INFLUENCE OF LIGHTING CONDITIONS ON DAILY RHYTHM OF BONE METABOLISM IN RATS AND POSSIBLE INVOLVEMENT OF MELATONIN AND OTHER HORMONES IN THIS PROCESS

Z. Ostrowska, B. Kos-Kudla[1], B. Marek[1], D. Kajdaniuk[1]

Department of Clinical Biochemistry, [1]Department of Pathophysiology and Endocrinology, *The Medical University of Silesia, Zabrze, Poland*
E-mail: ozdrasiek@poczta.onet.pl

Objective. To evaluate the influence of lighting conditions on daily fluctuations of biochemical markers of bone and mineral metabolism such as the activity of serum alkaline phosphatase (ALP), the concentration of carboxyterminal propeptide of type I procollagen (PICP), cross-linked carboxyterminal telopeptide of type I collagen (ICTP) and inorganic phosphorus (iP) as well as urinary excretion of hydroxyproline (HYP) and calcium (Ca). In addition, possible involvement of daily secretion of melatonin (MEL), GH/IGF-I axis activity and parathyroid, thyroid, adrenocortical and gonadal function in this process.

Methods. Three series of 48 adult male Wistar rats weighing 180±10 g were conditioned to different lighting conditions for 4 weeks: 1. control group (LD 12:12 h, light on from 7.00 to 19.00); 2. short-day group (LD 0.5:23.5 h, light on from 7.00 to 7.30) ; 3. long-day group (LD 23.5:0.5 h, light on from 19.30 to 19.00). The blood was obtained by decapitation. Material for studies was collected every 3 hours during a day. The concentration of hormones, PICP and ICTP was determined with the use of RIA method, whereas ALP, iP, HYP and Ca levels were estimated spectrophotometrically.

Results. A significant influence of different lighting conditions on the daily profile of investigated markers of bone metabolism in rats was confirmed. Thus a short day was found to have an inducing effect upon the level of these markers, while a long day showed a suppressing effect. Furthermore, substantial changes in the values of amplitude and phase of their daily rhythms were found. Also some distinct anomalies in daily oscillations of ALP, PICP, ICTP, HYP and Ca were found which were dependent on the time of the day and showed a negative correlation with the changes in endogenous MEL concentrations. Moreover, ICTP, HYP and Ca concentrations correlated positively with daily fluctuations of IGF-I and triiodothyronine.

Conclusions. Lighting conditions can influence daily fluctuations of studied bone metabolism markers in rats, and in the mechanism of this dependence the changes in endogenous MEL concentrations seem to play an important role. Secondary changes in daily IGF-I and triiodothyronine oscillations, caused by short- and long-day conditions, result in altered daily bone resorption rhythmicity.

Key Words: Lighting conditions – Bone metabolism – Hormones – Daily rhythm – Male rats

Biochemical parameters of mineral and bone metabolism in rats exhibit daily fluctuations with a peak during the light period (Milhaud et al. 1972; Simmons et al. 1979; Staub et al. 1979; Russel et al. 1983, 1985; Shinoda and Seto. 1985; Shinoda and Okada 1988; Ostrowska et al. 2002). The phasing of their rhythms appears to be constrained by the feeding and/or light:dark (LD) schedules (Shinoda and Seto 1985; Russel et al. 1993, 1994, 1995; Shinoda and Stern 1992). Most of the data support the

concept that the feeding schedule is a powerful synchronizer of the daily changes in calcium (Ca) metabolism and bone resorption pattern that can override the influence of the LD cycle (Milhaud et al. 1974; Talmage 1975; Phillippens et al. 1977; Russel et al. 1983; Shinoda and Stern 1992). Nevertheless, the LD cycle remained the predominant synchronizer for the rhythms of cartilage and bone collagen and non-collagen proteins synthesis. These rhythms were effectively abolished, however, when rats were fed during the early light span (Simmons et al. 1979; Russel et al. 1985). It has been observed that the exposure of rats to a constant lighting schedule for a period exceeding 10 days markedly influences the amplitude and phase of circadian oscillations of Ca, inorganic phosphorus (iP) and blood alkaline phosphatase (ALP) (Shinoda and Seto 1985). Besides exogenous factors, an important role in determining the daily rhythms of bone metabolism markers seems to be played by endogenous factors, mainly hormonal. It has been assumed that parathormone (PTH), melatonin (MEL), growth hormone (GH), insulin-like growth factor-I (IGF-I), corticosterone (B) and thyroid hormones are important synchronizers of these rhythms in rats (Staub et al. 1979; Russel et al. 1984; Canalis et al. 1988; Kream et al. 1980, 1990; Calvo et al. 1991; Ostrowska et al. 2002). On the other hand, these hormone rhythms in rats appear to be constrained by the LD schedule, and in the mechanism of this dependence the changes in endogenous MEL concentrations seem to play an important role (Krieger and Hauser 1978; Niles et al. 1979; Illnerova et al. 1983, 1989; Meunier et al. 1988; Reiter 1986; Cardinali 1991; Ostrowska et al. 1993 a,b, 1994, 2002; Vaughan et al. 1994).

This study was performed to investigate: 1. the influence of lighting conditions on daily fluctuations of selected biochemical parameters of bone and mineral metabolism (serum ALP activity and carboxy-terminal propeptide of type I procollagen – PICP, cross-linked carboxyterminal telopeptide of type I collagen – ICTP and iP concentrations as well as urinary excretion of hydroxyproline – HYP and Ca); 2. possible involvement of daily secretion of melatonin (MEL), GH/IGF-I axis activity and parathyroid, thyroid, adrenal cortex and gonads function in this process.

Materials and Methods

Animals. Three groups of 48 adult male Wistar rats, weighing 180 ± 10 g were conditioned to different lighting conditions for 4 weeks: 1. control group (LD 12:12 h, light on from 7.00 to 19.00 h), 2. short-day group (LD 0.5:23.5 h, light on from 7.00 to 7.30 h), 3. long-day group (LD 23.5:0.5 h, light on from 19.00 to 19.30 h). During the experiment all animals were housed under the conditions of uniform temperature (20-22 °C) and air humidity (80-85 %). 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*.

Experiments. After the end of experiment, the 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. First urine samples were collected between 6.30 and 9.30 hr. Six rats were sacrificed by decapitation 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, Germany) in order to measure ALP, PICP, ICTP, iP, MEL, IGF-I, total and free triiodothyronine (T3, FT3), total and free thyroxine (T4, FT4), corticosterone (B) and testosterone (T) concentrations; b) 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 also to the presence of all personell participating at the procedure of sacifice. 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), T3, FT3, T4,

Table 1

Mean values of melatonin (MEL) and chosen biochemical markers of bone metabolism (alkaline phosphatase – ALP, carboxyterminal propeptide of type I procollagen – PICP, cross-linked carboxyterminal telopeptide of type I collagen – ICTP, inorganic phosphorus – iP all determined in serum and hydroxyproline – HYP and total calcium – Ca, determined in urine) in rats exposed to different light conditions.

		Time of day (hours)							
Variables	Groups	2.00	5.00	8.00	11.00	14.00	17.00	20.00	23.00
	12 : 12	70.00 ± 1.69	49.88 ± 6.38	19.83 ± 3.47	18.31 ± 1.88	6.92 ± 0.37	5.87 ± 0.53	13.70 ± 1.36	23.33 ± 1.15
MEL	0.5 : 23.5	67.54 ± 4.76	47.81 ± 3.82	20.79 ± 0.96	26.04 ± 1.27	**18.59 ± 2.27***	**22.70 ± 1.65***	**39.40 ± 3.69***	**55.89 ± 2.89***
(pg/ml)	23.5 : 0.5	**5.96 ± 0.64***	**8.22 ± 0.67***	**8.45 ± 1.68***	**8.97 ± 0.83***	10.80 ± 2.04	**19.18 ± 1.89***	**22.32 ± 2.13***	**27.98 ± 1.30***
	12 : 12	239.90± 15.02	235.75± 30.33	215.95± 15.29	295.80± 7.63	337.90± 16.58	307.20± 15.81	252.80± 18.36	230.00± 18.83
ALP	0.5 : 23.5	**158.60 ± 9.19***	**124.80± 9.19***	180.37± 13.90	302.44± 18.34	**262.17± 14.06***	**174.38± 10.14***	**189.45± 16.96***	201.49± 11.13
(U/l)	23.5 : 0.5	**194.79± 9.33***	238.51± 15.60	223.91± 18.55	322.82± 23.15	400.31± 35.07	**441.62± 26.05***	276.22± 21.21	**429.53± 31.65***
	12 : 12	10.08 ± 1.04	13.30 ± 1.76	13.92 ± 0.79	18.10 ± 1.47	20.58 ± 0.58	18.06 ± 1.82	12.22 ± 1.23	13.18 ± 1.04
PICP	0.5 : 23.5	11.51 ± 0.33	9.63 ± 1.12	**3.16 ± 0.36***	**7.27 ± 1.04***	**9.04 ± 1.00***	**5.87 ± 1.08***	13.42 ± 1.35	**17.69 ± 1.07***
(µg/l)	23.5 : 0.5	**17.97 ± 0.57***	14.79 ± 1.23	15.00 ± 1.13	20.77 ± 1.53	19.90 ± 0.84	15.85 ± 0.44	11.78 ± 0.64	15.80 ± 0.86
	12 : 12	10.48 ± 0.61	15.94 ± 0.83	15.20 ± 1.60	18.95 ± 1.85	20.32 ± 1.04	18.51 ± 0.65	14.66 ± 0.67	11.80 ± 1.37
ICTP	0.5 : 23.5	**16.34 ± 1.65***	**18.11 ± 1.00***	17.08 ± 1.96	**10.67 ± 1.38***	**12.53 ± 0.47***	**12.71 ± 1.18***	14.74 ± 0.48	10.75 ± 1.10
(µg/l)	23.5 : 0.5	**19.71 ± 2.89***	19.32 ± 1.00	15.35 ± 1.12	14.61 ± 1.72	**12.93 ± 0.75***	**15.52 ± 0.41***	14.46 ± 1.38	**20.29 ± 1.37***
	12 : 12	2.30 ± 0.20	2.99 ± 0.12	2.70 ± 0.19	2.68 ± 0.35	3.17 ± 0.14	2.78 ± 0.09	2.61 ± 0.15	2.60 ± 0.08
iP	0.5 : 23.5	**3.42 ± 0.29***	**2.63 ± 0.04**	2.99 ± 0.06	2.48 ± 0.11	**2.63 ± 0.17***	2.55 ± 0.16	2.52 ± 0.15	**3.54 ± 0.17***
(mmol/l)	23.5 : 0.5	2.20 ± 0.09	**2.31 ± 0.19***	2.34 ± 0.17	2.37 ± 0.21	**1.81 ± 0.10***	**1.42 ± 0.09***	**1.78 ± 0.11***	**1.53 ± 0.03***
		Time of day (hours)							
		0.30 - 3.30	3.30 - 6.30	6.30 - 9.30	9.30 - 12.30	12.30 - 15.30	15.30 - 18.30	18.30 - 21.30	21.30 - 0.30
	12 : 12	12.94 ± 0.98	14.43 ± 0.46	15.36 ± 0.45	16.71 ± 0.52	16.50 ± 0.61	15.61 ± 0.90	14.09 ± 0.41	13.40 ± 0.44
HYP	0.5 : 23.5	13.34 ± 1.01	14.72 ± 0.99	15.32 ± 0.43	**12.99 ± 0.62***	**11.63 ± 0.42***	12.45 ± 1.11	**12.10 ± 0.64***	11.99 ± 1.07
(µmol/l)	23.5 : 0.5	16.01 ± 0.35	**16.90 ± 0.65***	14.77 ± 0.77	**13.19 ± 0.52***	**12.70 ± 0.55***	14.52 ± 0.34	14.00 ± 0.29	**15.80 ± 0.34***
	12 : 12	2.17 ± 0.27	2.04 ± 0.17	2.41 ± 0.26	3.40 ± 0.22	3.43 ± 0.13	2.75 ± 0.28	2.86 ± 0.28	2.50 ± 0.29
Ca	0.5 : 23.5	2.42 ± 0.24	2.35 ± 0.19	**3.22 ± 0.24***	2.96 ± 0.16	2.85 ± 0.33	2.57 ± 0.37	2.80 ± 0.18	2.85 ± 0.22
(mmol/l)	23.5 : 0.5	2.40 ± 0.19	**3.22 ± 0.20***	2.54 ± 0.02	**2.58 ± 0.14***	**2.21 ± 0.06***	2.51 ± 0.09	3.16 ± 0.18	2.70 ± 0.20

* $p \leq 0.05$ vs control group

Table 2
Mean concentrations of plasma parathormone (PTH), calcitonin (CT) and serum insulin-like growth factor (IGF-I), total and free triiodothyronine (T3, FT3), total and free thyroxine (T4, FT4), corticosterone (B) and testosterone (T) in rats exposed to different light conditions.

		Time of day (hours)							
Variables	Groups	2.00	5.00	8.00	11.00	14.00	17.00	20.00	23.00
	12 : 12	143.65 ± 13.52	163.35± 5.01	139.36± 5.50	100.43± 2.47	85.80± 3.38	97.57± 4.03	92.95± 1.24	104.78± 0.72
PTH	0.5 : 23.5	157.95 ± 3.32	**132.60± 3.18***	**85.93± 4.23***	**66.95± 5.98***	**73.52± 3.97***	**62.40± 2.80***	93.67± 5.14	113.75± 7.09
(pg/ml)	23.5 : 0.5	122.20 ± 2.67	**196.37± 10.53***	**189.80± 3.84***	**205.53± 9.43***	**163.87± 4.10***	**143.52± 7.80***	114.01± 4.70	107.90± 5.14
	12 : 12	19.36 ± 0.79	24.06 ± 0.66	19.07 ± 1.49	17.47 ± 1.08	12.92 ± 0.58	14.54 ± 1.89	15.21 ± 1.03	17.34 ± 0.94
CT	0.5 : 23.5	20.05 ± 0.81	22.11 ± 1.07	**14.76 ± 0.75***	**11.47 ± 0.70***	13.30 ± 0.68	**10.86 ± 0.76***	**10.87 ± 1.02***	**13.89 ± 0.78***
(pmol/l)	23.5 : 0.5	**13.40 ± 1.01***	**16.38 ± 1.53***	**26.80 ± 1.11***	**22.72 ± 1.09***	**23.75 ± 0.56***	17.52 ± 1.33	15.61 ± 0.61	15.61 ± 0.62
	12 : 12	112.79 ± 4.60	66.05 ± 7.13	82.20 ± 7.02	150.53 ± 8.82	178.04 ± 13.75	125.42 ± 6.81	103.03 ± 2.51	126.05 ± 7.61
IGF-I	0.5 : 23.5	**168.36 ± 4.67***	**102.95 ± 2.77***	**132.97 ± 6.12***	**183.40± 17.41***	152.05 ± 5.04	129.81 ± 4.48	**163.39± 17.67***	140.02 ± 6.78
(nmol/l)	23.5 : 0.5	**69.66 ± 3.23***	**96.10 ± 10.42***	**72.41 ± 2.88***	147.23 ± 5.41	**120.08 ± 6.84***	122.15 ± 8.75	91.21 ± 10.01	**98.50 ± 8.27***
	12 : 12	0.82 ± 0.14	0.81 ± 0.06	1.00 ± 0.05	1.48 ± 0.26	1.23 ± 0.05	0.88 ± 0.09	0.70 ± 0.06	0.62 ± 0.04
T3	0.5 : 23.5	0.67 ± 0.05	0.89 ± 0.02	**0.84 ± 0.04***	**0.59 ± 0.09***	**0.51 ± 0.04***	**0.57 ± 0.09***	0.58 ± 0.04	0.70 ± 0.04
(nmol/l)	23.5 : 0.5	0.85 ± 0.09	**1.40 ± 0.07***	1.27 ± 0.14	1.04 ± 0.09	1.04 ± 0.09	0.85 ± 0.13	**1.03 ± 0.08***	**1.08 ± 0.11***
	12 : 12	4.20 ± 0.22	4.80 ± 0.44	6.20 ± 0.43	8.07 ± 0.16	6.91 ± 0.55	5.31 ± 0.58	4.72 ± 0.14	5.24 ± 0.14
FT3	0.5 : 23.5	4.57 ± 0.23	**6.70 ± 0.88***	5.38 ± 0.34	**4.40 ± 0.28***	**4.33 ± 0.24***	4.38 ± 0.07	4.67 ± 0.17	**4.63 ± 0.17***
(pmol/l)	23.5 : 0.5	**5.94 ± 0.09***	**8.20 ± 0.61***	6.64 ± 0.20	**6.06 ± 0.25***	6.55 ± 0.82	**7.22 ± 0.21***	**6.37 ± 0.14***	**6.19 ± 0.27***
	12 : 12	36.70 ± 2.45	34.20 ± 4.86	40.63 ± 2.95	51.99 ± 1.25	43.62 ± 2.35	33.30 ± 2.49	36.00 ± 1.63	34.27 ± 1.22
T4	0.5 : 23.5	**27.75 ± 1.35***	32.71 ± 0.95	38.53 ± 4.63	**30.45 ± 3.20***	**32.43 ± 1.57***	36.40 ± 2.48	**24.72 ± 0.97***	**19.08 ± 1.35***
(nmol/l)	23.5 : 0.5	41.87 ± 1.51	42.88 ± 1.27	**47.78 ± 1.07***	**36.18 ± 2.30***	**32.12 ± 2.26***	**42.05 ± 1.34***	39.68 ± 2.92	**40.81 ± 1.84***
	12 : 12	27.86 ± 0.83	27.00 ± 2.95	20.30 ± 1.16	40.51 ± 2.50	33.50 ± 1.52	22.70 ± 1.16	27.39 ± 0.87	29.17 ± 1.21
FT4	0.5 : 23.5	**12.55 ± 0.58***	**12.30 ± 0.74***	**16.73 ± 0.61***	**23.29 ± 1.05***	22.10 ± 1.16	24.08 ± 0.44	**12.97 ± 0.53***	**13.56 ± 1.48***
(pmol/l)	23.5 : 0.5	**35.58 ± 1.56***	**43.55 ± 1.83***	**35.10 ± 4.03***	**17.67 ± 0.68***	**17.42 ± 1.79***	17.50 ± 3.09	29.77 ± 2.84	**36.14 ± 3.42***
	12 : 12	225.32± 18.65	223.15± 31.77	135.78± 4.51	181.08± 25.63	285.72± 32.34	685.20± 16.11	769.22± 18.31	262.01± 22.77
B	0.5 : 23.5	**142.12± 3.38***	240.82± 25.50	**469.24± 24.06***	244.26± 17.60	254.26± 12.35	**155.17± 9.05***	**242.66± 15.35***	217.93± 22.00
(nmol/l)	23.5 : 0.5	**501.57± 49.63***	**532.82± 30.46***	**774.00± 39.09***	**945.00± 92.31***	**417.00± 17.25***	**276.58± 38.41***	**113.60± 10.84***	168.54± 42.99
	12 : 12	0.92 ± 0.13	0.68 ± 0.11	0.70 ± 0.04	0.96 ± 0.15	1.31 ± 0.17	2.69 ± 0.36	1.88 ± 0.18	0.97 ± 0.11
T	0.5 : 23.5	**0.28 ± 0.03***	**1.14 ± 0.06***	**1.21 ± 0.08***	**1.51 ± 0.19***	**0.42 ± 0.03***	**0.55 ± 0.12***	**0.19 ± 0.01***	**0.36 ± 0.09***
(nmol/l)	23.5 : 0.5	1.00 ± 0.11	0.77 ± 0.02	0.87 ± 0.15	**1.85 ± 0.23***	**2.37 ± 0.10***	**1.63 ± 0.28***	**1.30 ± 0.09***	1.45 ± 0.23

* $p \leq 0.05$ vs control group

FT4, T, PICP, ICTP (FARMOS, Finland). Serum ALP activity, iP levels and urinary excretion of Ca were determined using ALPHA DIAGNOSTICS kits (Poland). Urinary excretion of HYP was determined according to Dróżdż et al. method (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, 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 Ca methods were up to: 1000 U, 15 mg/dl and 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%, 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%, Ca – 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 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

In rats kept under short-day conditions an increase of MEL concentration between 2 pm and 11 pm was shown. These changes were accompanied by a decrease of bone formation markers (ALP at the majority of time points; PICP from 8 am until 5 pm), of bone resorption markers (ICTP, HYP and Ca at all time points) and of iP at 2 am and 11 pm (Tables 1 and 2). The concentration of the majority of studied hormones was suppressed (PTH and CT mainly from 5 am until 5 pm or 11 pm; triiodothyronine from 8 am until 5 pm; thyroxine at the majority of time points; B and T from 2 pm until 2 am). However, IGF-I concentrations were higher between 8 pm and 11 am. At the remaining time points of the 24-hour period an opposite effect was generally seen with the studied bone turnover markers, hormones and IGF-I.

The circadian MEL rhythm in rats kept under short-day conditions was changed; an increase of the average of MEL concentration was observed and an acrophase shift by 32°. These changes were accompanied by a decrease of the mesor value of certain

Table 3
Chronobiologic parameters (M - mesor, A - aplitude, φ - acrophase) of the mean rhythms of melatonin (MEL) and chosen biochemical markers of bone metabolism (ALP, PICP, ICTP, iP all determined in serum and HYP and Ca, determined in urine) in rats exposed to different light conditions

		Groups		
Variables	Chronobiologic parameters	LD 12 : 12	LD 0.5 : 23.5	LD 23.5 : 0.5
MEL	M (pg/ml)	25.98	↑ **37.35**	↓ **13.99**
	A (pg/ml)	25.85	↓ **22.63**	↓ **8.77**
	φ (h.min)	3.16	**1.04** ←	→ **20.06**
	p	0.029*	0.005*	**0.060**
ALP	M (U/l)	264.41	↓ **199.21**	↑ **315.96**
	A (U/l)	49.82	52.94	↑ **96.36**
	φ (h.min)	14.47	13.30	→ **16.57**
	p	0.032*	**0.185**	**0.130**
PICP	M (mg/l)	15.00	↓ **9.70**	↑ **16.43**
	A (mg/l)	4.45	↑ **4.88**	↓ **2.15**
	φ(h.min)	13.41	→ **22.39**	**11.04** ←
	p	0.009*	**0.069**	**0.399**
ICTP	M (mg/l)	15.73	↓ **14.12**	16.52
	A (mg/l)	4.28	↓ **2.45**	↓ **3.37**
	φ (h.min)	13.09	**4.33** ←	**1.52** ←
	p	0.005*	**0.260**	0.013*
HYP	M (mmol/l)	14.88	↓ **13.07**	14.73
	A (mmol/l)	1.82	↓ **1.52**	1.72
	φ (h.min)	12.30	**6.19** ←	**2.20** ←
	p	0.001*	0.031*	0.016*
Ca	M (mmol/l)	2.67	2.75	2.66
	A (mmol/l)	0.56	↓ **0.19**	↓ **0.20**
	φ (h.min)	14.32	**12.12** ←	**0.59** ←
	p	0.030*	**0.516**	**0.603**
iP	M (mmol/l)	2.73	2.84	↓ **1.97**
	A (mmol/l)	0.21	↑ **0.40**	↑ **0.46**
	φ (h.min)	12.37	**1.28** ←	**7.06** ←
	p	0.330	0.161	**0.014***

↑ – 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)
significant differences, compared to control, are marked by bold

Table 4
Chronobiologic parameters (M - mesor, A - aplitude, φ - acrophase) of the mean rhythms of chosen hormonal factors in rats exposed to different light conditions

	Chronobiologic	Groups		
Variables	parameters	LD 12 : 12	LD 0.5 : 23.5	LD 23.5 : 0.5
PTH	M (pg/ml)	115.96	↓ **98.35**	↑ **155.42**
	A (pg/ml)	35.03	↑ **41.86**	↑ **49.08**
	φ (h.min)	4.36	**2.12** ←	→ **9.28**
	p	0.007*	0.006*	0.003*
CT	M (pmol/l)	17.50	↓ **14.87**	↑ **19.09**
	A (pmol/l)	4.22	↑ **4.82**	↑ **5.35**
	φ (h.min)	4.37	3.51	→ **11.06**
	p	0.009*	0.035*	0.035*
IGF- I	M (nmol/l)	118.01	↑ **146.62**	↓ **102.17**
	A (nmol/l)	33.15	↓ **9.57**	↓ **25.83**
	φ (h.min)	15.20	15.57	14.06
	p	0.188	0.811	0.137
T3	M (nmol/l)	0.94	↓ **0.67**	↑ **1.07**
	A (nmol/l)	0.34	↓ **0.16**	↓ **0.16**
	φ (h.min)	11.25	**4.45** ←	**6.39** ←
	p	0.014*	0.033	**0.260**
FT3	M (pmol/l)	5.68	↓ **4.88**	↑ **6.64**
	A (pmol/l)	1.49	↓ **0.75**	↓ **0.22**
	φ (h.min)	11.54	**5.01** ←	**7.14** ←
	p	0.029*	**0.183**	**0.875**
T4	M (nmol/l)	38.84	↓ **30.26**	40.43
	A (nmol/l)	6.47	6.15	↓ **3.66**
	φ (h.min)	11.06	10.01	**3.27** ←
	p	0.108	0.141	0.339
FT4	M (pmol/l)	28.55	↓ **17.20**	29.09
	A (pmol/l)	4.83	↑ **6.22**	↑ **12.71**
	φ (h.min)	12.39	13.40	**2.50** ←
	p	0.732	**0.011***	**0.006***
B	M (nmol/l)	345.94	↓ **245.82**	↑ **466.14**
	A (nmol/l)	267.38	↓ **78.80**	↑ **354.80**
	φ (h.min)	18.49	**8.51** ←	**8.46** ←
	p	0.047*	**0.337**	0.006*
T	M (nmol/l)	1.25	↓ **0.71**	↑ **1.41**
	A (nmol/l)	0.79	↓ **0.58**	↓ **0.60**
	φ (h.min)	17.34	**8.54** ←	**15.13** ←
	p	0.040*	0.028*	**0.051**

↑ – 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)
significant differences, compared to control, are marked by bold

bone metabolism markers, i.e. ALP, PICP, ICTP, HYP, all studied hormones and an increase of IGF-I average circadian concentration. A lowering of HYP, IGF-I, T3 and T amplitude was observed along with the abolition of ALP, PICP, ICTP, Ca, FT3 and B and a peak shift for PICP, ICTP, HYP, Ca, iP, PTH, triiodothyronine, B and T from 32 up to 166° (Tables 3 and 4).

In animals kept under long-day conditions a decrease of MEL secretion between 2 am and 11am was noted, while between 5 pm and 11 pm the secretion was increased (Tables 1 and 2). These changes were accompanied by an increase of bone formation markers (ALP at the majority of time points; PICP from 8 pm until 11 am), of bone resorption markers (ICTP from 11 pm until 5 am; HYP from 9.30 pm until 6.30 am; Ca from 3.30 am until 9.30 am). There was also a decrease of iP concentration from 2 pm until 11 pm. The opposite effect was noted at the remaining time points of the 24-hour period. An increase of studied hormones' concentrations was observed (from 8 am until 5 pm for PTH and CT; from 8 pm until 8 am for thyroid hormones; from 2 am until 2 pm for B; at 11am, 2 pm and 5 pm for T). IGF-I levels were lowered at 2 am, 2 pm and 11 pm. At the remaining time points of the 24-hour period a generally opposite effect was seen with respect to the examined hormones.

In rats kept under long-day conditions a lowering of the average circadian MEL concentration was observed along with rhythm abolition that followed levelling off of the nighttime secretion peak. These changes were accompanied by a mesor increase of the studied bone formation markers and hormones (except thyroxine) and by a decrease of average circadian IGF-I and iP levels. Lowering of the ICTP rhythm amplitude, abolition of ALP, PICP, Ca, T3, FT3 and T rhythms, intensified circadian oscillations of iP and FT4 and peak shift for ALP, PICP, ICTP, HYP, Ca, iP, PTH, CT, thyroid hormones, B and T from 32 to 206° were also observed (Tables 3 and 4).

In rats kept under short-day conditions a significant negative correlation was demonstrated between circadian changes in the concentration of all studied bone turnover markers and changes in MEL secretion. Under long-day conditions a correlation was statistically significant only with respect to PICP, ICTP, HYP, Ca and iP. Additionally, in rats kept under short-day conditions, circadian changes in the concentration of bone resorption markers show a significant positive

correlation with changes in IGF-I and triiodothyronine levels. However, in rats exposed to long-day conditions the relationship was significant only with respect to HYP and Ca (Table 5).

Table 5

Assessment of relationship between circadian changes in melatonin (MEL) concentration and changes in values of chosen biochemical markers of bone metabolism (ALP, PICP, ICTP, iP all determined in serum as well as HYP and Ca, determined in urine) in rats exposed to different light conditions. Table includes only those variables that show significant correlation in at least one study group.

Dependent variables	Independent variables	Groups LD 0.5 : 23.5	Groups LD 23.5 : 0.5
ALP (U/l)	MEL (pg/ml)	**-0.286***	NS
PICP (μg/l)	MEL (pg/ml)	**-0.289***	**-0.634****
ICTP (μg/l)	MEL (pg/ml)	**-0.576****	**-0.424****
	IGF-I (nmol/l)	**0.315***	NS
	T3 (nmol/l)	**0.600****	**0.491****
	FT3 (pmol/l)	**0.735****	**0.450****
HYP (μgmol/l)	MEL (pg/ml)	**-0.689****	**-0.370****
	IGF-I (nmol/l)	**0.806****	**0.288****
	T3 (nmol/l)	**0.744****	**0.700****
	FT3 (pmol/l)	**0.800****	**0.747****
Ca (mmol/l)	MEL (pg/ml)	**-0.567****	**-0.396****
	IGF-I (nmol/l)	**0.678****	**0.641****
	T3 (nmol/l)	**0.744****	**0.801****
	FT3 (pmol/l)	**0.560****	**0.851****
iP (mmol/l)	MEL (pg/ml)	**-0.330***	**-0.840****

* $p \leq 0,05$; ** $p \leq 0,01$; *** $p \leq 0,001$ statistically significant value of correlation coefficient

Discussion

The etiology of the daily rhythmicity of 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 feeding schedule, LD cycle and endocrine relationships (Milhaud et al. 1972; Talmage et al. 1975; Phillippens et al. 1977; Simmons et al. 1979; Staub et al. 1979; Kream et al. 1980, 1990; Russel et al. 1983, 1984, 1985; Canalis et al. 1988; Calvo et al. 1991; Shinoda and Stern 1992). Several published observations suggest that PTH and glucocorticoids might play a role in the synchronisation of the bone and mineral metabolism periodicity (Staub et al. 1979; Kream et al. 1980, 1990; Russel et al. 1984; Canalis et al. 1988; Calvo et al. 1991). In our preliminary investigations in rats, we demonstrated that the main candidate hormones are PTH, MEL, GH, IGF-I and thyroid hormones (Ostrowska et al. 2002), which affect bone metabolism *in vitro* (Badurski et al. 1994; Galus 1995; Nakade et al. 1999; Roth et al. 1999), and have a daily rhythm (except IGF-I, T4 and FT4) *in vivo* (Calvo et al. 1991; Wong et al. 1983; Lausson et al. 1985; Persengiev et al. 1991; Ostrowska et al. 1995, 2001). These hormone rhythms in rats appear to be constrained by the LD cycle (Ostrowska et al.1993a, b), and in mechanism of this dependence, changes in endogenous MEL concentrations seem to play an important role (Krieger and Hauser 1978; Niles et al. 1979; Illnerova et al. 1983, 1989; Maunier et al. 1988; Reiter 1986; Cardinali 1991; Ostrowska et al. 1993a, b, 1994, 2002; Vaughan et al. 1994; Pevet and Pitrosky 1996).

Little is known about the effects of lighting schedules on biochemical parameters of bone and mineral metabolism. Most of the data support the conception that the feeding schedule (meal timing) is a powerful "Zeitgeber" that can override the influence of the LD cycle (Milhaud et al. 1972; Talmage et al. 1975; Phillippens et al. 1977; Russel et al. 1983; Shinoda and Stern 1992). Nevertheless, the LD cycle remained the predominant synchronizer for the bone matrix formation rhythm (Simmons et al. 1979, 1983; Russel et al. 1983, 1985; Phillippens et al. 1977). The data reported herein suggest that lighting conditions can influence daily fluctuations of studied bone metabolism markers in rats, and that the changes in endogenous MEL concentrations seem to play an important role in this mechanism. In rats kept under short-day conditions an increase of MEL concentration between 14.00 and 23.00 h was shown; these changes were accompanied by a decrease of bone formation (ALP at the majority of time points; PICP from 8.00 until 17.00 h) and resorption markers (ICTP, HYP and Ca at all time points).

Furthermore, substantial changes in the values of chronobiologic parameters of these markers daily rhythmicity were found. A significant negative correlation between circadian changes in the concentration of all studied bone metabolism markers and changes in MEL secretion (values of correlation coefficients ranged from -0.286 to -0.689) appears to

confirm the concept of probable MEL participation in the mechanism of bone metabolism markers' circadian rhythm dependence on lighting conditions. One cannot exclude, however, the participation of other pineal gland derived substances, apart from MEL, in the mechanism of the above relationship. In the pineal glands of mammalians, the presence of indole substances other than MEL was revealed (KARASEK 1997; KARASEK and REITER 2002). The presence of polypeptide substances including those synthesized by the pineal gland (potential pineal hormones) was discovered. However, their chemical structure and potential participation in regulatory mechanisms underlying the control of the pineal gland are still unknown (REITER 1987, 1991; KARASEK 1997; KARASEK and REITER 2002).

Extension of the dark phase (i.e. with significant dominance of dark phase over light phase) caused not only significant disturbances in the circadian profile of MEL and recognized markers of bone turnover, but also of calciotropic and thyroid hormones, B, T and IGF-I. However, only circadian changes in the concentration of IGF-I and triiodothyronine showed significant correlation with the changes of bone tissue resorption markers. This relationship was directly proportional and the values of correlation coefficients oscillated between 0.315 and 0.806. The obtained results suggest that, for the studied hormones only, circadian changes in the concentration of IGF-I and triiodothyronine may mediate the MEL effect on the circadian rhythm of bone tissue metabolism.

It is worth observing that dark phase extension causes a certain discoordination of the mutual relationship between circadian MEL oscillations on one side and the studied markers of bone metabolism and hormonal factors on the other. There may be different mechanisms of mutual relationship between the LD phase ratio and circadian fluctuations of studied markers and secreted hormones. Current hypotheses regarding an explanation of the dark phase extension effect upon the sexual system have been put forward in several reviews (REITER 1987, 1991; KARASEK 1997; KARASEK and REITER 2002). Some authors (HOFFMAN 1981) believe that the duration of the nocturnal peak of MEL secretion is the critical parameter in the transmission of photoperiodic information (now referred to as the duration hypothesis). Administration of MEL to pinealectomized animals to produce a daily MEL peak corresponding to either a long photoperiod or a short photoperiod have validated Hoffmann's concept. Other authors (CARTER and GOLDMAN 1983) demonstrated that a 12-hour MEL infusion, like a short photoperiod, inhibited testicular development in juvenile Siberian hamsters, whereas 4- or 6-hour infusions failed to exhibit any inhibitory effect. This experiment approach has also been used in studies with different photoperiodic species, and in all situations, infusion of MEL induced the species-specific gonadal response characteristic of either a long or short photoperiod, depending on the duration of the infusion (PEVET and PITROSKY 1997). This mechanism might be the cause of distinct suppression of average circadian concentrations of examined hormones as well as changes in amplitude and phase of hormonal rhythms seen in our own studies on rats kept under short-day conditions. Other investigators (acc. to REITER 1987, 1991) suggest that a higher nocturnal MEL secretion peak might be the signal to which the endocrine system is responsive. However, in our own studies in rats kept under short-day conditions the amplitude did not change significantly despite of distinct increase of average circadian MEL concentration and extension of its nocturnal secretion peak; it was by 13 % lower than control. Still other authors (acc. to REITER 1987, 1991) believe that it is synchronizing endogenous MEL rhythm with cyclic sensitivity of MEL receptors which warrants the responsiveness of target organ to MEL. Partial discoordination of these rhythms, caused for example by an extended dark phase, may determine a weaker impact of endogenous MEL, while total dissociation results in the lack of MEL effect. Based on these hypotheses one might attempt to explain the mechanisms which lead in rats exposed to short-day conditions to partial disregulation of the relationship between the circadian rhythm of MEL and (most probably MEL-controlled) the rhythm of bone metabolism markers as well as circadian oscillations of the examined hormones.

The data reported herein also demonstrates that an extended light cycle does have a substantial impact on circadian fluctuations of examined bone metabolism markers. This may be related to the pineal gland dysfunction and/or decrease of MEL synthesis under these conditions (ILLNEROVÁ et al. 1983;

KARASEK 1997; REITER 1986, 1991; CARDINALI 1991). It has been known that an extended light cycle (i.e. with significant dominance of light phase over dark phase), like continuous light, may lead to the abolition of endogenous MEL circadian rhythm and, in effect, to a decrease of MEL blood concentration (PANG and ALLEN 1986). Light inhibits MEL synthesis not only in the pineal gland but also in the retina, Harderian gland and intestines (BUBENIK et al. 1978; PANG and ALLEN 1986; PANG and YIP 1988; KARASEK 1997; HUETHER 1993) and this also may be reflected in the blood concentration of this hormone. Our own rat studies have shown that long-day conditions induce circadian changes in MEL concentration. These changes show a negative correlation with changes of examined bone metabolism markers (significant with respect to PICP, ICTP, HYP, Ca and iP). The values of correlation coefficients oscillated between -0.288 and -0.840. These results seem to confirm the earlier formulated concept of MEL participation in the mechanism linking circadian oscillations of bone metabolism markers with lighting conditions applied. An average circadian MEL concentration decrease (due to rats' exposure to long day conditions) and circadian MEL rhythm abolition (due to a marked value decrease between 2 am and 11 am) were both associated with increased markers of bone formation (ALP, with the exception of the 11 am time point, and PICP from 20.00 until 11.00 h), resorption (ICTP from 23.00 until 5.00 h, HYP from 21.30 to 6.30 and Ca from 3.30 am until 9.30 h) and decreased iP concentration (most pronounced between 14.00 and 23.00 h). At the remaining time points of the 24-hour period the observed effect was usually the opposite. A consequence of these disturbances was a decrease of ICTP rhythm amplitude, abolition of ALP, PICP and Ca rhythms and a peak shift for all studied markers from 32 up to 206°. An increase of mesor value was observed only with respect to bone formation markers and a decrease only in the case of iP. Similar disturbances in the circadian rhythmicity of serum ALP, Ca and iP (appearing as decreased amplitude and/or rhythm phase shift) were noted by SHINODA and SETO (1985) yet this followed rats' exposure to constant lighting for a period exceeding 10 days.

Our own studies also demonstrate that the changes in IGF-I and triiodothyronine levels, triggered by long-day conditions and most probably mediated by MEL, may be of importance in inducing disturbances of circadian fluctuations of bone resorption markers. This is supported by the positive correlation, existing under such lighting conditions, between the changes in the concentration of ICTP, HYP or Ca and changes in the concentration of IGF-I and triiodothyronine; values of correlation coefficients ranged from 0.285 to 0.851.

In considering the mechanisms of generating and synchronizing circadian rhythmicity of bone formation and resorption processes one cannot disregard the fact that light may modify circadian rhythms in mammals not only *via* MEL (NILES et al. 1979; ARMSTRONG et al. 1986; REITER 1986; 1987, 1991; JOHN et al. 1990; KARASEK 1997) but also directly (MIKKELSEN and SERVIERE 1992; CUI et al. 1997). The majority of data point to the retinal-hypothalamic pathway, ending at the suprachiasmatic nucleus, as the main source of circadian rhythms modification, also including hormonal ones (MIKKELSEN and SERVIERE 1992; SCOTT et al. 1995; KARASEK 1997). It has been proven that the suprachiasmatic nucleus in the hypothalamus, playing the role of an endogenous biological clock, may modulate directly circadian hormonal secretion rhythms (MIKKELSEN and SERVIERE 1992). The interaction between the light and endocrine system may be also mediated by MEL receptors localized in the suprachiasmatic nucleus (GAUER et al. 1993, 1994 a,b). Similarly, as in the case of pinealectomy, the receptors' density probably changes under the influence of light (GAUER et al. 1994a). One cannot exclude that light, *via* similar mechanisms, may synchronize circadian oscillations of the examined biochemical bone metabolism markers.

In conclusion, our results suggest that lighting conditions considerably influence the circadian rhythm of bone metabolism in rats and that an important role in the mechanism of this dependence is played by changes in the level of endogenous melatonin. Secondary changes in daily IGF-I and triiodothyronine oscillations, caused by short and long day conditions, result in altered daily bone resorption rhythmicity.

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Corresponding author: Zofia Ostrowska, M.D.
Department of Clinical Biochemistry,
The Medical University of Silesia
Zabrze, Poland
E-mail: ozdrasiek@poczta.onet.pl