ORIGINAL ARTICLE

Diurnal and Day-to-Day Variations of Urinary Type 1 Collagen Crosslinked N-Telopeptides

Shimpei Fujita[1)], Kenta Wakamatsu[1)], Keishoku Sakuraba[2)], Yoshio Suzuki[2)]

ABSTRACT

Objectives: **This study investigated the diurnal and day-to-day variations of levels of Urinary type 1 collagen crosslinked N-telopeptides (NTX) /Cre and NTX to determine the availability of these markers in voluntary urine.**

Design: **Cross-sectional study**

Materials and Methods: **Seven healthy adult men participated in this study and collected their urine over a 24-hr period. Six participants collected their second-void morning urine for an additional 2 days.**

Results: **The mean coefficient of variance of diurnal NTX/Cre and NTX was 32.4% and 38.9%, respectively. NTX/Cre was highest from 06:00 to 10:00 hrs, and then consistently decreased thereafter. In contrast, NTX was lowest from 06:00 to 10:00 hrs, increased to 274.4 ± 7.5 nmol BCE/L from 10:00 to 14:00 hrs ($p < 0.001$), and then remained almost constant thereafter. The 3-day mean day-to-day variations of NTX/Cre and NTX in the second-void morning urine were 24.1% and 37.6%, respectively.**

Conclusions: **This study demonstrated that levels of NTX/Cre and NTX in voluntary urine are expected to be within approximately two-fold of the diurnal means. Therefore, the levels of NTX/Cre and possibly NTX in voluntary urine could be used for monitoring bone status.**

KEY WORDS

creatinine, diurnal variation, day-to-day variation, osteopenia, osteoporosis

INTRODUCTION

Type 1 collagen crosslinked N-telopeptides (NTX), metabolites of type 1 collagen, are the N-terminal telopeptides of the collagen molecule containing pyridinoline cross-links[1)]. Urinary NTX is a bone resorption marker as NTX is released into the blood during bone resorption and then excreted in the urine[2)]. Urinary NTX corrected for dilution by urinary creatinine (NTX/Cre) is nearly four times higher than normal NTX/Cre in patients with osteopenia/osteoporosis[3)].

In Japan, several markers of bone metabolism have been approved for use to determine insurance coverage for patients diagnosed with osteoporosis. They are used as indices for selecting drugs used to treat osteoporosis as well as for evaluating and determining drug efficacy. Serum tartrate-resistant acid phosphatase 5b (TRACP-5b), which has less diurnal and day-to-day variation and is not affected by renal function, is often selected as a bone metabolism marker for outpatients[4,5)]. In contrast, urinary NTX, which is covered by insurance, is not frequently used in clinical practice because of its greater intra- and inter-day variability than those of TRACP-5b and the influence of renal function.

Ju *et al*. reported that diurnal and day-to-day variations of urinary NTX are 26.7% and 23.1%, respectively[6)]. It reaches a peak early in the morning and then gradually decreases to the lowest levels during the evening[6-9)]. The second-void morning urine is recommended because of its correlation with the mean over a 24-hr period[10)].

At present, serum TRACP-5b is the primary choice for determining the status of bone resorption; however, it requires an invasive collection procedure and can only be analyzed in a hospital. In contrast, urinary NTX can be collected noninvasively, yet is considered limited to the second-void morning urine because of the large diurnal variation. Therefore, this study investigated the diurnal and day-to-day variations of NTX/Cre and NTX aimed at assessing their availability for diagnosing bone status.

METHODS

Participants

Seven healthy adult men were recruited for this study. Exclusion criteria were: (1) having cancers/tumors, diabetes mellitus, hypertension, cardiac disease, respiratory disease, or hepatic disease; (2) undergoing dialysis; (3) using immunosuppressants; or (4) using agents that affect bone metabolism or urinary creatinine excretion. Prior to this study, participants were given an oral and written explanation of the test and consent was obtained via a consent form. All seven men participated in the study. The characteristics of the participants are shown in Table 1. One participant (ID: G) did not collect the second-void urine after waking up on the second and third days.

This study was conducted at Oberlin University (Tokyo, Japan).

This study was approved by the Ethics Committee of Oberlin University (approved number: 19048) and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Received on March 24, 2023 and accepted on April 7, 2023

1) College of Health and Welfare, J.F. Oberlin University
3758, Tokiwa-machi, Machida, Tokyo 194-0294, Japan
2) Graduate School of Health and Sports Science, Juntendo University
1-1, Hiragagakuendai, Inzai, Chiba 279-1695 Chiba, Japan
Correspondence to: Shimpei Fujita
(e-mail: fujita_s@obirin.ac.jp)

ORCID ID:
Shimpei Fujita: 0000-0002-6930-4403
Yoshiko Suzuki: 0000-0003-4982-5591

Table 1: Characteristics of the participants

participant	Age (y)	Height (cm)	Body weight (kg)	BMI (kg/m^2)
A	34	171	62	21.2
B	33	178	65	20.5
C	35	176	70	22.6
D	47	181	72	22.0
E	38	165	80	29.4
F	42	172	65	22.0
G	41	170	78	27.0
mean	38.6	173.3	70.3	23.5
s.d.	5.1	5.4	6.8	3.3

s.d., standard deviation; BMI, body mass index

Table 2: Summary of the diurnal variation of urinary NTX concentrations

	Participant	n	mean	s.d.	s.e.m	min	Max	CV (%)
NTX/Cre	A	4	14.6	4.6	2.3	10.7	21.2	31.2
(nmolBCE/mmol・Cre)	B	5	22.3	9.3	4.1	13.4	36.3	41.5
	C	7	25.5	5.0	1.9	20.5	35.0	19.5
	D	9	35.1	11.6	3.9	22.2	61.8	33.0
	E	5	30.8	15.6	7.0	14.5	51.4	50.7
	F	5	9.9	1.1	0.5	8.6	11.6	11.6
	G	9	35.2	13.7	4.6	14.9	56.1	39.0
	mean	6.3	24.8	9.9	3.7	15.0	39.1	32.4
NTX	A	4	379.9	100.4	50.2	291.9	468.4	26.4
(nmolBCE/L)	B	5	246.6	131.2	58.7	79.6	437.8	53.2
	C	7	254.8	105.4	39.8	131.4	437.8	41.4
	D	9	292.6	118.3	39.4	159.1	519.7	40.4
	E	5	135.6	32.5	14.6	105.9	190.1	24.0
	F	5	225.4	52.7	23.6	165.4	307.9	23.4
	G	9	306.7	193.9	64.6	116.1	696.9	63.2
	mean	6.3	263.1	75.7	28.6	149.9	436.9	38.9

n, the number of samples from each participant; s.d., standard deviation; s.e.m., standard error of means; CV, coefficient of variation; BCE, bone collagen equivalents; NTX, crosslinked N-telopeptides; NTX/Cre, Urinary NTX adjusted by creatinine

Study design

This study was a cross-sectional study to assess the diurnal and day-to-day variations of urinary NTX. The number of the participants was set at 8, as in the report by Popp-Snijders *et al*[11], but only 7 subjects participated in the study.

Participants collected their urine for 3 days consecutively. On the first day, they collected all their urine beginning with their first void after waking up and recorded their void times. On the second and third days, the second-void urine after waking up was collected. Approximately 5 ml of urine was collected in a Spitz tube. The sample was kept at 3℃-6℃ and analyzed within 72 hrs.

During the experiment, participants collected voluntary urine without being instructed to urinate in order to avoid bias from the examiner.

Analysis

Urinary type 1 collagen crosslinked N-telopeptide (u-NTX) and creatinine (Cre) were analyzed using an ELISA kit Osteomark (Alere Medical, Chiba, Japan) and a Bio Majesty JCA-BM8060 automatic analyzer (Japan Electron Optics Laboratory Ltd., Tokyo, Japan) using the reagent CicaLiquid-S CRE (Kanto Chemical, Tokyo, Japan), respectively. Urinary NTX was expressed in terms of urine concentration (nmol bone collagen equivalents [nmol BCE/L]) and was adjusted based on creatinine (nmol BCE/mmol Cre). Analyses were conducted by Hoken Kagaku, Inc. (Yokohama, Japan)

Statistical analysis

To investigate the diurnal variations of NTX/Cre and NTX, the sampling time of the urine was divided into six ranges of 4 hrs each beginning at 06:00 hrs. As only one specimen was collected during 02:00 -06:00 hrs, we combined the two nighttime ranges, 22:00-02:00 and 02:00-06:00 hrs, and classified the sampling times into a total of five categories. The number of samples in each time category was 8, 9, 9, 7, and 11 for 06:00-10:00, 10:00-14:00, 14:00-18:00, 18:00-22:00, and 22:00-06:00 hrs, respectively. Diurnal variation was examined using generalized estimating equation. The model included the subject's identification as a subject variable, sampling times (five ranges) and the sampling order as within-subject variables, and both models with or without the interaction "sampling time x sampling order" were examined. In both NTX/Cre and NTX, the model including the interaction was adopted because of the smaller quasi-likelihood under the independence model criterion (QIC).

Diurnal and daily variations of NTX and NTX/Cre were analyzed using analysis of variance (ANOVA). SPSS ver. 19 (IBM Japan, Tokyo, Japan) was used for the analyses. The level of significance was set at $p < 0.05$.

RESULTS

Diurnal variation

The mean coefficient of variance of NTX/Cre and NTX was 32.4% (range: 11.6% 0.7%) and 38.9% (range: 23.4% 3.2%), respectively (Table 2).

Among the individual participants, the coefficient of variation decreased with the Cre correction in five out of the seven participants (ID: B, C, D, F, and G), but increased in the remaining two participants (ID: A and E).

Table 3: Analysis of variance table of diurnal urinary NTX and creatinine concentrations

		SS	df	MS	F value	p	
NTX (Cre adjusted)	Between group	3472	6	579	5.201	< 0.001	***
	Within group	4117	37	111			
	sum	7589	43				
NTX	Between group	169115	6	28186	1.756	0.136	
	Within group	594003	37	16054			
	sum	763118	43				

SS, sum of squares; df, degrees of freedom; MS, mean squares; NTX, crosslinked N-telopeptides; NTX/Cre, Urinary NTX adjusted by creatinine

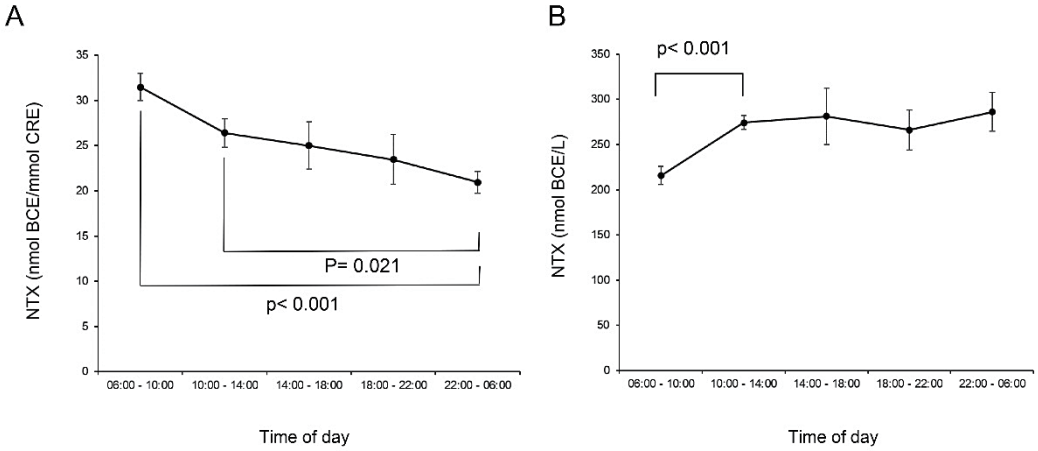

Figure 1: Diurnal variations of urinary NTX concentrations
(A) Creatinine adjusted NTX (nmol BCE/mmol Cre) and (B) NTX (nmol BCE/L)

Table 4: Summary of the daily variation of second-void urinary NTX concentrations

	Participant	n	mean	s.d.	s.e.m	min	max	CV (%)
NTX/Cre	A	3	18.9	5.7	3.3	13.2	24.5	29.9
(nmolBCE/mmol・Cre)	B	3	22.9	11.7	6.7	15.1	36.3	51.1
	C	3	30.5	5.0	2.9	25.1	35.0	16.4
	D	3	25.6	1.9	1.1	23.6	27.3	7.3
	E	3	25.6	4.0	2.3	21.7	29.6	15.5
	F	3	13.5	3.3	1.9	9.7	15.8	24.6
	mean	6	22.8	5.9	2.4	18.1	28.1	24.1
NTX	A	3	320.6	141.1	81.4	187.4	468.4	44.0
(nmolBCE/L)	B	3	254.6	156.5	90.4	79.6	381.2	61.5
	C	3	311.9	157.4	90.8	143.1	454.5	50.4
	D	3	321.0	122.4	70.7	186.8	426.6	38.1
	E	3	158.2	25.2	14.6	133.5	183.9	15.9
	F	3	187.8	29.3	16.9	165.4	221.0	15.6
	mean	6	259.0	71.7	29.3	149.3	355.9	37.6

n, the number of samples from each participant; s.d, standard deviation; s.e.m., standard error of means; CV, coefficient of variation; BCE, bone collagen equivalents; NTX, crosslinked N-telopeptides; NTX/Cre, Urinary NTX adjusted by creatinine

Table 5: Analysis of variance of second-void urine for NTX and creatinine concentrations (day-to-day variation)

		SS	df	MS	F value	p
NTX (Cre adjusted)	Between group	529	5	106	2.837	0.064
	Within group	447	12	37		
	sum	976	17			
NTX	Between group	77088	5	15418	1.080	0.419
	Within group	171290	12	14274		
	sum	248378	17			

SS, sum of squares; df, degree of freedom; MS, mean squares; NTX, crosslinked N-telopeptides; NTX/Cre, Urinary NTX adjusted by creatinine

The ANOVA showed that NTX concentrations were not significantly different among the participants, but Cre correction reduced the mean square (MS) of the within-group relative to between-group, and a significant difference among the participants was observed. ($p < 0.001$, Table 3).

Participants urinated four to nine times within 24 hrs after waking. When diurnal variation was analyzed using generalized linear estimating equations by dividing urination time into five segments, NTX/Cre was highest during 06:00 0:00 hrs (31.5 ± 1.5 nmol BCE/mmol Cre) and then consistently decreased thereafter; the nighttime period (22:00-06:00 hrs) was significantly lower than 06:00-10:00 ($p < 0.001$) and 10:00-14:00 hrs ($p = 0.021$). NTX concentrations were lowest during 06:00-10:00 hrs (215.7 ± 10.1 nmol BCE/L), increased to 274.4 ± 7.5 nmol BCE/L during 10:00-14:00 hrs ($p < 0.001$), and then remained nearly constant thereafter (Figure 1).

Day-to-day variation

The 3-day mean day-to-day variation of NTX/Cre and NTX regarding the second-void urine was 24.1% (range: 7.3% 1.1%) and 37.6% (range: 15.6% 1.5%), respectively (Table 4). With the exception of participant F, all the participants had a smaller coefficient of variation (CV) after Cre correction.

ANOVA results for day-to-day variation showed no significance among the participants for both NTX/Cre and NTX (Table 5).

DISCUSSION

Diurnal variation of NTX/Cre has been reported to peak early in the morning and to be at its lowest around 20:00 hrs in adult women[7,9], men and women over 65 years of age[8], and healthy adult men and women (25-44 years of age)[6]. NTX, unadjusted by Cre, was reported to be nearly constant during the day in two boys and five girls with an average age of 12.2 yrs (range: 10.4-14.4 yrs)[12]. In the present study, NTX/Cre peaked during 06:00-10:00 hrs and gradually decreased thereafter, whereas NTX remained at a constant value after 10:00 hrs. Therefore, urinary NTX remained almost constant during the day, and NTX/Cre peaked early in the morning and then gradually decreasing thereafter in Japanese adult males (33-47 yrs of age), with these results in accordance with those reported previously regarding other ethnic and aged/sex groups.

The magnitude of the diurnal variation in elderly women was reported to be 24.12 (nmol BCE/mmol Cre) with a diurnal mean of 46.94 (nmol BCE/mmol Cre)[8]. In elderly men, the diurnal variation was 10.31 (nmol BCE/mmol Cre) with a mean of 27.35 (nmol BCE/mmol Cre)[8]. Another study reported that the diurnal variation was 26.7% relative to the mean in healthy men and women[6]. The present study showed that the mean CV (s.d. /mean) was 32.4% for NTX/Cre and 38.9% for NTX. Therefore, despite diurnal variation, approximately 95% (mean ± 2 s.d.) of the measured values in urine are expected to be within ± 64.8% for NTX/Cre and ± 77.8% for NTX from the diurnal means with the assumption that the concentrations is normally distributed.

As urinary NTX concentration is affected by renal function, this value was adjusted by the creatinine concentration. In this study, the Cre adjustment was confirmed to reduce the intraindividual variation to make interindividual differences significant. Meanwhile, the mean CVs for diurnal variation were 32.4% for NTX/Cre and 38.9% for NTX. In addition, NTX/Cre gradually decreased from early morning to evening, whereas NTX was fairly constant during the day. As previously discussed, > 95% of the voluntary urine measurements can be expected to be within two-fold of the diurnal mean. Therefore, if voluntary urine should be analyzed, NTX could be more useful when compared with NTX/Cre as it has less systematic diurnal error.

The day-to day mean CV for NTX/Cre was 12.3% for two consecutive days and 14.2% for 2 months in postmenopausal women with a mean age of 63.6 yrs, without hormone replacement therapy[13]. The intraindividual variation of NTX/Cre was also reported to be 10%-24% in healthy men, 20% in premenopausal women, and 18%-20% in early menopausal women[11]. The diurnal variation of NTX/Cre was also reported to be 17.5%-19.0% regarding the second-void urine of healthy adult males (50.8 ± 18.7 yrs) [14] and 23.1% in the first-void urine of healthy adult males and females (25-44 yrs)[6]. The amount of NTX produced per hour is affected by environmental factors such as sleep-wake times, light-dark cycles, posture, and meal timing, which affects the overall concentration and timing of the peak[15]. Therefore, the day-to-day variation was greater in healthy adult males than females, especially for the elderly. In the present study, the mean CV of NTX/Cre in the second-void urine over 3 days was 24.1%, which was almost the same level as that reported for healthy men[11]. The CV for NTX was 37.6%. Therefore, approximately > 95% (mean ± 2 s.d.) of the measured values were expected to be within two-fold of the day-to-day mean for both NTX/Cre and NTX.

The present study showed that the concentrations of NTX/Cre and NTX in voluntary urine were within two-fold of the diurnal means in both NTX/Cre and NTX. The intraindividual day-to-day variations were also within two-fold of the mean in both NTX/Cre and NTX. Regarding osteopenia/osteoporosis, the mean NTX/Cre has been reported to be four times higher than in individuals without accelerated bone loss[3]. Therefore, the levels of NTX/Cre and possibly NTX in voluntary urine could be used for monitoring bone status.

This study has some limitations. We discussed the possibility of using NTX/Cre and NTX in voluntary urine for monitoring bone status, but we did not compare the urinary concentrations in patients with osteopenia/osteoporosis to those of healthy individuals. Moreover, although the participants in this study were healthy active adult males (age: 33-47 yrs), future studies should include other age/sex group.

CONCLUSION

This study demonstrated that levels of NTX/Cre and NTX in voluntary urine are expected to be within approximately two-fold of the diurnal means. Therefore, the levels of NTX/Cre and possibly NTX in voluntary urine could be used for monitoring bone status.

ACKNOWLEDGEMENTS

The authors thank all the participants.

ETHICAL STATEMENT

Ethical approval was obtained from the Ethics Committee of Oberlin University (approved number: 19048).

INFORMED CONSENT

Written informed consent was obtained from all participants.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

This study was funded by ROHTO Pharmaceutical Co., Ltd (Osaka, Japan).

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