Osteoporosis Int (1995) 5:472–477


**Osteoporosis International**

*Original Article*

# Circadian Rhythm in Type I Collagen Formation in Postmenopausal Women With and Without Osteopenia

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**Abstract.** A circadian rhythm in the serum concentration of the procollagen type I carboxyl-terminal propeptide (sPICP) has previously been demonstrated in premenopausal women. This study was performed to investigate the circadian rhythm in sPICP in healthy and osteopenic postmenopausal women. Blood samples were taken every third hour for 27 h from three groups of women: 12 early postmenopausal women (aged 55 ± 2 years; mean ± SD); 12 late postmenopausal women (aged 73 ± 1 years); and 12 osteopenic but otherwise healthy late postmenopausal women (aged 73 ± 1 years). A circadian rhythm in sPICP was found in all three groups, as shown by cosinor analysis ($p$ = 0.000003–0.03). The circadian rhythm in sPICP was significantly different between the osteopenic group and the age-matched healthy group ($p$ <0.008). The amplitude of the circadian rhythm in sPICP was about twice as high in the osteopenic group, and the time of the maximum tended to be about 3 h later, as compared with the age-matched healthy group. The plasma concentration of osteocalcin, as measured by a recently developed two-site enzyme-linked immunosorbent assay, also showed a circadian rhythm in all three groups ($p$ = 0.0001–0.05), with no significant differences between groups. In conclusion, we have found a significant circadian rhythm in sPICP in both early and late postmenopausal women. In osteopenic women the nightly peak in sPICP is larger and persists later into the night as compared with non-osteopenic women.

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**Keywords:** Circadian rhythm; Cosinor analysis; Osteopenia; Postmenopause; Procollagen type I carboxyl-terminal propeptide (PICP)

## Introduction

Procollagen type I carboxyl-terminal propeptide (PICP) is a globular protein with relative molecular weight of about 100 000. The protein is cleaved from its precursor molecule procollagen by specific enzymes in a 1:1 stoichiometric ratio during the formation of mature type I collagen [1,2]. Type I collagen constitutes about 90% of the organic matrix of bone [3], and the circulating level of PICP is thus used as a biochemical marker of bone formation. The rate of bone formation as measured histomorphometrically correlates with the circulating level of PICP [4–6]. However, PICP is not an entirely specific marker of bone formation, since type I collagen is also formed in varying amounts in many other tissues of the body, such as skin, tendon and teeth [2].

Circadian rhythms have been reported in most biochemical markers of bone turnover such as deoxypyridinoline/creatinine (D-Pyr/Cr) [7–9], osteocalcin [7,10,11], and the carboxyl-terminal pyridinoline cross-linked telopeptide of type I collagen (ICTP) [12]. A circadian rhythm in the serum concentration of PICP has been reported in premenopausal women [12]; postmenopausal women have not been investigated in this respect.

It is well known that women with osteopenia or osteoporosis have an increased bone turnover compared with non-osteopenic women. D-Pyr/Cr excretion is higher in elderly osteopenic women than in age-matched non-osteopenic women [13]. Women with

osteoporosis and vertebral fractures have a nightly peak in D-Pyr/Cr which is larger and persists longer into the morning than in healthy women [14]. The effect of osteoporosis or osteopenia on the circadian rhythm in serum PICP is not known.

The aim of the present study was to investigate the circadian rhythm in the serum concentration of PICP in postmenopausal women with and without osteopenia.

## Materials and Methods

*Participants*

The study comprised 36 women, divided into three groups:

*Group A*: Twelve healthy postmenopausal women who had recently passed menopause (age, 55 ± 2 years (mean ± SD); menopausal age, 5.6 ± 0.6 years; height, 164 ± 7 cm; weight, 67 ± 8 kg).

*Group B*: Twelve healthy elderly postmenopausal women (age, 73 ± 1 years; menopausal age, 25.5 ± 3.5 years; height, 162 ± 4 cm; weight, 70 ± 12 kg). These women were randomly selected from an initial group of 512 women, recruited by a questionnaire sent to 2009 women. The women were selected for this group on the basis of a forearm bone mineral content (BMC; measured by single photon absorptiometry with an $^{125}I$ source (3.7 GBq) and photopeak at 27 keV) less than 2 SD below the mean premenopausal level.

*Group C*: Twelve elderly osteopenic but otherwise healthy postmenopausal women (age, 73 ± 1 years; menopausal age, 21.3 ± 2.1 years; height, 157 ± 6 cm; weight, 63 ± 10 kg). Osteopenia was defined by a forearm BMC more than 2 SD below the mean premenopausal level. The women were randomly selected from the same initial grup of 512 women as the previous group.

Groups B and C were selected to be age-matched. In each of these two groups, 2 women were found to have wedge fractures by radiography of the spine. None of the women in the three groups was found to have a crush fracture. For details see Schlemmer et al. [9], where measurements of the urinary excretion of pyridinium cross-links from the same study are also reported. None of the women had any history of endocrine, renal or metabolic diseases or was taking any medications or oral contraceptives at the time of the study. The research protocol was approved by the ethics committee of Copenhagen County. The women received thorough information and gave their written consent before entering the study, in accordance with the Helsinki Declaration II.

*Procedures*

Normal day-time activities and normal eating and sleeping patterns were permitted. During the circadian study, starting at 1400 hours blood samples were taken every third hour until the following day at 1700 hours. Serum and plasma samples were stored at −20 °C until assayed.

*Biochemical Markers*

The serum concentration of procollagen type I carboxyl-terminal propeptide (sPICP) was determined by a radioimmunoassay recently developed in our laboratory. The assay was based on PICP purified from the cell culture medium of human fetal fibroblasts; this PICP was cleaved as in vivo from procollagen. The assay used polyclonal rabbit antibodies and had a measurement range of 0.15–3.75 nmol/l. The detection limit was 0.03 nmol/l, and the intra- and inter-assay coefficients of variation for duplicates were 2% and 4%, respectively. The reference interval for the serum concentration of PICP in healthy women above 30 years of age was 0.36–1.44 nmol/l [15].

The plasma concentration of osteocalcin (pOC) was determined by a recently developed two-site enzyme-linked immunosorbent assay (ELISA). The assay was based on two highly specific monoclonal antibodies against human osteocalcin, recognizing the midregion (amino acids 20–43) and the N-terminal region (amino acids 1–19), respectively; the assay thus detected intact osteocalcin (1–49) as well as the N-terminal/mid fragment (1–43). The assay had a measurement range of 5.0–80.0 μg/l and a detection limit of 2.0 μg/l. The intra- and inter-assay coefficients of variation for duplicates were 4% and 6%, respectively. The reference interval for postmenopausal women was 9.2–48.0 μg/l.

The serum concentration of alkaline phosphatase (sAP) was measured enzymatically according to Scandinavian recommendations [16]. The serum concentration of albumin (sALB) was measured by a standard routine laboratory method.

*Statistical Analysis*

The results of the biochemical assays were analyzed using the procedures of the SAS Institute [17]. The mean and standard error of mean were calculated for each time point of the day and night for each marker in each group of women. The study mean value was calculated for each woman as the mean of the ten samples obtained during the study. Differences in the study mean values between groups were tested using one-way analysis of variance. Furthermore, the data were linearly transformed for each participant so that the mean of the pair of values from the overlapping time points at the start and at the end of the study (i.e. the two set of values at 1400 hours and 1700 hours) were identical, and then corrected for each individual's study mean value (i.e. $y_{dev,i} = 100\% \cdot (y_i - \Sigma^{10}_{i=1}[y_i/10]) / \Sigma^{10}_{i=1}[y_i/10]$). The mean and standard error of the mean of the corrected data were calculated for each time point of the

day and night in each group of women. The corrected data were subjected to cosinor analysis: The circadian change in the parameter was calculated for each woman by multiple regression analysis, with sine and cosine as independent variables (i.e. $y = \beta_s \cdot \sin(t) + \beta_c \cdot \cos(t) + \alpha$, where $t = 2\pi \cdot \text{time(h)}/24$). The coefficients ($\beta_s$,$\beta_c$) to sine and cosine were then used as summary measures of the circadian variation and compared with zero by multiple analysis of variance. Differences in circadian rhythms between groups were tested using two-sample comparison of population rhythms according to Nelson et al. [18]. Combined rectangular and polar representation of the circadian rhythm parameter estimates ($\beta_s$,$\beta_c$) with joint 95% confidence regions were plotted as vectors for sPICP and pOC. In this representation the length and direction of the vector indicates the amplitude of the calculated circadian rhythm and the time of maximum, respectively, while the point (0,0) being outside or inside the confidence region indicates whether the circadian rhythm is significant or not, respectively [18].

## Results

Figure 1 shows the mean and standard error of mean of the values of sPICP, pOC, sAP and sALB during the circadian studies. The data are shown both as measured (absolute) values, and as drift-corrected percentage deviation from each individual's study mean value (relative values). Significant circadian rhythms in all markers were found for all three groups of women ($p \leq 0.05$). The overall group mean values (Table 1) were not significantly different in any of the markers between any of the groups.

For sPICP in absolute as well as relative values, there was a trend towards the osteopenic women having the highest values and the age-matched non-osteopenic women having the lowest values of the three groups, especially during the nightly peak; if the samples taken at 0500 hours were seen in isolation, there would be a significant difference in sPICP between the osteopenic and age-matched non-osteopenic group ($p < 0.02$). The peak in sPICP also tended to last later into the night for the osteopenic group compared with the two other groups. A statistically significant difference in the circadian rhythm in sPICP was found between the osteopenic group and the age-matched non-osteopenic group ($p < 0.008$).

A vector representation of the circadian rhythm parameter estimates with joint 95% confidence regions is shown in Fig. 2 for sPICP and pOC.

For pOC the circadian rhythms were very similar between groups, when looking at the relative values (Figs 1, 2). For sAP and sALB the circadian rhythms were virtually indistinguishable, when looking at the relative values (Fig. 1). No significant differences in circadian rhythms between any of the groups were found in pOC, sAP or sALB.

**Table 1.** Overall group mean values (± 1 SD) of the biochemical markers

| Marker | Early postmenopausal women | Late postmenopausal women | Osteopenic late postmenopausal women |
|---|---|---|---|
| sPICP (mmol/l) | 0.82±0.25 | 0.79±0.19 | 0.87±0.20 |
| pOC (μg/l) | 32.5±9.2 | 31.4±6.7 | 37.9±9.2 |
| sAP (u/l) | 182±43 | 167±39 | 184±36 |
| sALB (mmol/l) | 0.67±0.03 | 0.66±0.03 | 0.65±0.03 |

## Discussion

In this study we found significant circadian rhythms in sPICP in both early and late postmenopausal healthy women, and in women with osteopenia. We believe the circadian rhythms in sPICP reflect true endogenous rhythms in the kinetics of release of PICP. To estimate the extent to which the observed circadian rhythms in the biochemical markers of bone turnover were influenced by possible hemoconcentration due to the variations in body posture during the day and night, we measured the serum concentration of albumin. Since the nadir of the circadian rhythm in serum albumin coincides with the zenith of the rhythm in sPICP, the rhythm in sPICP is not caused by hemoconcentration. Furthermore, the biological half-life of circulating PICP is less than 10 min (in rats) [19], so the release of PICP from the tissues into the blood stream is significant compared with the pool of circulating PICP.

The osteopenic women generally tended to have higher levels of sPICP, pOC and sAP than the age-matched healthy women (Table 1), although the differences were not statistically significant. This is an indication of a higher rate of bone turnover in the osteopenic women.

The major finding in this study is that the nightly peak in sPICP is larger and persists later into the night in osteopenic women than in age-matched non-osteopenic women. The differences in the circadian rhythms in D-Pyr/Cr between the groups of the present study were not statistically significant [9]. However, in a previous larger study the urinary excretion of pyridinium cross-links has been reported to be higher in women with osteopenia as compared with healthy women [13]. Thus the *turnover* of type I collagen is increased in osteopenia, especially at night. The increase in type I collagen formation may be caused by the increased type I collagen degradation, mediated by a coupling effect.

All three groups of women had similar circadian rhythms in pOC. The nightly peak at 0200 hours in pOC was 10%–15% higher than the 24-h mean values in all three groups (Fig. 1). This corresponds well with the range of 5%–20% for the circadian variation in circulating OC reported by other investigators using other immunoassays for OC [7,10,11].

The biological half-life of circulating skeletal alkaline

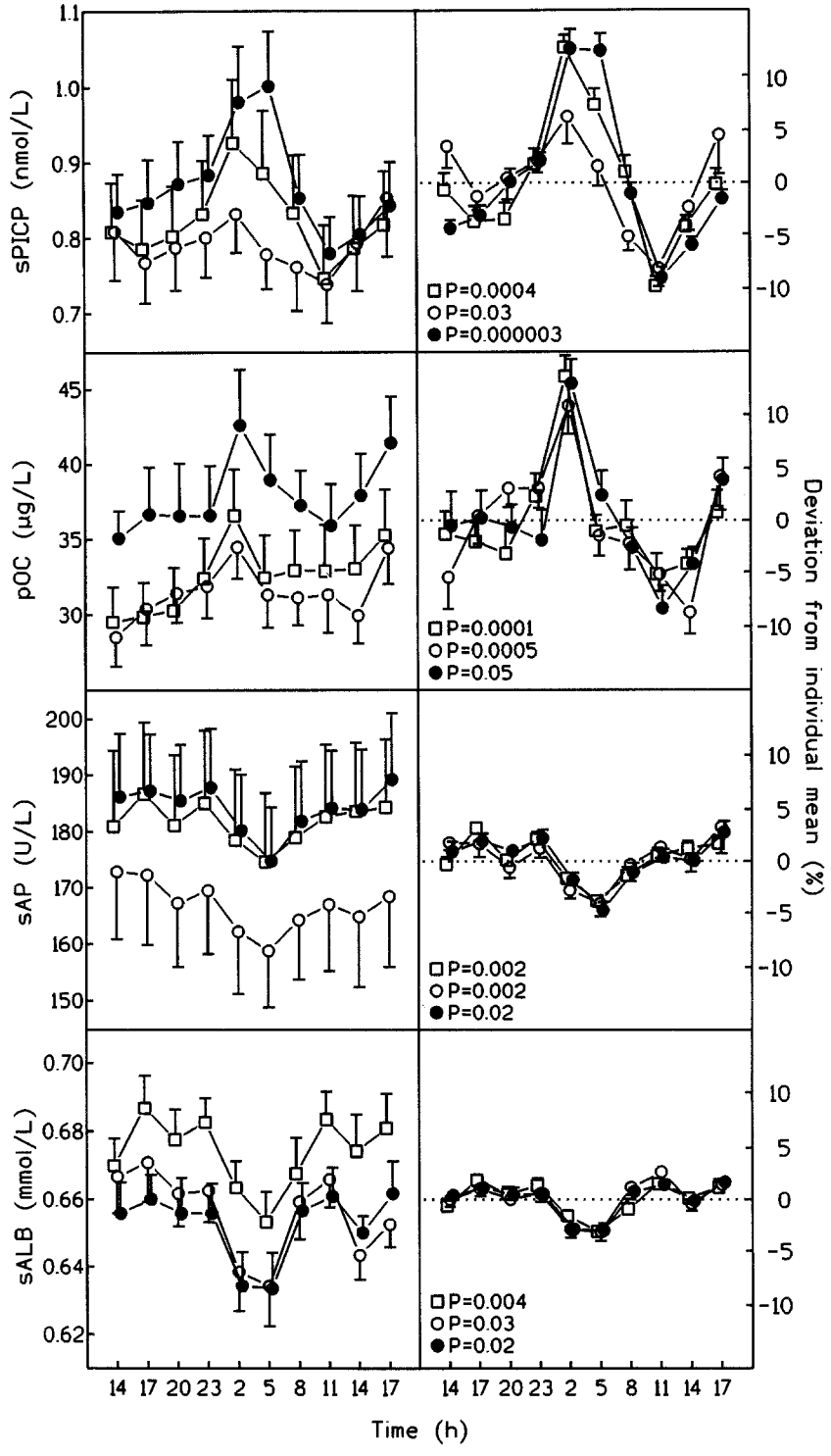


**Fig. 1.** The values of the four markers measured during the circadian studies of the healthy early postmenopausal (*open squares*), healthy late postmenopausal (*open circles*) and osteopenic late postmenopausal women (*filled circles*). *Left-hand column*: the values for each time point of the day and night. *Right-hand column*: the drift-corrected deviation from each individual's mean value of the circadian study for each time point of the day and night. Data are given as mean values with 1 standard error of the mean. The *p*-values indicate the significance of the circadian rhythm.

phosphatase has been reported to be in the order of 1–2 days [20]. Thus, any true endogenous circadian variation in the kinetics of release of alkaline phosphatase will be buffered by a large circulating pool. Furthermore, in this study we measured total alkaline phosphatase, which means that the liver isoenzyme could blur any possible rhythm in the skeletal isoenzyme. We did find a small but significant circadian rhythm in sAP in accordance with previous findings [8]. However, a major part of the rhythm seen in sAP can probably be ascribed to hemoconcentration effects, as reflected in the albumin data.

The etiology of the circadian rhythms in the various biochemical markers of bone turnover is not known. In the case of sPICP, the rate of synthesis of type I collagen in bone can be influenced by a large array of systemic and local hormones, growth factors and cytokines [21]. While continuous administration of parathyroid hor-

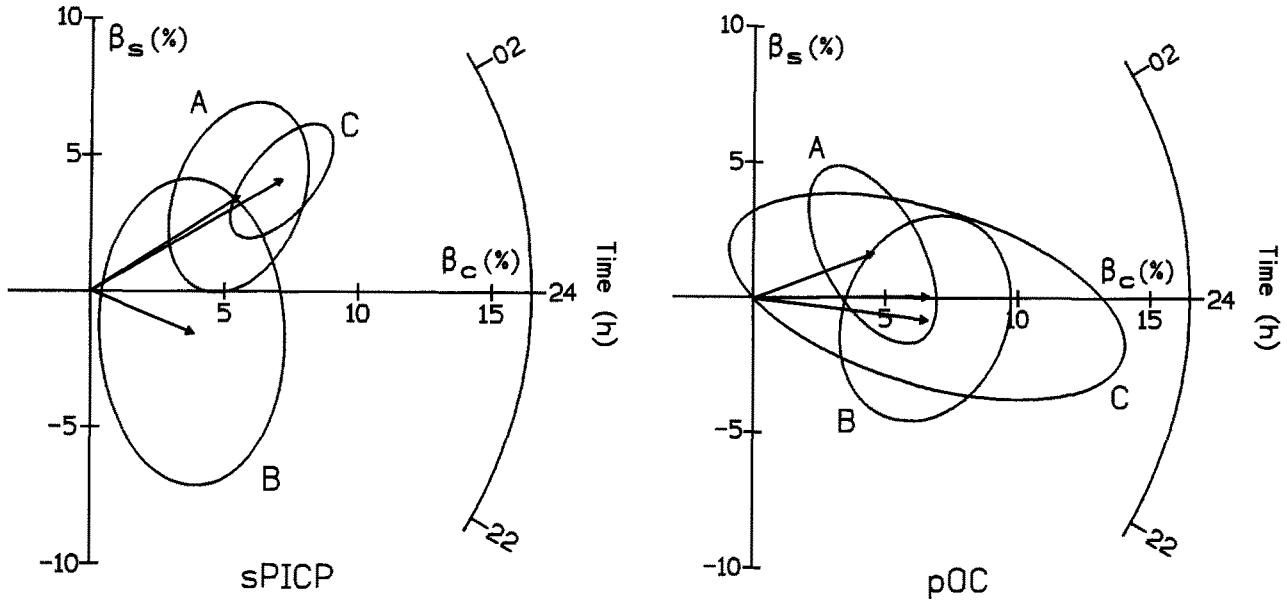


**Fig. 2.** Combined rectangular and polar representation of circadian rhythm parameter estimates with joint 95% confidence region for sPICP (*left*) and pOC (*right*). $\beta_s$ and $\beta_c$ denote the coefficients (in units of percentage deviation from individual study mean value) to sine and cosine, respectively, in the cosinor analysis. The letters denote the healthy early postmenopausal (*A*), the healthy late postmenopausal (*B*) and the osteopenic late postmenopausal women (*C*), respectively. Two-sample comparison of population rhythms (amplitude-acrophase test) [18] showed a significant difference in the circadian rhythm in sPICP between group B and group C ($p<0.008$).

mone (PTH) inhibits the synthesis of type I collagen in cultured rat calvariate, intermittent administration has a stimulatory effect, possibly mediated by a stimulation of the release of insulin-like growth factor I [22]. Since serum PTH has a circadian rhythm with peak concentrations in the afternoon and at night [23,24], PTH could be associated with the mechanism of circadian rhythm in type I collagen synthesis. Another candidate hormone is cortisol, which also affects collagen synthesis in vitro [25], and has a circadian rhythm in vivo [26].

In conclusion, we have found a circadian rhythm in sPICP in both early and late postmenopausal women. In osteopenic women the nightly peak in sPICP is larger and persists later into the night as compared with non-osteopenic women.

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*Received for publication 20 October 1994*
*Accepted in revised form 6 April 1995*