Acute fasting diminishes the circadian rhythm of biochemical markers of bone resorption

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Abstract

Objective: Biochemical markers of bone turnover exhibit circadian rhythms with the peak during the night/early morning and the nadir in the late afternoon. The nocturnal increase in bone resorption could theoretically be caused by the absence of food consumption which brings about a decrease in net calcium absorption and an increase in parathyroid hormone (PTH), followed by increased bone resorption in response to the body's demand for calcium. The aim of the present study was to assess the influence of a 33-h fast on the circadian variation in biochemical markers of bone turnover.

Design: Eleven healthy premenopausal women (age: 24 ± 5 years) participated in a randomised, cross-over study consisting of two periods: either 33 h of fasting (fasting) followed 1 week later by a 33-h period with regular meals eaten at 0800–0830 h, 1130–1230 h and 1800–1900 h (control) or vice versa.

Methods: Urinary CrossLaps (U-CL/Cr) corrected with creatinine, as a marker of bone resorption; serum osteocalcin (sOC) as a marker of bone formation; serum intact PTH (iPTH); serum phosphate; and serum calcium corrected with albumin.

Results: Both the fasting and the control periods showed a significant circadian rhythm in U-CL/Cr ($P < 0.001$), but the decrease was significantly less pronounced in the morning hours during the fasting period. Fasting resulted in a significant decrease in serum iPTH (throughout the study period) as compared with the control period ($P < 0.05$ – 0.001). No change was observed in sOC by fasting.

Conclusion: Food consumption has a small influence on the circadian variation in bone resorption, independent of PTH. The fall in iPTH during fasting may be secondary to an increased bone resorption produced by fasting.

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Introduction

A well-known circadian variation with the peak during the night/early morning and the nadir late in the afternoon has been described in biochemical parameters of bone resorption such as the urinary excretion of pyridinium cross-links (1–4), urinary cross-linked N-telopeptide of type I collagen (NTx) (5), urinary CrossLaps (6) and serum carboxy-terminal pyridinoline cross-linked telopeptide of type I collagen (7). A less pronounced circadian variation is also known in biochemical markers of bone formation, such as serum osteocalcin (sOC) (4, 8) and serum procollagen type I carboxy-terminal propeptide (7, 9).

Whereas the circadian variation in bone formation may be dependent on the circadian fluctuation in serum cortisol (10–12), the aetiology of the circadian variation in bone resorption remains unknown. Earlier studies have found the variation in bone resorption to be independent of age, menopausal status, posture (2), and the circadian fluctuations in serum cortisol

(12). However, the influence of calcium is ambiguous. Suppression of intact parathyroid hormone (iPTH) with a constant infusion of calcium did not influence the circadian pattern of the urinary Ntx (5), whereas the nocturnal peak in the urinary Ntx was suppressed by 1000 mg calcium administered at 2300 h for 14 days (13). A recent study demonstrated, in rats, that when solid food and liquid intake were divided into portions, the peak in bone resorption seen after food ingestion was blunted and the 24-h bone resorption decreased. After 30 days on this schedule an increase in bone mass was observed (14).

Ebeling *et al.* (15) had previously shown that the total 24-h urinary excretion of pyridinium cross-links increased with 4 days of calcium deprivation (dietary calcium (230 mg/day) and treatment with a calcium-binding agent). Grinspoon *et al.* (16), however, failed to find any significant response in the 24-h urinary excretion of pyridinium cross-links with 4 days of fasting. Neither of these studies, however, was designed to examine the effect of fasting on the circadian rhythm

in biochemical markers of bone resorption. Theoretically, therefore, the nocturnal peak in bone resorption may be caused by the absence of food consumption, followed by an increased bone resorption in response to the body's demand for calcium. The aim of the present study was to investigate this hypothesis.

Participants and methods

Participants

Eleven healthy premenopausal women (age: 24 ± 5 years; height: 168 ± 6 cm; weight: 63 ± 6 kg (mean ± 1 s.d.)) participated in a randomised, cross-over study consisting of two periods of either 33 h of fasting (water, coffee and tea permitted) followed 1 week later by a 33-h period with regular meals eaten at 0800–0830 h, 1130–1230 h and 1800–1900 h or vice versa. Normal daytime activities and sleeping pattern were permitted. None of the women had known endocrine or renal disease, or were taking any medication at the time of the study.

All the women fasted from 2300 h on the evening before the start of the two 24-h periods. The participants emptied their bladder at 0500 h in the morning, and urine was collected in 3-h aliquots starting at 0500 h until the following day at 0800 h. Blood samples were taken at 3-h intervals starting at 0800 h. The blood and urine samples were stored at -20°C until assayed.

The research protocol was approved by the Ethical Committee of Copenhagen County (KA95114g). All the participants gave their informed consent, in accordance with the Helsinki II Declaration.

Methods

Urinary CrossLaps were measured by an ELISA assay based on an antiserum (rabbit) reaction with a sequence derived from type I collagen (EKAHDGGR). The average within-run and total coefficients of variation of the assay were 5.3% and 6.6% respectively (17). The results were then corrected with creatinine (Cr) and expressed as mg/mol creatinine.

The sOC was measured by a two-site enzyme-linked immunosorbent assay (N-MID hOsteocalcin; Osteometer BioTech A/S, Herlev, Denmark). The assay is based on two highly specific monoclonal antibodies against human osteocalcin, recognising the mid region (amino acids 20–43) and the N-terminal region (amino acids 1–19) respectively, and the assay thus detected intact osteocalcin (1–49) as well as the N-terminal mid fragment (1–43). The assay had a measuring range of 5.0–75.0 $\mu\text{g/l}$ and a detection limit of 2.0 $\mu\text{g/l}$. The intra- and interassay coefficients of variation were less than 7% (18).

Serum iPTH was measured by a commercially available IRMA (Coat-A-Count Intact PTH IRMA; Diagnostic Products Corporation, Los Angeles, CA,

USA). The detection limit was 0.1 pmol/l and the intra-assay coefficient of variation was 6.0%.

Serum calcium, serum phosphate, urinary creatinine, and serum albumin were measured by standard laboratory methods.

Statistical analysis

The results of the biochemical assays were analysed using the procedures of the SAS Institute (19). To obtain normality and homogeneity of variance, urinary CrossLaps/Cr and sOC were logarithmically transformed (log concentration) before analysis of the circadian rhythm. These serial measurements were analysed according to concepts described by Matthews *et al.* (20). The 24-h cyclical changes were calculated for each woman by multiple-regression analysis, with sine and cosine as independent variables (e.g. $y = \beta_1 \sin(t) + \beta_2 \cos(t) + \alpha$, where $t = 2\pi \text{ time (h)}/24$). The coefficients (β_1 and β_2) to sine and cosine were then used as summary measures of the circadian variation and compared with zero by multiple analysis of variance (19). Student's paired *t*-test was used for comparison within groups. For visualising the effect of fasting, a delta value of urinary CrossLaps/Cr and sOC is given as mean of control value–fasting value for each individual at each time-point.

Serum calcium was corrected by albumin (e.g. total Ca + $1.1(0.70 - \text{serum albumin})$).

Results

Figure 1 shows the 24-h fluctuation in the urinary excretion of CrossLaps/Cr and sOC. A highly significant circadian variation in urinary CrossLaps/Cr was observed during both the fasting and the control period ($P < 0.001$). A significantly less pronounced decrease during the morning and afternoon hours ($P < 0.05$ – 0.01) was observed in the fasting group as compared with the controls. sOC showed a circadian rhythm during the control period ($P < 0.01$) and the fasting period ($P = 0.05$). Fasting did not change the circadian pattern in sOC significantly.

Figure 2 shows the 24-h fluctuation in the urinary excretion of CrossLaps/3 h. The pattern of the curves was similar to the CrossLaps/Cr curves, with a significantly less pronounced decrease observed in the fasting period between 1100 and 1400 h ($P < 0.01$). One value was dismissed at the first time-point because of collection error.

Figure 3 shows the 24-h fluctuation in serum iPTH, serum calcium (corrected for albumin) and serum phosphate. Fasting significantly decreased ($P < 0.05$ – 0.001) iPTH and abolished the circadian rhythm in iPTH. Albumin-corrected serum calcium was significantly higher during the daytime in the fasting period than in the control period. In serum phosphate no significant differences between the fasting and the control period was seen at any time-point.

Figure 1 Mean values of the urinary (U) excretion of CrossLaps/Cr and serum (S) osteocalcin during the two 24-h periods (left-hand figures). Fasting is shown as solid circles and control as solid triangles. Mean delta value of urinary CrossLaps/Cr and sOC is given as control value–fasting value for each individual at each time-point (right-hand figures). Values are means $\pm$ S.E.M. * $P < 0.05$; ** $P < 0.01$.

The mean (average of eight time-points) increased significantly in serum calcium (mmol/l) (corrected for albumin) (2.31 ± 0.05 vs 2.36 ± 0.03 ; $P < 0.05$) and decreased significantly in serum iPTH (4.4 ± 1.6 vs 3.1 ± 1.0 pmol/l; $P < 0.01$) whereas no significant differences was observed in the urinary CrossLaps/Cr (220 ± 59 vs 246 ± 71 ng/μmol; not significant) or serum phosphate (1.36 ± 0.12 vs 1.34 ± 0.09 mmol/l; not significant). All values are given as control vs fasting.

Discussion

Food consumption alone cannot explain the circadian rhythm in the biochemical markers of bone resorption – even though fasting significantly changes the circadian pattern. However, part of the circadian variation seen in healthy individuals is probably caused by food.

The circadian variation in the biochemical markers of bone resorption seen in this study was more pronounced in the control period, when regular

Figure 2 Mean value of the urinary (U) excretion of Crosslaps/3h during the two 24-h periods. Fasting is shown as solid circles and control as solid triangles. Values are means $\pm$ S.E.M. ** $P < 0.01$.

Figure 3 Mean value of serum (S) iPTH, serum calcium (S-Ca) (alb. corr., corrected for albumin) and serum (S) phosphate during the two 24-h periods. Fasting is shown as solid circles and control as solid triangles. Values are means $\pm$ S.E.M. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

meals were served, than in the fasting period. Urinary CrossLaps/Cr fell more significantly after food ingestion in the daytime, whereas the curves for the evening and the following night (when none of the participants were given any meals) were indistinguishable.

This effect on urinary CrossLaps/Cr was not caused by an increase in PTH in order to maintain the serum calcium at an acceptable physiological level, because serum iPTH actually fell and the circadian pattern was abolished, whereas serum calcium increased during fasting. It is more likely that a direct demineralisation took place during fasting, which increased serum calcium, followed by a decrease in serum iPTH as the outcome. These changes may be mediated by the acidosis caused by fasting. An earlier study by Kocian & Brodan (21) demonstrated that short-term starvation of obese subjects induces metabolic acidosis and increased calciuria, resulting in a severe negative calcium balance. As most body calcium resides in bone mineral, the elevated urinary excretion of calcium is likely to originate from bone. Experimental studies with neonatal mouse calvariae cultured in medium with a reduced pH (an *in vitro* model of metabolic

acidosis) have further shown that there is a net efflux of calcium from the bone during acute 3-h culture (22) and chronic culture (24 h or longer) (23, 24). Furthermore, Krieger *et al.* (25) demonstrated increased osteoclastic activity and decreased osteoblastic activity in an experimental model of metabolic acidosis. Hence the well-known circadian variation in bone resorption with the peak during the night could, theoretically, be caused in part by the nightly fast – although the present study showed that this can only explain a small part of the circadian variation in bone resorption. In serum phosphate no significant difference was observed between the fasting and control period at any time-point, suggesting that the effect of fasting is mediated through calcium metabolism rather than phosphate.

When the present study was planned there were no data in the literature regarding the influence of food intake on the circadian variation of bone resorption. We thus did not know whether a change in food intake would affect the overall circadian variation in bone resorption or whether it would have an acute effect just during daytime or no effect at all. The present study was therefore designed to have 80%

power (at a 5% significance level) to detect an elimination of the circadian effect on urinary excretion of CrossLaps at a specific time-point. We found a significant effect of fasting only during the early day time which was not reflected in the 24-h urinary excretion of CrossLaps. We therefore believe that this finding needs to be confirmed before a final conclusion can be drawn regarding the magnitude of the effect of food intake on the circadian variation of bone resorption.

We did not include the participants at a specific time in their menstrual cycle in this study. However the time-span between the two study periods is short (1 week) and the study was performed as a randomised, cross-over study for which reason we do not believe that the fluctuations in hormones during the menstrual period has any influence on the results presented in this article.

Serum iPTH peaked at about 2000 h in our control period, whereas it usually occurs between 0200 and 0600 h (26–28). The participants were up in order to empty their bladder completely. This practice has no influence on the circadian variation in biochemical markers of bone resorption (2), but perhaps it affected the time at which serum PTH peaked. A shift in sleeping time is known to blunt the start of the rise in serum PTH (27), although the theoretical changes in sleeping pattern caused by the nightly awakening in this study are of less magnitude than in the study of Logue *et al.* (27).

We and others have shown the circadian rhythm in bone resorption to be independent of sex steroids, posture (2), and fluctuations in cortisol (12) and PTH (5). The present acute fasting study provides the first evidence to explain parts of the changes in the circadian variation in biochemical markers of bone resorption. However, it explains only a small part, the greater part of the aetiology of the circadian rhythm in bone resorption still remains unsolved. Further studies are needed – especially a longer period of fasting – in order to assess whether the observed changes continue in the following days, and to establish which particular components of the food are able to induce a further decrease in the urinary excretion of CrossLaps. The latter is probably not caused by calcium intake, based on the response of serum PTH to fasting.

In conclusion, we found that food consumption in the morning potentiates the morning/early afternoon decrease in the urinary excretion of CrossLaps, a new marker of bone resorption. This effect seems to be independent of PTH.

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