Assessment of the relationship between circadian variations of salivary melatonin levels and type I collagen metabolism in postmenopausal obese women

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Abstract

BACKGROUND: Few experimental and clinical studies show that melatonin (MEL) can play a significant part to modulate circadian bone metabolism. On this basis it was suggested that MEL secretion which altered during 24-h in obese women could be of importance to regulate bony mass defect after menopause.

OBJECTIVE: The aim of the study was to prove if there were any connection between changes in 24-h profile of serum MEL levels and circadian metabolism of type I collagen in postmenopausal women with visceral obesity.

METHODS: The relationship of 24-h profile of salivary MEL and circadian metabolism of type I collagen (as assessed by measuring saliva concentrations of carboxyterminal propeptide of type I procollagen - PICP and cross-linked carboxyterminal telopeptide of type I collagen - ICTP) was investigated in 26 women with visceral obesity (33.5 < BMI < 42.1 kg/m²) and 18 healthy volunteers with correct body mass (21 < BMI < 24.5 kg/m²; 0.73 < WHR < 0.76). The specimens were collected at subjects’ home at 3 h intervals during a 24 h span. The age range of all subjects was 52–60 years.

RESULTS: In all the obese women studied a tendency to suppress circadian levels of tested biochemical markers of bone metabolism was observed (especially regarding ICTP); those alterations were accompanied by substantial increment in MEL concentrations during the day. Significant and negative correlation was found between values of acrophase MEL and PICP rhythms and both amplitude and acrophase of MEL and ICTP rhythms.

CONCLUSION: Our results confirm hypothesis that alterations in MEL concentrations might have a protective effect against postmenopausal loss of bone mass.
In elderly people, because of sex hormones and growth [1, 2]. During development bone growth it undergoes simultaneously processes of resorption and formation and resorption [4]. The correlation was stronger with the increase of bone anabolism [6, 7]. Based on experimental and clinical studies [3, 4] pointing at the substantial role of melatonin in the modulation of bone metabolism it might be speculated that the changed circadian secretion of melatonin in obese women is of importance in regulating bone mass loss after the menopause.

The aim of our study was to demonstrate whether there exists a relationship between changes of the circadian profile of melatonin (MEL) concentration in saliva and the circadian metabolism of type I collagen, the latter based on saliva determination of carboxyterminal propeptide of type I procollagen (PICP) and cross-linked carboxyterminal telopeptide of type I collagen (ICTP) in postmenopausal women with visceral obesity.

Material and Methods

Twenty six postmenopausal women (33.5 < BMI < 42.1 kg/m²) with visceral distribution of adipose tissue (0.80 < WHR < 0.87) were investigated. The control group consisted of 18 healthy postmenopausal women with normal body mass (21 < BMI < 24.5 kg/m²; 0.73 < WHR < 0.76). The subjects’ age ranged from 52 to 60 (mean 56.9 ± 3.1). The study was conducted with the permission of Ethics Committee at the Silesian Academy of Medicine in Katowice, and all patients gave their written informed consent to participate in the study.

All participants, from both the study and control groups, took saliva samples at 3-hour intervals during a 24-hour span, starting at 8 a.m. Samples were collected at home by subjects themselves, who were first instructed how to proceed. Special Salivette test tubes (Sarstedt) were used. During the dark period a weak (25W) red light was lit immediately prior to saliva sampling and was switched off again within 2 minutes. The saliva samples were secured in the refrigerator at –2°C until furnished to the laboratory where they were centrifuged (450 g, 10 min.) and stored frozen (–75°C) until determining MEL, PICP and ICTP concentrations with the help of commercially available RIA kits.

MEL concentration in saliva was determined using kits from DRG Instruments GmbH (USA), while PICP and ICTP were measured with the help of kits from FARMOS (Finland). Sensitivity of the method and intra- and interassay coefficients of variations were as follows: 1pg/sample, 8.6% and 9.2% for MEL; 1.2µg/l, 3.1% and 5.8% for PICP; 0.34 µg/l, 4.5% and 6% for ICTP.

Introduction

The bone tissue remains in a dynamic state since it undergoes simultaneously processes of resorption and growth [1, 2]. During development bone growth predominates while in adults equilibrium is reached. In elderly people, because of sex hormones’ loss and aging, resorption becomes dominant and bone loss starts to occur. The speed of osseous metabolic turnover, which is a result of bone tissue formation and its resorption is inversely proportional to the concentration of estrogens which determines its effect upon bone mass during postmenopausal period. The elevated bone metabolic turnover and lowered bone mass, when occurring together, markedly increase the probability of occurrence of osteoporotic fractures in postmenopausal women [1, 2].

Lately, there have been suggestions that bone mass changes occurring in postmenopausal women may also remain related to melatonin [3]. It has been shown that decrease in melatonin concentration in the early postmenopausal period may be an important cofactor in osteoporosis development [3]. An investigation of postmenopausal osteoporosis model obtained by ovary removal in rats led to the observation that successive lowering of melatonin concentration correlated negatively with changes in concentration of biochemical markers of bone tissue formation and resorption [4]. The correlation was stronger with respect to resorption markers.

It has been pointed out that in obese postmenopausal women (at least 20% overweight) the decrease in bone tissue mineral density as well as increased urine excretion of total calcium are less marked compared to postmenopausal women with normal body mass [5, 6]. This may result from the endocrinological role of the adipose tissue as the source of estrogens as well as from increased burden upon the bone tissue which leads to an increase of bone anabolism [6, 7]. Based on experimental and clinical studies [3, 4] pointing at the substantial role of melatonin in the modulation of bone metabolism it might be speculated that the changed circadian secretion of melatonin in obese women is of importance in regulating bone mass loss after the menopause.

Abbreviations

A amplitude
BMI body mass index
C control group
f (T) average hormone concentration at the given time point
ICTP cross-linked carboxyterminal telopeptide of type I collagen
M mesor
MEL melatonin
O obese women
PICP carboxyterminal propeptide of type I procollagen
PR percentage of the total variance attributed to the circadian rhythm
PTH parathormone
RIA radioimmunological method
SE standard error
WHR waist to hip circumference ratio
φ acrophase
ω angular frequency
25-OHD3 25-hydroxy vitamin D
The results were analyzed statistically using variance analysis for Kruskal-Wallis nonparametric tests. After rejecting the variance uniformity hypothesis, further analysis of statistical significance was conducted using “U” Mann-Whitney test.

Codependency between MEL concentration and values of PICP and ICTP was assessed by the Spearman correlation test.

Statistical analysis of circadian rhythms of MEL concentration and saliva-contained examined exponents of bone metabolism was carried out using the cosinor method according to Halberg et al. [8]. Cosinor analysis was carried out for a fixed average time group value by fitting the main cosinor function $f(T) = M + A \cos (\omega T + \phi)$, where $f(T)$ is the average hormone concentration at the given time point; $M$ is the mesor; arithmetic average of actual values describing oscillations within the cycle; $A$ is the amplitude, the difference between maximum (or minimum) value and the sinusoidal average; $\phi$ is the acrophase, angle ($360^\circ = 24$ h) corresponding to maximum value of a given hormone concentration within 24 hours; $\omega$ is angular frequency. The appearance of a rhythm was deducted following rejection of zero amplitude hypothesis.

**Results**

The multifactor variance analysis carried out initially showed that obese postmenopausal women with visceral distribution of adipose tissue show substantial day and night dependent changes in MEL concentration as well as in the concentration of type I collagen metabolism markers studied ($p < 0.05$); this is shown in Figs. 1, 2 and 3.

All women from the control group demonstrated a characteristic circadian rhythm of MEL concentration in saliva with acrophase reached at 00:57, mesor value of 12.97 pg/ml and amplitude reaching 9.15 pg/ml. Obese postmenopausal women had a markedly lower amplitude of MEL rhythm with unchanged mesor value and a small shift in acrophase value towards later time points (average 28 min). The significant increase in average MEL concentrations in saliva, with respect to control group, was found at 08:00, 11:00, 14:00 and 17:00 while the decrease occurred at 23:00 and 02:00 (Fig.1).

PICP concentration in saliva in postmenopausal women with normal body weight was subject to characteristic circadian fluctuations with a maximum occurring at 05:41. The average circadian concent-

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**Fig. 1.** Mean levels of melatonin (MEL; pg/ml ± SE) in saliva and the chronobiologic parameters of MEL circadian rhythm in postmenopausal obese women ($33.5 <$ BMI < 42.1 kg/m²) with visceral type of adipose tissue distribution ($0.80 <$ WHR < 0.87) - group O and in healthy volunteers ($21 <$ BMI < 24.5 kg/m²; $0.73 <$ WHR < 0.76) - group C ($^*P < 0.05$ indicates statistically significant 24 h rhythm).
Fig. 2. Mean levels of carboxyterminal propeptide of type I procollagen (PICP; µg/l ± SE) in saliva and the chronobiologic parameters of PICP circadian rhythm in postmenopausal obese women (33.5 < BMI < 42.1 kg/m²) with visceral type of adipose tissue distribution (0.80 < WHR < 0.87) - group O and in healthy volunteers (21 < BMI < 24.5 kg/m²; 0.73 < WHR < 0.76) - group C (* P = less than 0.05 indicates statistically significant 24 h rhythm).

<table>
<thead>
<tr>
<th>Groups</th>
<th>M µg/l</th>
<th>A µg/l</th>
<th>φ h:min</th>
<th>p</th>
<th>PR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>15.55</td>
<td>7.91</td>
<td>5.41</td>
<td>0.014^</td>
<td>81.73</td>
</tr>
<tr>
<td>O</td>
<td>13.61*</td>
<td>7.02*</td>
<td>5.02</td>
<td>0.005^</td>
<td>88.30</td>
</tr>
</tbody>
</table>

Time of day (hours)

Fig. 3. Mean levels of cross-linked carboxyterminal telopeptide of type I collagen (ICTP; µg/l ± SE) in serum and the chronobiologic parameters of ICTP circadian rhythm in postmenopausal obese women (33.5 < BMI < 42.1 kg/m²) with visceral type of adipose tissue distribution (0.80 < WHR < 0.87) - group O and in healthy volunteers (21 < BMI < 24.5 kg/m²; 0.73 < WHR < 0.76) - group C (* P = less than 0.05 indicates statistically significant 24 h rhythm)

<table>
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<tr>
<th>Groups</th>
<th>M µg/l</th>
<th>A µg/l</th>
<th>φ h:min</th>
<th>p</th>
<th>PR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.14</td>
<td>0.29</td>
<td>5.20</td>
<td>0.006^</td>
<td>87.35</td>
</tr>
<tr>
<td>O</td>
<td>0.93</td>
<td>0.36*</td>
<td>4.38</td>
<td>0.002^</td>
<td>90.97</td>
</tr>
</tbody>
</table>

Time of day (hours)
Conclusion was 15.55 µg/l while amplitude 7.91 µg/l. In patients with visceral type of obesity, lowering of the mesor value and PICP rhythm amplitude was demonstrated together with a small shift of the peak concentration towards earlier time points (average 39 minutes, Fig. 2). A significant decrease of average PICP concentrations in saliva with respect to control group was observed at 05:00, 08:00, 11:00, 17:00 and 20:00 while increase was noted at 02:00 and 14:00.

A characteristic circadian ICTP rhythm was demonstrated in the saliva of women from the control group with acrophase reached at 05:20. Mesor value was 1.14 µg/l, while the amplitude 0.29 µg/l. The circadian ICTP rhythm in obese women examined was changed; an increase of amplitude was observed as well as acrophase shift towards earlier time points (average 42 min.) accompanied by simultaneous lowering of average circadian concentration (Fig. 3). Average ICTP concentrations in saliva were significantly lowered with respect to control group at 02:00 and from 08:00 until 23:00.

The results pertaining to the assessment of the relationship between chronobiological parameters of MEL rhythms and type I collagen metabolism markers examined in postmenopausal women with visceral type of obesity are shown in Table 1. In the obese women examined, a significant correlation is demonstrated between changes in acrophase values of MEL and PICP rhythms (r = -0.464) and both amplitude and acrophase values of MEL and ICTP rhythms (r = -0.430 and -0.630, respectively).

**Discussion**

Clinical studies show that obesity is not a risk factor in the development of osteoporosis. It turns out that augmentation of fat deposits may even have a preventive effect against the loss of bone mass in postmenopausal women. Studies of Johnston et al. [5] and Ribot et al. [6] proved that a decrease in mineral density of osseous tissue and an increase of total calcium excretion in urine are much lower in obese postmenopausal women than in persons of the same age having normal body mass. According to some authors [6, 7] this may result from an increased burden placed on supporting bones in obese persons; this may lead, in turn, to increased bone anabolism. Others suggest that this is related to the endocrinological role of adipose tissue as a source of estrogens [7]. It is known that estrogens exhibit multidirectional effects upon bone cells, both directly (via receptors) and indirectly (via other hormones, growth factors and cytokines) [1, 9]. It has been shown that premenopausal women who were obese are protected after menopause from the loss of trabecular bone owing to relative hyperestrogenism. Its source is, as mentioned before, the “third ovary” i.e. the adipose tissue capable of converting adrenal androstendione into estrone.

It is conceivable that changes in bone mass in obese postmenopausal women might be caused by less manifested changes in calcitropic hormones, compared to women with normal body mass. This concept requires, however, confirmation in clinical studies. The assessment of PTH and 25-OHD3 concentrations was carried out mainly in obese women of reproductive age. Bell et al. [10] found decreased concentrations of 25-OHD3 and increased PTH secretion in persons awaiting surgical body mass reduction; these parameters became normalized following this kind of surgery. Also, Compston et al. [11] found that average 25-OHD3 concentrations are markedly lower in obese persons awaiting jejunostomy, as compared to control group.

Based on our own studies, a presumption comes to mind that the lessened bone mass loss that was found by other investigators in obese postmenopausal women may also be related to changes in melatonin concentration. A few studies point to possible melatonin participation in modulating bone tissue metabolism [3]. Our own investigations carried out on male

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Mesor Δ (µg/l) vs control group</th>
<th>Amplitude Δ (µg/l) vs control group</th>
<th>Acrophase Δ (h.min) vs control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEL (µg/ml)</td>
<td>PICP (µg/l)</td>
<td>NS</td>
<td>NS</td>
<td>- 0.464</td>
</tr>
<tr>
<td></td>
<td>ICTP (µg/l)</td>
<td>NS</td>
<td>-0.430</td>
<td>-0.630</td>
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<td>&lt; 0.05</td>
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rat model [unpublished data] show that melatonin is an important determinant of circadian bone metabolism. It acts as a suppressor both on processes of bone formation as on its resorption; the effect is, however, much stronger with respect to resorptive processes. In starving rats [12] an inversely proportional relationship was demonstrated between melatonin concentration and blood-determined PICP concentration as well as urine-excreted hydroxyproline and total calcium.

Also, it has been known that melatonin secretion decreases with age [13, 14, 15] and the menopause is accompanied by significant decrease of melatonin secretion and augmented rate of pineal calcification [13, 14, 15, 16]. Probably, the lowering of melatonin secretion in early stages of menopause may be an important factor accompanying the development of osteoporosis [3]. In an experimental model of postmenopausal osteoporosis (obtained via removal of ovaries in female rats) a successive lowering of nocturnal melatonin levels was observed [4]. These changes correlated negatively with the concentration of markers of osseous bone formation, and, particularly, resorption. This is why it has been suggested that the melatonin deficiency, showing as a result of switching off female sex hormone functions, may be a cofactor in inducing bone mass changes in female rats with removed ovaries.

In obese women of postmenopausal age a significant correlation was noticed between chronobiological rhythm parameters of investigated bone turnover markers and melatonin. Thus the concept that increased melatonin secretion during daytime - such phenomenon is observed in obese postmenopausal women [17, 18, 19, 20, 21, 22] – might have a protective effect against bone mass loss following menopause. In postmenopausal women with visceral obesity a marked increase in average MEL concentrations was shown in saliva during the daytime with a decrease occurring during nighttime. This resulted in decreased amplitude of this hormone rhythm with a slightly increased mesor value and small acrophase shift towards later time points (28 min.). These changes were accompanied by a lowering in mesor value and in the amplitude of PICP rhythm in saliva with a small shift of peak concentration towards earlier time points (39 min.). Changes in chronobiological parameters of the circadian ICTP rhythm in saliva were affirmed as small acrophase shift toward earlier time points (42 min.), increase in amplitude and lowering of average circadian concentration. The tendency to suppress circadian concentrations of type I collagen metabolism markers studied, as observed in obese women with visceral obesity (particularly clear with respect to ICTP), may indicate a decreased bone turnover in these patients, while the tendency towards increased daily MEL concentrations, showing simultaneously, points out towards possible participation of this hormone in generating changes in the extent of type I collagen metabolism. Our results indicate that not only relative hyperestrogenism and/or increased burden placed on supportive bones, but also increased melatonin production may probably have a protective effect upon the loss of bone mass in obese postmenopausal women.

Conclusions

1. In postmenopausal women with a visceral type of obesity there is a tendency to suppress the circadian concentrations of PICP and ICTP relative to controls which may indicate a decreased bone turnover in these patients. Such changes are accompanied by a marked increase of melatonin concentration during daytime.

2. A statistically significant correlation between the values of acrophase of MEL and PICP rhythms and values of amplitude and acrophase of MEL and ICTP rhythms, proven in postmenopausal women with visceral obesity, suggests that changes in melatonin concentration may probably have a protective effect against bone mass loss after menopause.

REFERENCES

Assessment of the relationship between circadian variations of salivary melatonin levels and type I collagen metabolism in postmenopausal obese women


