Journal of the American College of Cardiology
© 2014 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 63, No. 17, 2014
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2014.01.040

Biomarkers

Circulating Cardiac Troponin T Exhibits a Diurnal Rhythm

Lieke J. J. Klinkenberg, MSc,* Jan-Willem van Dijk, MSc,† Frans E. S. Tan, PhD,‡ Luc J. C. van Loon, PhD,† Marja P. van Dieijen-Visser, PhD,* Steven J. R. Meex, PhD*

Maastricht, the Netherlands

Objectives The goal of this study was to test the unverified assumption that chronically elevated cardiac troponin T (cTnT) levels fluctuate randomly around a homeostatic set point.

Background The introduction of high-sensitivity cardiac troponin (cTn) assays has improved sensitivity for acute myocardial infarction (AMI). However, many patients with a single positive cTn test result do not have AMI. Therefore, the diagnosis of AMI relies strongly on serial testing and interpretation of cTn kinetics. Essential in this regard is a profound understanding of the biological variation of cTn.

Methods Two studies were conducted to assess biological cTnT variation and to investigate the presence of a diurnal rhythm of cTnT. Study 1 comprised 23 male subjects with type 2 diabetes, with no acute cardiovascular disease. Serial venous blood samples were drawn over an 11-h period (8:30 AM to 7:30 PM). In study 2, the presence of a diurnal cTnT rhythm was investigated by hourly sampling of 7 subjects from study 1 over 25 h.

Results In study 1, we observed a gradual decrease in cTnT concentrations during the day (24 ± 2%). This decrease was present in all participants and was most prominent in subjects with the highest baseline cTnT values (Pearson's R 0.93). Diurnal variation of cTnT, as assessed in study 2, was characterized by peak concentrations during morning hours (8:30 AM, 17.1 ± 2.9 ng/l), gradually decreasing values during daytime (8:30 PM, 11.9 ± 1.6 ng/l), and rising concentrations during nighttime (8:30 AM the next day, 16.9 ± 2.8 ng/l).

Conclusions A diurnal cTnT rhythm substantiates the recommendation that all dynamic changes in cTnT should be interpreted in relation to the clinical presentation. Epidemiological studies and risk-stratification protocols with the use of cTnT may benefit from standardized sampling times. (Exercise and Glycemic Control in Type 2 Diabetes; NCT00945165) (J Am Coll Cardiol 2014;63:1788–95) © 2014 by the American College of Cardiology Foundation

Cardiac troponin (cTn) is a sensitive marker of cardiac injury and is the preferred biomarker for the diagnosis or exclusion of acute myocardial infarction (AMI). Since the introduction of the high-sensitivity cTn assays, detectable cTn levels are no longer restricted to cardiac patients but can also be reliably assessed in apparently healthy subjects. Persistently elevated cTn concentrations near or above the 99th percentile of a healthy reference population are frequently observed in patients with stable coronary artery disease, elderly subjects, and patients with various chronic diseases such as type 2 diabetes (1–4). Some have suggested that a higher decision limit (3-fold the 99th percentile limit) might be advisable for diagnosing AMI in these patient groups (3,5).

Regardless of the cutoff value used, the critical tool for discriminating between AMI-induced cTn release and relatively stable cTn elevations in chronic diseases is serial testing and the assessment of kinetic changes in cTn. Guidelines advocate a rise and/or fall of cTn in patients with evidence of myocardial ischemia, with at least 1 cTn value exceeding the 99th percentile of the reference population (6). It should be recognized, however, that there is no perfect diagnostic threshold, and the application of any change criteria thus far is a trade-off, directed by the need for a high sensitivity or a high specificity. Therefore, a profound understanding of the naturally occurring biological variation of cTn is essential when interpreting dynamic changes in serial testing. By

From the *Department of Clinical Chemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Center (MUMC), Maastricht, the Netherlands; †Department of Human Movement Sciences, School for Nutrition, Toxicology and Metabolism (NUTRIM), Maastricht University Medical Center (MUMC), Maastricht, the Netherlands; and the ‡Department of Methodology and Statistics, Maastricht University, Maastricht, the Netherlands. This study was supported by a research grant from Stichting De Weijerhorst (Maastricht, the Netherlands) to Dr. van Dieijen-Visser. The authors have reported that they have no relationships relevant to the contents of this paper to disclose. The first 2 authors contributed equally to this work, and the Departments of Clinical Chemistry and Human Movement Sciences, Maastricht University Medical Center, contributed equally as well.

Manuscript received October 11, 2013; revised manuscript received December 17, 2013, accepted January 22, 2014.

definition, biological variation is measured by serially sampling healthy subjects at regular intervals (7). Thus far, studies in healthy subjects have reported short-term (hourly) biological variation of cTn ranging from 3% to 48%, which translates into reference change values (RCVs) of 45% to 85% (8–11). This finding means that a difference in serial cTn measurements which exceeds this threshold is unlikely due to analytical and biological variation only and is therefore considered a "true" change at a p value <0.05. However, it should be noted that healthy subjects may not be truly representative of the typical patient who requires clinical evaluation for chest pain.

The present study comprised a highly standardized assessment of within-day, diurnal, and between-week biological variation of cardiac troponin T (cTnT) levels in subjects with increased odds of requiring hospital examination for chest pain.

Methods

To examine within-day, diurnal, and between-week variation in cTnT levels, 2 distinct studies were conducted. Both studies complied with the principles of the Declaration of Helsinki and were approved by the institutional review board and the ethics committee at Maastricht University Medical Center. All participants provided written informed consent.

Study 1: within-day and between-week variation. The study group consisted of 23 male subjects (body mass index [BMI] 30 ± 4 kg/m^2; mean age 63 ± 7 years) who were diagnosed with type 2 diabetes for a mean duration of 7 ± 5 years (Online Table 1). According to their BMI, 16 subjects were classified as overweight (BMI 25 to 30 kg/m^2) and 7 subjects as obese (>30 kg/m^2). Subjects were recruited by using advertisements in the local newspaper. Exclusion criteria were self-reported renal failure, liver disease, morbid obesity (BMI >40 kg/m^2), history of severe cardiovascular problems (AMI or stroke in the past year), hypertension (>160 mm Hg systolic blood pressure and/or >100 mm Hg diastolic blood pressure), and exogenous insulin therapy. This study is part of a more extensive project investigating glycemic control and lifestyle interventions (12).

Participants visited the laboratory by car or public transportation on 3 occasions (experiments A to C) in randomized order (Fig. 1). Subjects were asked to refrain from exhaustive physical labor and exercise training for 2 days before each test day. Experiment A estimated within-day cTnT variation under sedentary conditions. From 8:30 AM until 7:30 PM, subjects were restricted to a sedentary laboratory environment and spent the day seated in a chair or couch, while reading, talking, watching television, or working on a laptop. Participants received standardized breakfast, lunch, and dinner at 8:30 AM, 12:30 PM, and 5:00 PM. Blood samples (8 ml) were collected from an antecubital venous catheter 5 min before each meal, as well as 90 and 150 min after each meal, resulting in a total of 9 blood samples collected within 11 h.

Experiment B was conducted to investigate whether within-day cTnT variation was affected by light physical activity. The test day was identical to experiment A, except for the addition of 15 min of slow-paced walking (total distance 800 to 1,000 m, including 2 staircases) after each meal (at 9:15 AM, 1:15 PM, and 5:45 PM) to mimic a day with low-to-moderate levels of physical activity. Experiment C was conducted to examine between-week variation of cTnT. Before breakfast at 8:30 AM, a blood sample was collected. Between-week variation was calculated by using the morning samples (8:30 AM) of experiments A through C. For all subjects, experiments A through C were performed on the same day of the week, within a total study period of 15 to 29 days.

Abbreviations and Acronyms

AMI = acute myocardial infarction
BMI = body mass index
CI = confidence interval
CK = creatine kinase
cTn = cardiac troponin
cTnT = cardiac troponin T
CV_i = within-person biological coefficient of variation
eGFR = estimated glomerular filtration rate
IQR = interquartile range
RCV = reference change value

Study 2: diurnal variation. After finalizing study 1, additional experiments were conducted to investigate the hypothesis that circulating cTnT exhibits a diurnal rhythm. Seven subjects who also participated in study 1 were sampled every hour (8 ml) by using an antecubital venous catheter over a time span of 25 h (Fig. 1). Participants were restricted to a sedentary laboratory environment from 8:30 AM till 9:30 AM the next day with standardized meals consumed at 8:30 AM, 12:30 PM, and 5:00 PM (breakfast, lunch, and dinner, respectively). Subjects went to bed at 11:30 PM, and the lights were off at 11:35 PM until 7:00 AM. During the night, polyethylene coiled extension lines (Vygon, Ecouen, France) were used for blood sampling to prevent disturbance of participants' sleep.

Laboratory measurements. Blood samples were collected in ethylenediaminetetraacetic acid–containing tubes. Immediately upon collection, the blood samples were centrifuged, and plasma was stored at −80°C until analysis. Hematology parameters were analyzed immediately in the diurnal samples on a Sysmex XE-5000 analyzer (Sysmex Corporation, Kobe, Japan). cTnT was measured in duplicate by using a high-sensitivity cTnT assay (Roche Diagnostics, Indianapolis, Indiana) on the cobas 6000 analyzer (lot number 167650). The limits of blank and detection of the assay were 3 and 5 ng/l, respectively; the 99th percentile among healthy subjects is 14 ng/l (13). Creatinine, creatine kinase (CK), and albumin were measured on the cobas 6000 analyzer. The estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration formula (14).

Statistical analysis. Between-person, within-person biological (CV_i), and analytical coefficients of variation were calculated by using a balanced analysis of variance with a nested random design in 2 levels; 95% confidence intervals

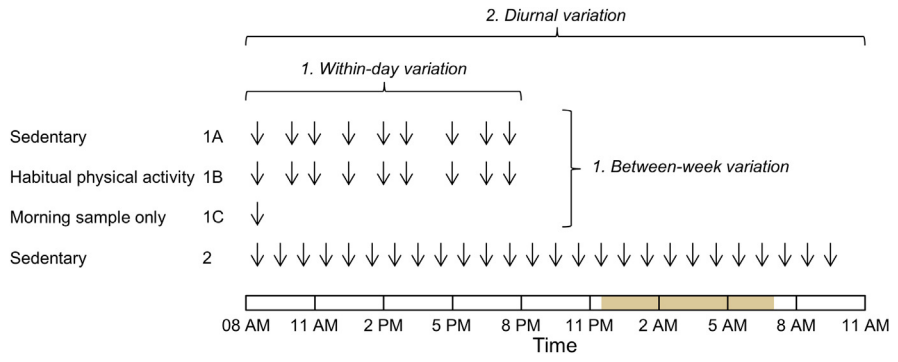

Figure 1 **Overview of Within-Day, Diurnal, and Between-Week Collection of Blood Samples**

Within-day variation was estimated under sedentary (1A Sedentary) or habitual physical activity (1B Habitual physical activity) conditions in 23 patients over an 11-h period. Between-week variation was estimated by using the 3 morning samples (8:30 AM) collected at the same day of the week within a total study period of 15 to 29 days (1A, 1B, and 1C morning sample only). Diurnal changes in cardiac troponin T were monitored in 7 subjects under sedentary conditions (2 Sedentary). Participants slept in supine position between 11:30 PM and 7:00 AM **(shaded area)**. All blood samples (indicated by **arrows**) were collected at standardized clock hours.

(CIs) of these estimated variance components were calculated according to the method of Burdick and Graybill (15). Outliers in terms of the CV_i were identified by using Cochran's C test. RCVs (z-score of 1.96) and the index of individuality were calculated according to the method of Fraser and Harris (16). RCVs were also evaluated after a log-normal transformation (17). The Wilcoxon signed rank test was applied to compare cTnT concentrations measured at 8:30 AM and 7:30 PM. Hemoglobin and hematocrit values were used to quantify possible plasma volume changes due to changes in hydration status and/or posture during the diurnal variation study (18).

Diurnal cTnT changes were analyzed by fitting individual data to a cosine curve with a 24-h period by using the method of cosinor-rhythmometry as described by Nelson et al. (19). Briefly, the cosinor model is described as: $Z(t) = M + A \cdot \cos(\omega t + \varphi) + e(t)$, where $Z(t)$ represents the measured cTnT concentration at a given time (t), M the mesor (average value of a cosine curve fitted to the data), A the amplitude (one-half the difference between the peak and the nadir value), ω the angular frequency (degrees per unit time, with 360° representing a complete cycle), φ the acrophase (timing of maximal value in degrees), and e(t) the error between the cosine model and the measurement. Rearrangement of the model by using trigonometric

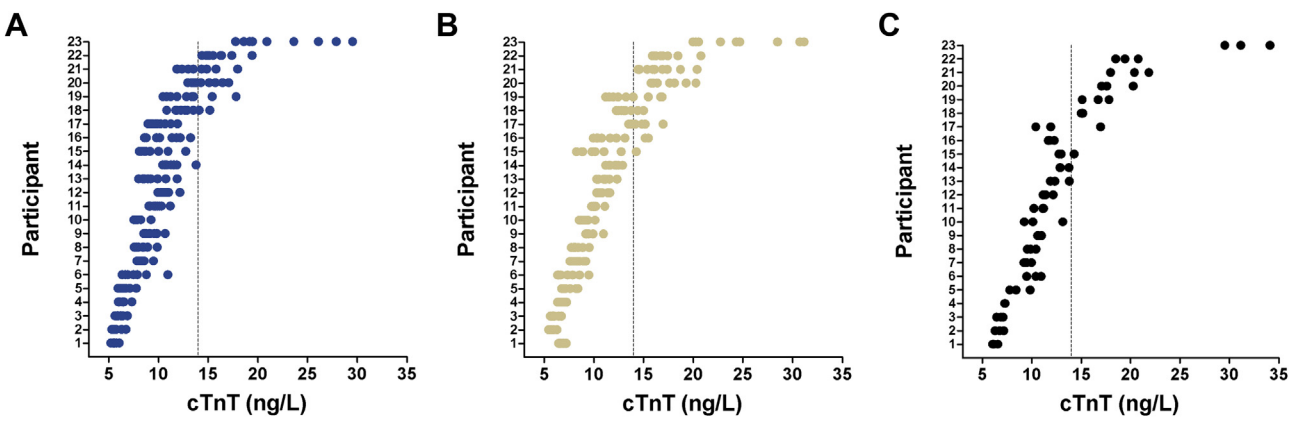

Figure 2 **Within-Day and Between-Week Distribution of Cardiac Troponin T**

(A) Within-day distribution of cardiac troponin T (cTnT) (8:30 AM to 7:30 PM) in 23 subjects under sedentary conditions. **(B)** Within-day distribution of cTnT (8:30 AM to 7:30 PM) in 23 subjects under standardized habitual physical activity. **(C)** Between-week distribution of cTnT assessed by using the 3 morning samples (8:30 AM). **Circles** represent mean cTnT values from each sampling point for a given individual. The 99th percentile (14 ng/l) is indicated by the **dotted line**. Subjects were ranked on the basis of their visual range of cTnT concentration during the sedentary test day: generally, high subject numbers correspond to a wider range in cTnT concentration. The ranking used for Figure 2A is extended to Figures 2B and 2C and Online Figure 1, allowing direct comparisons between figures throughout the paper.

Table 1 Within-Day and Between-Week Variation in Cardiac Troponin T Levels

	Within-Day		
	Sedentary	Habitual Physical Activity	Between-Week
Samples/subjects	207/23	207/23	69/23
Mean concentration, ng/l	10.3	11.3	12.8
Variance components			
CV_G	38.4 (29.5–54.5)	40.4 (31.1–57.4)	43.8 (33.6–62.2)
CV_I	14.2 (12.9–15.9)	12.7 (11.5–14.2)	9.7 (8.1–12.3)
CV_A*	2.0 (1.8-2.2)	1.9 (1.8–2.1)	1.8 (1.5–2.1)
RCV†			
Normal	39.8	35.7	27.4
Log-normal	48.6, −32.7	42.7, −29.9	31.4, −23.9
Index of individuality	0.37	0.32	0.23

Values are N/n, % (95% CI), or %. *On the basis of duplicate measurements. †On the basis of a *z*-score of 1.96.
CI = confidence interval; CV_A = analytical coefficient of variation; CV_G = between-person coefficient of variation; CV_I = within-person biological coefficient of variation; RCV = reference change value.

identities produces a linear model in the coefficients. Evidence of a diurnal rhythm was indicated by a significant cosine model fit ($p < 0.05$). A mean cosine model was computed by using the rearranged cosine models in a linear mixed model with a random subject term. All statistical calculations were performed by using SPSS version 20 (IBM SPSS Statistics, IBM Corporation, Armonk, New York).

Results

Within-day biological variation of cTnT is larger than between-week biological variation. The within-day distribution of cTnT (8:30 AM to 7:30 PM) in 23 subjects under sedentary conditions is shown in Figure 2A. All values (range 5.2 to 29.5 ng/l) were above the detection limit of the assay, and 6 subjects had at least 1 measurement above the 99th percentile of 14 ng/l. To verify the results obtained under completely sedentary conditions, the same subjects were also measured during a second test day at a 1-week interval that included habitual physical activity (see Methods section). Results were highly comparable to the sedentary test day: cTnT concentrations ranged from 5.4 to 31.2 ng/l, and 9 subjects had at least 1 measurement above the 99th percentile (Fig. 2B). To estimate between-week variability of cTnT, an additional morning sample (8:30 AM) was collected at another 1-week interval. cTnT concentrations at 8:30 AM on 3 separate visits were between 6.0 and 34.1 ng/l (Fig. 2C). Eight subjects had at least one 8:30 AM measurement above the 99th percentile.

Table 1 lists the within-day and between-week cTnT variation components and their 95% CIs. The power of this study to detect a CV_i different from 0 was >0.8, taking into account the number of subjects, samples, and replicates and the observed ratio between the analytical variation and the biological variation (20). Within-day biological variability of cTnT was not affected by habitual physical activity (CV_i 14.2% [95% CI: 12.9% to 15.9%]) under sedentary conditions compared with 12.7% (95% CI: 11.5% to 14.2%) under habitual physical activity. Surprisingly, the within-day biological variation of cTnT under sedentary conditions was significantly higher than the between-week variation, as indicated by nonoverlapping 95% CIs (CV_i 14.2% [95% CI: 12.9% to 15.9%] vs. CV_i 9.7% [95% CI: 8.1% to 12.3%]). A similar trend was present between within-day biological variation of cTnT under habitual physical activity and between-week biological variation. Identification and exclusion of subjects who were considered outliers in terms of their within-person biological variation provided similar results (data not shown). We further examined the individual pattern of cTnT during the day to gain additional insight in the relatively high within-day biological variation of cTnT.

cTnT concentrations fluctuate nonrandomly and exhibit a gradual decrease during the day. cTnT concentrations exhibited a gradual decrease during the sedentary test day, with the highest concentration at 8:30 AM (median 11.8 ng/l [interquartile range [IQR]: 9.3 to 15.2 ng/l) and the lowest concentration at the end of the test day (7:30 PM; median 8.6 ng/l [IQR: 6.7 to 10.8 ng/l]; $p < 0.001$) (mean cTnT decrease of 24%) (Fig. 3A). A similar decrease has been previously reported for CK levels under constant resting conditions, and this decrease could be reversed to stable concentrations when light physical activity was introduced during the day (21). To investigate whether the same mechanism underlies the continuous decrease of cTnT, we examined the cTnT pattern during the second test day, which included 3 short walking sessions. Figure 3A shows that the decline in cTnT was only marginally affected by the 3 walking sessions during the day: a median of 11.4 ng/l (IQR: 9.2 to 16.7 ng/l) at 8:30 AM and a median of 9.8 ng/l (IQR: 6.8 to 12.3 ng/l) at 7:30 PM ($p < 0.001$) (mean cTnT decrease of 18%). In contrast, the gradually decreasing pattern of CK observed under sedentary conditions was completely reversed by habitual physical activity (Fig. 3B), consistent with previous observations (21). The decreasing pattern of cTnT was an evident phenomenon in all subjects, and it was most prominent in subjects with the highest basal cTnT levels (Fig. 3C, Online Fig. 1).

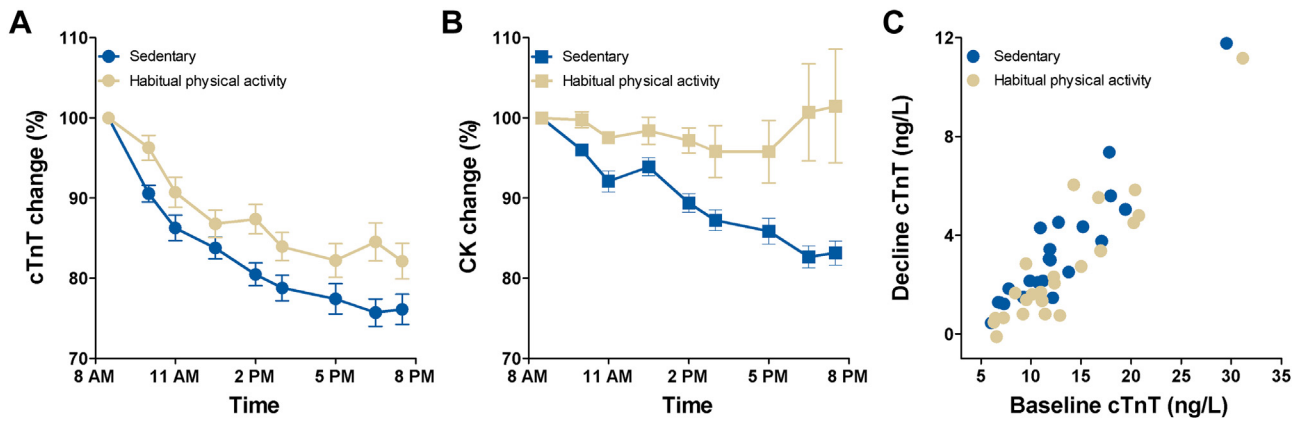

Figure 3 Cardiac troponin T Exhibits a Gradual Decrease During the Day, Which Is Not Affected by Habitual Physical Activity

(A) Cardiac troponin T (cTnT) change from baseline value under sedentary and habitual physical activity conditions (mean ± SEM). **(B)** Creatine kinase (CK) change from baseline value under sedentary conditions and habitual physical activity (mean ± SEM). **(C)** Correlation between baseline cTnT value and magnitude of cTnT decrease under sedentary conditions and habitual physical activity (Pearson's R 0.93 and 0.91; both $p < 0.001$).

The kinetics of cTnT are not due to changes in kidney function. To verify that the decreasing cTnT pattern was not related to changes in kidney function or plasma volume, we measured the concentrations of creatinine and albumin during both test days. No time-of-day changes in eGFR or albumin were observed under sedentary conditions or habitual physical activity (Online Fig. 2).

Circulating cTnT exhibits a diurnal rhythm. To investigate whether the decrease in cTnT during daytime was followed by a rise during night hours, we collected blood samples from 7 subjects every hour over a time span of 25 h (from 8:30 AM to 9:30 AM the next day). In all participants, the cTnT decline during daytime was accompanied by an increase during night hours, with peak concentrations in the morning (Fig. 4A). The intraindividual difference between the peak and the nadir cTnT concentrations ranged from 3.4 to 11.8 ng/l. Correction for posture-induced changes in plasma volume (18) did not abrogate the diurnal rhythm (data not shown). Consistent with the sedentary test day, CK values decreased continuously throughout the 25-h period, whereas eGFR and albumin levels fluctuated randomly around the subject's homeostatic set point (data not shown). On the basis of this oscillation in cTnT, we fitted individual cTnT data to a cosine function to model the

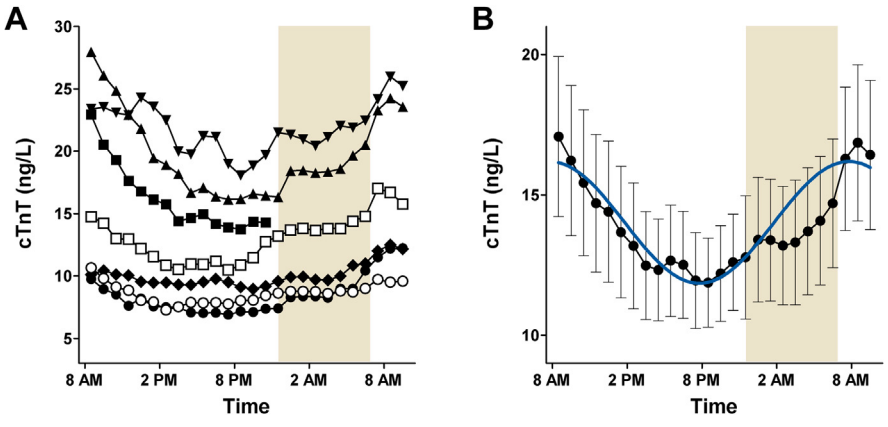

Figure 4 Cardiac Troponin T Exhibits a Diurnal Rhythm

(A) Individual 25-h cardiac troponin T (cTnT) profiles under sedentary conditions. Subjects slept in a supine position between 11:30 PM and 7:00 AM **(shaded area)**. Sampling in 1 subject **(closed squares)** was terminated at 10:30 PM due to technical problems with the intravenous cannula that hindered sampling throughout the night. **(B)** Mean ± SEM 25-h cTnT profile and fitted cosine curve.

Table 2 The Individual Diurnal Variation of Cardiac Troponin T Is Significant, as Described by Using a Cosine Curve

Subject*	Mesor (ng/l)	Amplitude (ng/l)	Acrophase (h)	R^2
▲	19.5	4.1	9:08 AM	0.80†
▼	21.7	2.3	9:18 AM	0.74†
■‡	17.5	4.0	6:37 AM	0.91†
□	12.9	2.4	6:03 AM	0.81†
○	8.6	0.9	6:30 AM	0.65†
◆	10.0	1.0	7:35 AM	0.55†
●	8.4	1.7	7:16 AM	0.63†

*Subject symbols correspond to the symbols used in Figure 4. †$p < 0.001$. ‡Sample collection until 10:30 PM (14 h).

variation in the observed cTnT concentration as a function of time. The fitted cosine function for each individual can be described by the mesor, amplitude, and acrophase (Table 2). Quantitatively, in all participants, the diurnal cTnT variation was significantly described by the fitted cosine curve, as indicated by the "goodness-of-fit" statistic (range R^2 0.55 to 0.91; all $p < 0.001$). In line with the individual cTnT curves, the group cosinor model exhibited a strong fit with the mean diurnal cTnT profile (Fig. 4B).

Discussion

The present study provides novel evidence that circulating cTnT levels exhibit a diurnal rhythm, characterized by peak concentrations during morning hours, gradually decreasing concentrations throughout daytime, and rising concentrations during nighttime. This diurnal oscillation is a general phenomenon but was most prominent in subjects with the highest baseline cTnT values.

cTnT and type 2 diabetes. We studied biological variability of cTnT in patients with type 2 diabetes without acute cardiovascular disease. Twenty percent of patients with type 2 diabetes have persistently elevated cTnT concentrations above the 99th percentile, in the absence of an acute cardiac event (22,23). Even in the pre-diabetic state (impaired glucose tolerance or impaired fasting glucose), cTnT elevations are frequently observed (23). Type 2 diabetes is a highly prevalent condition (25%) among patients who present at the emergency department with symptoms suggestive of AMI (5,24). A thorough understanding of the biological variation of cTnT levels near or above the 99th percentile is therefore a timely, and relatively unexplored, diagnostic issue.

Diurnal oscillation of cTnT: effect on reference change values. The extended timeline and highly standardized protocol for blood sampling formed the basis for the key finding of this study: the presence of a diurnal rhythm of cTnT. A remarkable consequence of the diurnal rhythm is that the within-day biological variation was significantly higher than the between-week biological variation, provided that the latter was measured in samples collected at the same hour of the day. Another important consequence of the diurnal rhythm relates to the violation of an important pre-condition when using RCV; that is, the assumption that biological variation is random. This point can be illustrated by using data from Figure 4. Under conditions of nonrandom variation (e.g., a diurnal rhythm), the calculated biological variation strongly depends on the time frame chosen. For example, when a time frame between 8:30 AM and 12:30 PM is considered, the calculated biological variation is more than twice the biological variation assessed in the time frame between 4:30 PM and 8:30 PM (CV_i 9.4% [95% CI: 7.4% to 12.7%] vs. CV_i 4.3% [95% CI: 3.1% to 6.2%], respectively). These differences translate directly to changes of similar magnitude for RCV. The use of different time frames across studies might have contributed to a relatively broad range of biological variations in cTnT, which have been previously assessed in healthy subjects and patients with stable cardiovascular disease (8–11,25,26). Altogether, the observation of a diurnal rhythm of cTnT, with the highest amplitude in patients with the highest baseline cTnT levels, may pose an additional challenge to diagnosing AMI in this patient group.

Circadian variation in cardiovascular physiology and pathology. The finding of a cardiac biomarker exhibiting rhythmic activity is in line with the described circadian variation in cardiovascular physiology, gene, and/or protein expression (27). For example, heart rate and blood pressure are lowest at nighttime and begin to rise before the time of awakening in anticipation of the demands of daytime activities, and they decrease again in the evening, anticipating sleep (28). In addition, it has been shown that enhanced amino acid incorporation into rat myocardial tissue takes place during the sleeping hours, with the least synthesis occurring 12 h later, indicating that myocardial growth and renewal processes occur especially during the night (29). In addition to physiology, cardiovascular pathology also exhibits diurnal variation. For example, the incidence of AMI displays a daily rhythmic pattern, with the highest incidence between 6:00 AM and 12:00 PM (30,31). Even infarct size exhibits a circadian dependence on the time-of-day onset of ischemia, with the largest infarct sizes observed in patients with symptom onset in the early morning hours (32,33).

Biological significance of a diurnal cTnT rhythm. The biological significance of a diurnal pattern in cTnT has yet to be established. Related to this matter is elucidation of the release mechanism of cTnT, as well as clearance from the bloodstream. Similar to findings in the published data (34), our eGFR findings were stable during the 25-h period, indicating that the fluctuations in cTnT could not be explained by intraindividual changes in eGFR. However, it is not clear if the fluctuations reflect a disease-specific mechanism (e.g., ischemia) or a physiological mechanism (e.g., protein turnover). Nevertheless, these findings provide support for the hypothesis that cTn can be applied to track the impact of a number of physiological and pathophysiological cardiovascular processes that are potentially unrelated to cardiac ischemia (35,36). Therefore, it would be of

interest to assess the rhythmicity of cTnT in healthy subjects. These studies will become feasible when even more sensitive assays become available, with higher precision in the lower range.

Study limitations. Because the study group consisted of overweight or obese type 2 diabetic patients treated with oral glucose-lowering medication, we cannot generalize these results directly to the cardio-healthy population. In addition, various factors specific for the studied group might have contributed to the diurnal rhythm of cTnT. The effect of antidiabetic medication on cTnT levels is unknown, although medication was stable for at least 3 months before patient inclusion and was continued as normal during the entire study period, including test days. We did not explicitly control for intermittent low oxygen saturation during the night (e.g., sleep apnea), which is often associated with subjects who are overweight or obese. In addition, the relationship between diurnal variation in heart rate and blood pressure and cTnT levels has not been explored. Finally, sample size, particularly for evaluation of diurnal variation ($n = 7$), was relatively low to exclude sample size error. However, the validity of the data is supported by the high consistency of the observations across all participants.

Conclusions

cTnT exhibited a diurnal rhythm in these study subjects with increased odds of requiring hospital examination for chest pain. The presence of a diurnal rhythm has direct consequences for epidemiological studies and stresses the necessity of interpreting all dynamic changes of cTnT in relation to the clinical presentation. Although the finding of a biomarker exhibiting circadian variation is in line with cardiovascular physiology and pathology, further studies are needed to understand the biological significance and to demonstrate the rhythmicity in various disease populations.

Reprint requests and correspondence to: Dr. Steven J. R. Meex, Department of Clinical Chemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Center (MUMC), P.O. Box 5800, 6202 AZ Maastricht, the Netherlands. E-mail: steven.meex@mumc.nl.

REFERENCES

1. Everett BM, Cook NR, Magnone MC, et al. Sensitive cardiac troponin T assay and the risk of incident cardiovascular disease in women with and without diabetes mellitus: the Women's Health Study. Circulation 2011;123:2811–8.
2. Omland T, de Lemos JA, Sabatine MS, et al. A sensitive cardiac troponin T assay in stable coronary artery disease. N Engl J Med 2009;361:2538–47.
3. Thygesen K, Mair J, Giannitsis E, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. Eur Heart J 2012;33:2252–7.
4. deFilippi CR, de Lemos JA, Christenson RH, et al. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. JAMA 2010;304:2494–502.
5. Mueller M, Biener M, Vafaie M, et al. Absolute and relative kinetic changes of high-sensitivity cardiac troponin T in acute coronary syndrome and in patients with increased troponin in the absence of acute coronary syndrome. Clin Chem 2012;58:209–18.
6. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. Eur Heart J 2012;33:2551–67.
7. Fraser CG. The nature of biological variation. In: Biological Variation: From Principles to Practice. Washington, DC: AACC Press, 2001:1–27.
8. Wu AH, Lu QA, Todd J, Moecks J, Wians F. Short- and long-term biological variation in cardiac troponin I measured with a high-sensitivity assay: implications for clinical practice. Clin Chem 2009;55:52–8.
9. Vasile VC, Saenger AK, Kroning JM, Jaffe AS. Biological and analytical variability of a novel high-sensitivity cardiac troponin T assay. Clin Chem 2010;56:1086–90.
10. Vasile VC, Saenger AK, Kroning JM, Klee GG, Jaffe AS. Biologic variation of a novel cardiac troponin I assay. Clin Chem 2011;57:1080–1.
11. Frankenstein L, Wu AH, Hallermayer K, Wians FH Jr., Giannitsis E, Katus HA. Biological variation and reference change value of high-sensitivity troponin T in healthy individuals during short and intermediate follow-up periods. Clin Chem 2011;57:1068–71.
12. van Dijk JW, Venema M, van Mechelen W, Stehouwer CD, Hartgens F, van Loon LJ. Effect of moderate-intensity exercise versus activities of daily living on 24-hour blood glucose homeostasis in male patients with type 2 diabetes. Diabetes Care 2013;36:3448–53.
13. Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin T assay. Clin Chem 2010;56:254–61.
14. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–12.
15. Burdick RK, Graybill FA. Confidence Intervals on Variance Components. New York, NY: Marcel Dekker, 1992.
16. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 1989;27:409–37.
17. Fokkema MR, Herrmann Z, Muskiet FA, Moecks J. Reference change values for brain natriuretic peptides revisited. Clin Chem 2006;52:1602–3.
18. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 1974;37:247–8.
19. Nelson W, Tong YL, Lee JK, Halberg F. Methods for cosinor-rhythmometry. Chronobiologia 1979;6:305–23.
20. Roraas T, Petersen PH, Sandberg S. Confidence intervals and power calculations for within-person biological variation: effect of analytical imprecision, number of replicates, number of samples, and number of individuals. Clin Chem 2012;58:1306–13.
21. Gutenbrunner C. Circadian variations of the serum creatine kinase level—a masking effect? Chronobiol Int 2000;17:583–90.
22. Hallen J, Johansen OE, Birkeland KI, et al. Determinants and prognostic implications of cardiac troponin T measured by a sensitive assay in type 2 diabetes mellitus. Cardiovasc Diabetol 2010;9:52.
23. Zheng J, Ye P, Luo L, Xiao W, Xu R, Wu H. Association between blood glucose levels and high-sensitivity cardiac troponin T in an overt cardiovascular disease-free community-based study. Diabetes Res Clin Pract 2012;97:139–45.
24. Reichlin T, Hochholzer W, Bassetti S, et al. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. N Engl J Med 2009;361:858–67.
25. Nordenskjold AM, Ahlstrom H, Eggers KM, et al. Short- and long-term individual variation in cardiac troponin in patients with stable coronary artery disease. Clin Chem 2013;59:401–9.
26. Scharnhorst V, Krasznai K, van't Veer M, Michels RH. Variation of cardiac troponin I and T measured with sensitive assays in emergency department patients with noncardiac chest pain. Clin Chem 2012;58:1208–14.
27. Martino TA, Sole MJ. Molecular time: an often overlooked dimension to cardiovascular disease. Circ Res 2009;105:1047–61.
28. Degaute JP, van de Borne P, Linkowski P, Van Cauter E. Quantitative analysis of the 24-hour blood pressure and heart rate patterns in young men. Hypertension 1991;18:199–210.
29. Rau E, Meyer DK. A diurnal rhythm of incorporation of L-[3H] leucine in myocardium of the rat. Recent Adv Stud Cardiac Struct Metab 1975;7:105–10.

30. Cohen MC, Rohtla KM, Lavery CE, Muller JE, Mittleman MA. Meta-analysis of the morning excess of acute myocardial infarction and sudden cardiac death. Am J Cardiol 1997;79:1512–6.
31. Muller JE, Stone PH, Turi ZG, et al. Circadian variation in the frequency of onset of acute myocardial infarction. N Engl J Med 1985; 313:1315–22.
32. Reiter R, Swingen C, Moore L, Henry TD, Traverse JH. Circadian dependence of infarct size and left ventricular function after ST elevation myocardial infarction. Circ Res 2012;110:105–10.
33. Fournier S, Eeckhout E, Mangiacapra F, et al. Circadian variations of ischemic burden among patients with myocardial infarction undergoing primary percutaneous coronary intervention. Am Heart J 2012;163: 208–13.
34. Larsson A, Akerstedt T, Hansson LO, Axelsson J. Circadian variability of cystatin C, creatinine, and glomerular filtration rate (GFR) in healthy men during normal sleep and after an acute shift of sleep. Chronobiol Int 2008;25:1047–61.
35. deFilippi CR, de Lemos JA, Tkaczuk AT, et al. Physical activity, change in biomarkers of myocardial stress and injury, and subsequent heart failure risk in older adults. J Am Coll Cardiol 2012;60:2539–47.
36. Lazzarino AI, Hamer M, Gaze D, Collinson P, Steptoe A. The association between cortisol response to mental stress and high-sensitivity cardiac troponin T plasma concentration in healthy adults. J Am Coll Cardiol 2013;62:1694–701.

Key words: biological variation ■ cardiac troponin ■ diurnal rhythm.

APPENDIX

For a supplemental table and figures, please see the online version of this article.