positive – with daily fluctuations of GH and IGF-I was observed. Changes in the values of ALP during the day correlated negatively with daily oscillations of CT. Daily fluctuations of ICTP, HYP and total calcium correlated negatively with 24-hours levels of MEL, and positively – with daily concentrations of GH, IGF-I and thyroid hormones. Changes in the concentrations of MEL during the day correlated negatively with daily oscillations of other hormones (Table 3).

Discussion

Biochemical parameters of bone metabolism exhibit daily rhythmic fluctuation both in experimental animals and in humans [1–11, 23]. *In vitro* studies in the rats indicate that bone matrix formation (specifically net collagen synthesis) and bone resorption progresses most actively during the daily light span and least actively during the daily dark span [7, 16]. The pattern of *in vivo* bone metabolism activity in rats is similar to that observed *in vitro*, and is confirmed by a distinct increase of biochemical markers of these processes during midday with the maximum usually between 11 am and 3 pm [3, 6, 23]. Similarly, in our studies in rats distinct daily oscillations of osteogenesis markers (ALP and PICP in serum) with a peak at 1.39 and 2.49 pm, and bone tissue resorption (ICTP in serum and HYP in urine) with a maximum at 12.38 and 1.26 pm were shown. The daily oscillations of urinary excretion of total calcium and serum iP levels in our study were similar to those found by others [6, 16]; markedly higher values were observed during the daily light span.

The etiology of the daily rhythms in the various biochemical parameters of bone and mineral metabolism is not completely understood. The timing and magnitude of these rhythms appears to be constrained by the LD cycle, feeding schedules (meal timing), and endocrine relationships. Numerous investigators have reported the influence of feeding schedules on the circadian changes in plasma calcium and iP concentrations [1, 6, 14, 15], and bone-resorbing activity in serum [16]. Most of the data support the concept that the feeding schedule is a powerful synchronizer that can override the influence of the LD cycle. Nevertheless, the LD cycle remained the predominant synchronizer for the rhythms of cartilage and both bone collagen and non-collagen protein synthesis [3, 6, 7, 15, 23]. These latter rhythms were effectively abolished, however, when rats fed during the early light span; this non-physiological feeding being a particularly unphysiological time of day [6].

There is some evidence that systemic and local hormones might play a role in the synchronization of the bone metabolism periodicity [4, 10, 17–20]. In this study, we demonstrated that the main candidates hormones are PTH, MEL, GH, IGF-I and thyroid hormones which affect bone metabolism *in vitro* [24, 25, 26, 27], and have a daily rhythm (except IGF-I, T4 and FT4) *in vivo* [10, 28–32]. However, daily fluctuation of CT, B and T levels have probably not got greater importance in determining daily bone metabolism periodicity.

Most investigators postulate that PTH might play a role in the synchronization of the daily rhythm of bone resorption. Some studies have indicated that oral calcium supplementation for 14 days in doses sufficient to reverse the nocturnal increase in PTH blunted the nocturnal increase in bone resorption and decreased overall daily bone resorption [33–35]. It is therefore likely that daily rhythm in PTH or calcium intake is an important modulator of the daily periodicity of bone resorption. However, doses of calcium that reversed the PTH rhythm blunted rather than reversed the daily rhythm of urinary excretion of deoxypyridinoline [33, 34]. Other studies have demonstrated a similar effect of calcium supplementation on serum bone-resorbing activity in rats [16]. The data reported herein suggest that the physiological alterations of PTH do not seem to regulate the daily rhythm of bone resorption in rats. No consistent correlation was observed between PTH and ICTP chronograms and those of HYP and total calcium. Numerous reports failed to demonstrate an association between the biorhythms of bone formation, plasma calcium or phosphorus, and PTH titers [3, 7, 19]. These studies indicated that adrenalectomy abolished the regional DNA synthesis rhythm without altering the phases of the plasma calcium and iP rhythms, and that parathyroidectomy abolished the regional rhythms for collagen and non-collagen protein synthesis and calcium rhythms, without altering the phase of the plasma iP and B rhythms. Dietary calcium-lactate supplements, which raised plasma calcium levels towards normal in parathyrectomized rats, were able to correct serum B values but did not normalize bone collagen and non-collagen protein synthesis values. These results suggest, that physiological alterations on B serve to modulate cartilage and bone cell proliferation but they do not seem to regulate the phasing of the collagen synthetic rhythm, and that PTH is essential to maintain normal collagen and non-collagen protein synthesis [6, 7, 19]. In our studies in rats, we demonstrated that the daily rhythmicity of studied markers of bone formation were dependent upon and antiphasal to the daily fluctuations of PTH. A nega-

tive correlation between PTH and ALP chronograms and those of PICP seem to corroborate the conception that physiological alterations of PTH serve to modulate daily rhythm of bone formation.

Although CT treatment has been shown to prevent bone loss in estrogen-deficient states, the function of endogenous CT in bone metabolism is not clearly established. In rats, endogenous CT has a protective effect against PTH-stimulated cancellous bone loss, but not cortical bone loss [36]. In our studies in rats, serum CT concentration rose during the daily dark period when the values of serum bone tissue metabolites, urinary excretion of calcium, and serum iP levels were minimal. No correlation was found between CT and ICTP chronograms and those of HYP, total calcium and iP. Obtained results suggest that CT does not contribute to daily rhythmicity of bone resorption. In normal rats, changes in endogenous CT secretion may actually increase rather than decrease daily fluctuations of plasma calcium and iP. Daily changes in theirs values are influenced by condition reflex developed due to the feeding habits of the rats [14, 29, 37]. It is suggested that intestinal hormones rather than calcium may be the primary control of CT secretion, and that the gastrointestinal tract plays a major role in both calcium and phosphate homeostasis [14].

It is known that CT stimulate of bone tissue formation [24, 25]. However, performed analysis of correlation indicated only small inversely proportional dependence between CT and ALP and PICP chronograms, significant only in relation to ALP. Hence there is a suggestion that this hormone can have only little importance in maintaining physiological rhythm of osteoblasts activity.

Another candidate hormone is cortisol, which seems to be important in determining the peak and nadir of the daily bone resorption rhythm [16, 38]. Other studies, however, indicate that only the daily fluctuations in osteocalcin can be controlled by the daily variations in serum cortisol, whereas this hormone does not control the daily oscillations in either the serum PICP or the urinary excretion in piridinum crosslinks [39]. A direct inhibitory effect of cortisol on osteoblast maturation and activity [40–43] may also lead to enhanced bone loss. This inhibitory effect is reflected by plasma osteocalcin, which is reduced in conditions accompanied by an excess of glucocorticoids [43, 44]. In the rats, no significant correlation was observed between daily oscillations of B and daily fluctuations of serum and urinary bone metabolites, urinary excretion of total calcium and serum iP levels. Obtained result suggest that physiological fluctuations in serum B levels have probably not got greater importance in maintaining daily rhythmicity of bone tissue metabolism.

Inversely proportional dependence between daily secretion of MEL in rats and daily rhythmicity of the bone metabolism markers observed in our studies deserves particular attention. High MEL levels during the night period was accompanied by low values of serum ALP, PICP and ICTP and urinary excretion of HYP and total calcium. Little is known about the effects of MEL on bone tissue metabolism in male rats. Our own studies performed previously on starved male rats have shown a suppressive effect of MEL on bone formation and resorption processes [45]. Furthermore, it has been found [unpublished data] that lighting conditions, pinealectomy and long-term MEL administration influence the daily bone metabolism in male rats, and the changes in endogenous MEL concentrations seem to play an important role in this mechanism. MEL is also an important modulator of experimental osteoporosis processes induced in male rats by way of orchidectomy [unpublished data]. There could be two possible explanations of the mechanism of bone metabolism modulation by MEL. It seems that MEL can influence bone metabolism indirectly by inducing changes in concentrations of endogenous factors, including hormones (such as PTH, B, IGF-I and thyroid hormones) that significantly influence these processes [unpublished data] and directly [26, 27]. Latest studies indicate that MEL may play an essential role in regulating bone growth. *In vitro* studies indicate that it stimulates osteoblast differentiation and mineralization of matrix in culture [27]. MEL enhances also synthesis of collagenic and non-collagenic proteins of bone matrix [26]. Obtained results in the rats allowed the supposition that maintaining normal MEL concentrations in blood during the day maintenance of physiological rhythm of studied markers of bone formation and resorption.

Little is known about the role of GH/IGF-I axis activity on the maintenance of normal daily rhythmicity of bone metabolism. The data reported herein suggest that serum GH and IGF-I concentrations might play a role in the synchronization of the bone metabolism markers rhythmicity. A positive correlation between daily oscillations of GH and IGF-I and daily fluctuations of studied markers of bone formation and resorption seems to corroborate this concept. It is known that GH has influence on bone tissue mainly by IGF-I [46, 47]. IGF-I has been shown to be an important stimulator of bone tissue formation and osteoblasts activities *in vitro* and *in vivo* [20, 46–50]. This hormone also stimulates bone tissue resorption *in vitro*, having an influence on mature osteoclasts and conditions forming new osteoclasts from precursor cells [20, 46, 48]. IGF-I treatment stimulated resorptive activity in ovariectomized rats [51]. It had no significant effect on bone formation but when combined with PTH, IGF-I blunted the response to PTH on the periosteal and endocortical surface. IGF-I and/or insulin-like growth factor binding protein-5 seemed to be involved in the estrogen-induced modulation of PTH action on osteoblast proliferation and function [52]. The administration of rhGH and rhIGF-I, like that of estradiol, into aged ovariectomized rats prevents further loss of bone mass and strength at sites containing trabecular bone. In addition, rhGH increases cortical bone mass above pretreatment values [53]. Positive correlation between daily concentrations of GH and IGF-I and daily levels of bone metabolism markers obtained in our studies additionally indicates possible participation of these hormones

in determining physiological bone metabolism rhythmicity.

We have shown in our studies in the rats significant convergence of rhythms of chosen markers of bone resorption with daily oscillations of thyroid hormones, which allows the conclusion that these hormones concentrations in blood can have importance in periodical synchronization of physiological rhythms of serum ICTP and HYP levels and urinary excretion of total calcium. Concentrations of thyroid hormones, similarly as concentrations of the bone resorption markers, start to increase at the beginning of the light phase and reach the highest values between 11am and 2 pm. The stimulating influence of thyroid hormones on bone tissue resorption has been well documented [24, 25, 54]. It is claimed that it is realized in the way of a direct influence on skeleton cells [24, 25]. The positive correlation between thyroid hormones and ICTP chronograms and those of HYP and total calcium shown in the rats additionally indicates the possibility of participation of these hormones in modulating daily rhythmicity of bone tissue resorption.

It is known that bone is an androgen-dependent tissue [55, 56]. Short-term androgen deficiency caused an increase in both serum osteocalcin and histomorphometric parameters of bone metabolism in male rats, but not a decrease in bone mass [56]. Long-term androgen deficiency resulted in a decrease in cortical thickness and density. Bone mass in male rats was decreased 4 months after orchidectomy, preceded by an early increase in bone metabolism. Both the early increase in bone metabolism and the later decrease in bone mass were prevented by aromatizable and nonaromatizable androgens and by estrogen [56]. Because T is a potent regulator of bone and mineral metabolism [24, 25, 56], it seemed likely that the increased T content of the serum was the important component of increased bone and mineral parameters. Performed analysis of correlation in the rats indicated only a slight dependence between daily T secretion and daily levels of serum bone metabolites, urinary excretion of total calcium and serum iP levels. Hence the suggestion that the daily alterations of T do not seem to regulate the physiological rhythm of rat bone metabolism.

In conclusion, our results suggest that physiological daily rhythmicity of PTH, MEL, GH, IGF-I and thyroid hormones most probably play an important role in the regulation of the circadian rhythm of bone metabolism markers.

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