Quantification of the Circadian Modulation of the Bone Resorption Marker CTX-I in Serum and Urine Under Controlled In-patient Conditions

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1 Introduction & Objectives

- Bone resorption is higher during the night compared to the daytime and this is reflected in serum and urine CTX-I levels [1]. However, there are a number of factors including food intake that may influence this diurnal profile [1, 2]. So, controlled in-patient lab conditions with control of food intake is important in designing studies investigating diurnal variability.
- This study investigated the characteristics of the CTX-I profile over 24h in post-menopausal women in an environment minimizing confounding influences of irregular sleep, food intake and exercise.

2 Methods

Study population

- Healthy post-menopausal women aged 55 to 75 years with body weight >50 kg and body mass index 19 to 30 kg/m^2.
- Medical history and laboratory examinations were conducted to ensure subjects were healthy and medication free.
- Subjects with sleep/wake disorders/complaints were also excluded.
- Subjects had a regular sleep history (for at least 1 month prior to screening) of bedtime between 21:30 to 00:00 on at least 5 nights per week, typical nightly sleep duration of 6.5 to 8.5 hours, typical night time awakening frequency for micturition of ≤2 and no habit of day time naps >1 hour.
- Subjects with serum CTX-I below the limit of quantification of the assay at the screening visit were excluded.

Study Design and Assessments

- A regular sleep/wake cycle (22:30-06:30) was scheduled 7 days prior to and during the 3 day lab study (sampling on last day). Subjective sleep diaries and actigraphy were used to confirm compliance with sleep/wake cycle instructions and exercise restrictions.
- The timing of food intake and blood/urine sampling was consistent throughout the study. Breakfast, lunch, and dinner were served at 07:00, 13:00, and 19:00 respectively during the in-clinic period and water could be taken at other times.
- Breakfast had the same nutritional content as dinner since these times were considered to influence resorption the most. Lunch had a higher nutritional content to ensure daily nutritional status.
- Serum sampling was obtained ~ every 2h. A longline indwelling cannula was used for overnight sampling to minimise disturbance.
- Subjects were asked to void at 08:00 and the urine discarded. Subjects were then asked to void at the end of each 2h (or 4h) collection period with all urine collected within each period.

Statistical analysis

- Characteristics of the 24h profiles for CTX-I in serum and urine were determined by non-linear regression fitting a cosine model to the data for each subject.
- Estimates of mesor (average), amplitude of the 24h variation and time of the fitted maximum of CTX-I (peak time) were calculated separately for each subject.
- The mesor, amplitude and peak time were analysed between subjects and were compared before and after clock change from summer to winter time (JUL - SEP vs. NOV).
- The correlations between mesor, amplitude and peak time were also calculated by Pearson's product-moment correlation coefficient.
- Statistical analysis tests were performed at the two-sided 5% significance level.

3 Results

Demographics and Baseline Characteristics

- 25 subjects were enrolled. Table 1 shows baseline demographics for all 25 subjects and 14 subjects for whom significant wave fits for CTX-I in urine were obtained (as described herein below).

Table 1. Baseline Demographics

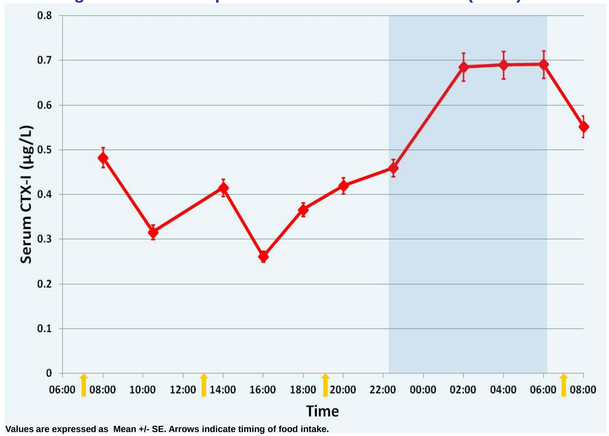
	All Subjects (n=25)	Sub-group for Urine CTX-I analysis (n=14)
Age (years)	65.2 ± 6.0	64.5 ± 6.5
Height (m)	1.63 ± 0.05	1.62 ± 0.06
Weight (kg)	65.8 ± 7.4	63.3 ± 7.0
Body mass index (kg/m^2)	24.8 ± 2.8	24.1 ± 2.8

Expressed as mean ± SD

Characteristics of the CTX-I Profile over 24h

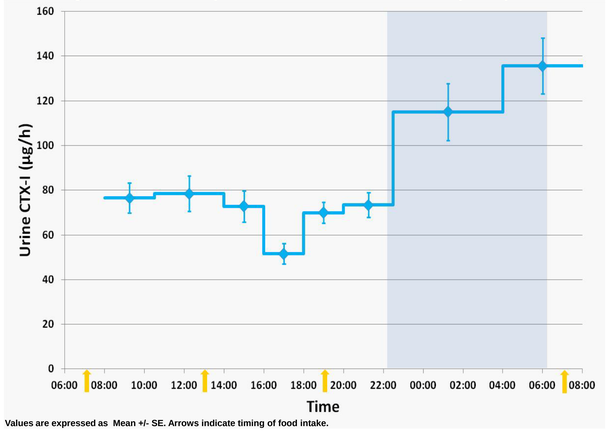
- Figures 1 and 2 show 24h time profiles for CTX-I in serum and urine, respectively.
- Figure 3 shows the 24h time profile for urine CTX-I in 14 subjects for whom significant wave fits were obtained.

Figure 1. 24h time profile for mean Serum CTX-I (N=25)



Values are expressed as Mean +/- SE. Arrows indicate timing of food intake.

Figure 2. 24h time profile for mean Urine CTX-I (N=25)



Values are expressed as Mean +/- SE. Arrows indicate timing of food intake.

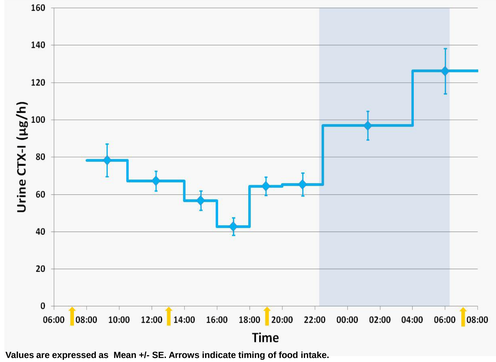
- Mesor, amplitude and peak time estimates are presented in Table 2.
- In each of the 25 subjects the amplitude of the 24h variation was significantly greater than 0 in serum ($p<0.05$).
- Significant ($p<0.05$) wave fits were obtained in 25 and 14 subjects for serum CTX-I and in urine CTX-I, respectively.
- The amplitude between subjects varied and correlated with the mesor in both serum and urine ($r=0.836$ and 0.824, respectively, both $p<0.001$).

Table 2. Mesor, Amplitude and Peak time of 24h time profile for serum and urine CTX-I

	Serum CTX-I (n=25)	Urine CTX-I (n=14)
Mesor	0.44 ± 0.13 µg/L	80.89 ± 25.40 µg/h
Amplitude	0.187 ± 0.078 µg/L	33.22 ± 12.84 µg/h
Peak Time (hh:mm)	03:17 ± 45min	04:06 ± 01:29

Expressed as Mean ± SD

Figure 3. 24h time profile for mean Urine CTX-I for subjects with significant cosine wave fits (n=14)



Values are expressed as Mean +/- SE. Arrows indicate timing of food intake.

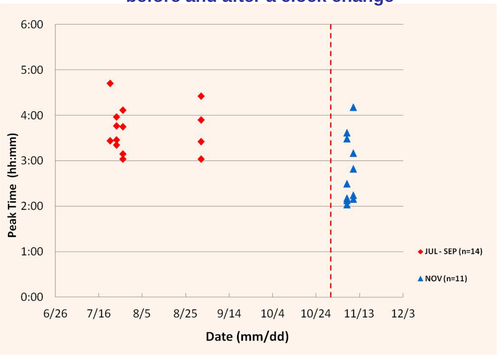
- The study covered ~5m period (Jul - Nov).
- Figure 4 shows the individual peak time in serum CTX-I across the 5-month collection period.
- A significantly ($p<0.01$) earlier peak time in serum CTX-I was obtained in NOV compared to JUL - SEP, Table 3.

Table 3. Comparison of sampling time (Mesor, Amplitude and Peak time of 24h time profile for serum CTX-I) before and after a clock change

		JUL – SEP (n=14)	NOV (n=11)	P-value
Mesor	(µg/L)	0.45 ± 0.12	0.43 ± 0.14	0.4355
Amplitude	(µg/L)	0.189 ± 0.082	0.184 ± 0.075	0.5791
Peak Time	(hh:mm)	03:41 ± 30min	02:47 ± 44min	0.0024

Expressed as Mean ± SD
t-test from ANOVA model

Figure 4. Individual Peak Times in Serum CTX-I before and after a clock change



Peak time : Time of the fitted maximum of Serum CTX-I (clock time)
Dotted-line represents the end of summer time (2011 in the UK).

4 Discussion & Summary

- The data show that in post-menopausal women, CTX-I has a clear circadian profile with mean peak levels at ~3am in serum and ~4am in urine. The amplitude is greater in post-menopausal women with higher CTX-I levels.
- Circadian characteristics can be assessed at the level of the individual. These data have implications for the assessments of bone resorption status based on a single sample.
- A significant earlier peak time in serum CTX-I was obtained in November compared to summer, but a similar effect was not observed for amplitude or mesor. Because timing of sleep and meals was identical across the periods, this effect may be related to the change from summer to wintertime.

5 Acknowledgements

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6 References

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