ported previously (11, 12, 16). The difference is probably attributable to the use of a particle-enhanced nephelometric immunoassay (14) in the present study in contrast to previous studies (11, 12, 16) using a particle-enhanced turbidimetric immunoassay (10). Method calibration is not yet established internationally but is required to allow direct comparison between methods (18).

In children >1 year, serum creatinine increases with age until adulthood. Serum creatinine concentrations were adjusted to height and body size using the Morris formula, and a better correlation was found. As a single-sample measurement for estimation of GFR, serum cystatin C concentrations do not require adjustments to height and body size, which assigns serum cystatin C measurements advantages for use in clinical practice.

Gender did not alter the serum concentrations of cystatin C in agreement with previous studies (12, 13) contrary to serum creatinine. No differences from hemoglobin, bilirubin, and lipemia have been demonstrated using the particle-enhanced nephelometric immunoassay method, making the assay suitable for measurements of pediatric samples (14).

In conclusion, the results demonstrate that serum concentrations of cystatin C in children without evidence of kidney diseases are constant after the first year of life, contrary to serum creatinine. The reference interval for serum cystatin C in children >1 year was calculated to be 0.51–0.95 mg/L. After the first year of life, serum cystatin C possesses the advantage of being independent of age, gender, and muscle mass, which facilitates the recognition of abnormal renal function.

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References
18. Diurnal Rhythm of CrossLaps in Human Serum, Maria Wichers,* Elke Schmidt, Frank Bidlingmaier, and Dietrich Klingmuller (Department of Clinical Biochemistry, University of Bonn, Sigmund-Freud-Strasse 25, 53105 Bonn, Germany; * author for correspondence: fax 49-228/287-5028)

Measurements of molecules of bone resorption or formation can be used for estimating the rate of bone turnover. Bone turnover, as assessed by several bone metabolic markers, has been reported to undergo a diurnal rhythm. Thus far, urinary pyridinoline and deoxypyridinoline, serum osteocalcin, bone specific alkaline phosphatase, serum type I collagen cross-linked N-telopeptides (NTx), the C-terminal pyridinoline cross-linked telopeptide of type I collagen, and urinary excretion of NTx were
reported to undergo circadian periodicities with high values at night (1-5).

Degradation products derived from a sequence (EKAHĐ-β-GGR) specific for a part of the C-telopeptide α1-chain of type I collagen (CTx) in urine and serum could be quantified by the enzyme-linked immunosorbent assay, CrossLaps™. It had been demonstrated that such fragments are sensitive markers of bone resorption (6-8). The purpose of this study was to determine whether there are diurnal fluctuations in the concentration of CTx in serum; if this proves true, it is important to specify the time of blood sampling.

Six healthy 24- to 27-year-old men (weight, 74–85 kg) were studied over a single 24-h period. Blood was sampled every 60 min from an intravenous catheter placed in each subject between 0800 and 0830. Meals were offered at 1000, 1400, and 1930. Activity was restricted to small movement within and around the study room, and lights were turned out between 0000 and 0700. Blood was centrifuged at 3000 g for 10 min, and serum was separated and stored at −20 °C until assay.

CTx was measured by a one-step enzyme-linked immunosorbent assay (Osteometer), which is based on one highly specific monoclonal antibody against a β-aspartate isomerized form of the epitope EKAHĐ-β-GGR derived from the C-terminal telopeptide region of the type I collagen α1-chain. According to the manufacturer, the detection limit of the assay is 92 pmol/L, the intra- and interassay CVs are 4.9% (mean, 3500 pmol/L) and 6.6% (mean, 3501 pmol/L).

Hormone concentrations are presented as median values and individual secretion profiles. The 24-h concentration profiles of CTx were tested for diurnal variation by cosinor rhythmometry, a computer program provided by Dr. W. Burr (Clinic of Epileptology, University of Bonn) as described previously (9). A significant fit of the curve was defined when the possibility of the data representing a horizontal line rather than a cosine curve was <5%. The acrophases, which were not necessarily the detected maximal concentrations, represented the time of occurrence of the maximal values of the best-fitting pattern; the amplitude was defined as 50% of the difference between the acrophase and the nadir concentrations. A value <0.05 was considered significant.

Individual serum CTx concentration profiles over 24 h in six healthy male volunteers are illustrated in Fig. 1. The highest concentrations were observed at night between 0130 and 0430, with peak values of 3948–9945 pmol/L; the lowest values were measured between 1100 and 1500 (443–2356 pmol/L). The concentrations fell quickly in the morning between 0800 and 1100 and came to a nadir between 1100 and 1500. After the nadir, they increased slowly to the initial concentrations between 0130 and 0430 at night. The peak was 66% greater than the 24-h mean, and the nadir was 60% below the 24-h mean. A significant (P <0.01) diurnal secretion in all six volunteers was validated by cosinor rhythmometry (Table 1).

The diurnal variation of markers of the bone mineral metabolism needs to be taken into account when interpreting the results of biochemical marker measurements. Most markers tend to be higher at night. This is most characteristic for the bone resorption markers.

A new direct immunoassay using antibodies that recognize serum type I C-telopeptide breakdown products, CrossLaps (CTx), is now available. CTx is a very important marker for the follow-up treatment of osteoporosis. A recent study showed that serum and urine CTx concentrations decrease significantly among women treated with clinically relevant doses of different antiresorptive agents. Furthermore, the annual percentage of change in bone mineral density correlated with the measured changes in CTx concentration (10). Unfortunately, the investigators did not take into account the timing of blood sampling.

The present investigation demonstrated a significant circadian rhythm of this bone resorption marker in serum with a peak value between 0130 and 0430 at night and a nadir between 1100 and 1500 during the day. The amplitude of variation is ~60-66% of the mean value. This finding is in agreement with a recent study that measured urinary CTx during 24 h in seven separate collections. A significant variation with a peak at night and a nadir at 1700 h was found (1).

The pattern of the circadian variation of CTx in serum was similar to those of other metabolic bone markers. It followed the rhythmicity of osteocalcin (2) and NTx (4) in serum. Urinary excretion of creatinine-corrected pyridinoline, deoxypyridinoline, and NTx showed a diurnal rhythm, with a peak between 0400 and 0700 and a nadir between 1300 and 1600. Their excretion at night was ~50% higher than during the day (11, 12). It should be noted that most patients usually are older than our subjects, and one study found that urinary NTx values in men of 20–30 years were higher than at subsequent ages (13).

Table 1. Circadian rhythms of the individual CrossLaps secretion profiles analyzed by cosinor rhythmometry.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesor</td>
<td>2587.21</td>
<td>3949.33</td>
<td>3521.04</td>
<td>4389.04</td>
<td>4005.08</td>
<td>5787.83</td>
</tr>
<tr>
<td>Amplitude</td>
<td>987.12</td>
<td>2374.63</td>
<td>2203.78</td>
<td>2517.50</td>
<td>1895.25</td>
<td>3698.61</td>
</tr>
<tr>
<td>Theta (h)</td>
<td>4.55</td>
<td>3.58</td>
<td>2.81</td>
<td>4.61</td>
<td>1.41</td>
<td>3.04</td>
</tr>
<tr>
<td>F statistic</td>
<td>26.72*</td>
<td>43.30*</td>
<td>41.65*</td>
<td>36.70*</td>
<td>16.98*</td>
<td>78.84*</td>
</tr>
</tbody>
</table>

*P <0.01.
secretion is very different between younger and older subjects.

In conclusion, CTx in serum demonstrated a marked circadian rhythm in all subjects. The peak value, which on an average is 66% higher than the mean value, highlights the importance of the timing of sample collection for appropriate interpretation of therapeutic response.

References


