Diurnal Variation in the Mechanical and Neural Components of the Baroreflex

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Abstract—Diminished baroreflex sensitivity in the morning negatively influences morning coronary blood flow and blood pressure control in hypertensive patients. Our aim was to determine the contribution of the mechanical and neural components of the cardiac baroreflex to diurnal variation in blood pressure control. In 12 healthy participants, we used the modified Oxford method to quantify baroreflex sensitivity for rising (G_{up}) and falling (G_{down}) pressures in the morning (7:00 AM) and afternoon (4:00 PM). Beat-to-beat blood pressure, R-R intervals, and carotid artery diameter measurements were recorded. Integrated sensitivity was determined by plotting R-R intervals against systolic blood pressure. The mechanical component was carotid artery diameter plotted against systolic blood pressure, and the neural component was R-R intervals plotted against carotid artery diameter. Linear mixed models were used to compare the integrated, mechanical, and neural sensitivities between morning and afternoon. We found significant diurnal variation in integrated sensitivity, with an attenuated response in the morning ($G_{up}=13.0\pm0.6$; $G_{down}=6.3\pm0.4$ ms/mm Hg) when compared with the afternoon ($G_{up}=15.1\pm0.6$; $G_{down}=12.6\pm0.4$ ms/mm Hg). For rising pressures, the diminished integrated sensitivity in the morning was caused by a reduction in mechanical sensitivity, whereas for falling pressures it was caused by a reduction in neural sensitivity. Our findings explicate the mechanisms underlying diurnal variation in baroreflex function. Pharmacological and lifestyle interventions targeted specifically at the diminished component of the cardiac baroreflex in the morning may lead to better management of hypertension. (*Hypertension*. 2011;58:51-56.)

Key Words: circadian rhythms ■ baroreflex ■ blood pressure ■ heart rate ■ carotid artery

Blood pressure (BP) exhibits a circadian rhythm, with the lowest pressures occurring during nocturnal sleep and a rapid "morning surge" after waking.[1] BP is most reactive to a given level of physical activity in the morning,[2] and risk of orthostatic intolerance is also greater at this time compared with the afternoon.[3] In a number of prospective studies (reviewed by Kario[4]), the morning surge in BP has been found to be an independent risk factor for cardiovascular and cerebrovascular events, which also peak between 6:00 AM and 12:00 PM.[5,6] There is a wealth of evidence that BP control varies significantly over a 24-hour period, with studies in the frequency domain indicating the presence of a circadian rhythm in sympathovagal balance.[7,8] However, investigations directed at unraveling the autonomic mechanisms governing these time-of-day differences are lacking.

The arterial baroreflex control of vagal neural outflow is quantified by the responses in R-R interval to changes in systolic BP.[9] Previous studies have identified diurnal variation in baroreflex sensitivity in both normotensive and hypertensive people, with reduced sensitivities after waking compared with during sleep.[10–13] Moreover, Hossmann et al[14]

reported 24-hour variations in the cardiac baroreflex response to rising BP after *l*-noradrenaline infusions, with the highest sensitivities reported at 3:00 AM and 12:00 PM and the lowest at 3:00 PM and 9:00 AM. These studies, however, only document diurnal changes in integrated baroreflex sensitivity; it remains unknown whether the differences in integrated sensitivity are attributed to altered mechanical transduction of pressure into barosensory vessel stretch and/or the neural transduction of barosensory stretch into efferent autonomic outflow.[9] Clear delineation of these mechanisms is critical for targeting clinical interventions at the site responsible for the diminished baroreflex response.

The aim of this study is to determine the relative contribution of these individual components of the baroreflex arc to diurnal variation in BP control, as quantified by the modified Oxford method. Given that there is evidence to suggest that distensibility and compliance of the carotid artery display diurnal variation,[15,16] it seems reasonable to speculate that circadian rhythms in these vascular properties observed in steady-state conditions may translate into time-of-day differences in barosensory vessel responsiveness to

changes in BP. Therefore, we hypothesized that differences in integrated baroreflex sensitivity in the morning versus afternoon are attributed to time-of-day effects on the mechanical component.

Methods

Participants

Based on the information presented in Tables 1 (healthy subjects) and 2 (heart diseased patients) of La Rovere et al,[17] we selected a 20% change in baroreflex sensitivity as being the minimum clinically significant value. Using data from our own laboratory, analyzed with the linear mixed modeling approach,[18] we have found trial-to-trial coefficients of variation of ≈14% (SD of differences: 19.8%) for integrated, mechanical, and neural sensitivities. Using the N-Query software, we estimated that a sample size of 10 would have 80% power to detect a change in sensitivity of 20%. Twelve healthy participants (7 men and 5 women) were recruited for the study to ensure high-quality data from a minimum of 10 individuals. All of the participants gave written informed consent. The mean±SD age was 24.7±4.0 years (range: 20 to 33 years), and body mass index was 22.3±2.3 kg/m². All of the participants abstained from caffeine and exercise on the day of the study, and dietary intake was controlled to be consistent before testing sessions. Individuals on regular medication or with a known history of respiratory, cardiovascular, or endocrine disease were excluded from participating. Ethical approval was obtained from the New Zealand Central Regional Ethics Committee, and the study conformed to the Declaration of Helsinki.

Measurements

Beat-to-beat BP via photoplethysmography (Finometer MIDI, Finapres Medical Systems, Arnhem, The Netherlands) and ECG (ECG lead CM5, Corometrics Neo-Trak 502) were recorded noninvasively. To account for potential drift, finger BP measures were verified at the brachial artery in the contralateral arm by sphygmomanometry. These measures were acquired continuously via an analog-to-digital converter (Powerlab/16SP ML795; ADInstruments, Colorado Springs, CO) at 200 Hz per channel. Analysis was performed offline using the arterial BP and ECG waveforms to determine the timing of the R waves and beat-to-beat values for systolic, diastolic, and mean arterial pressures. Ultrasound imaging (Terason t3000, Burlington, MA) was used to measure beat-to-beat carotid artery diameter. A longitudinal section of the left carotid artery <2 cm proximal to the bifurcation was imaged and recorded (Camtasia Studio, TechSmith Co, Ltd, Okemos, MI) for offline analysis using custom edge tracking software as has been described previously.[19] All of the offline data processing was performed using custom written software in LabView 8.2 (National Instruments, Austin, TX) on a Macintosh 2.26 GHz MacBook Pro computer.

Experimental Protocol

All of the participants were studied in the supine position in a temperature-controlled laboratory (22°C to 23°C). A venous cannula was inserted into the right antecubital vein and, after a 15-minute stabilization period, baseline data were recorded for 5 minutes. Participants then underwent baroreflex testing using the modified Oxford method technique. Oxford tests were repeated until a valid trial was completed, that is, the drop and rise in BP were >15 mm Hg relative to baseline levels. Participants completed a morning (7:00 AM) and an afternoon (4:00 PM) trial, which were separated by a minimum of 48 hours and completed in a counterbalanced fashion. For the morning trial, a standardized carbohydrate meal was consumed at 5:00 AM, and for the afternoon trial 2 identical meals were consumed at 5:00 AM and 2:00 PM to keep dietary intake as consistent as possible between testing sessions.
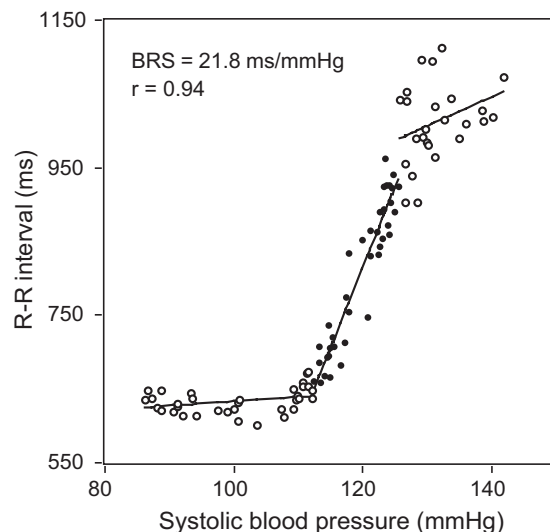
Figure 1. Piecewise regression model for elimination of threshold and saturation regions of the integrated baroreflex response to rising pressures. ○, The threshold and saturation regions; ●, the linear portion of the baroreflex sensitivity (BRS).

Baroreflex Sensitivity

The modified Oxford method involves sequential intravenous bolus injections of 50 to 250 μg of sodium nitroprusside (SNP) followed by 150 to 300 μg of phenylephrine hydrochloride (PE), as described previously.[9,20] Once the recording of hemodynamic measurements had begun, BP was allowed to stabilize, after which the injection of SNP was administered. This was followed 60 seconds later by the injection of PE. Recording ceased when systolic BP began to plateau after the rise after the PE injection. Oxford trials, therefore, typically lasted 120 to 180 seconds. Doses given for SNP and PE were typically 150 and 250 μg, respectively, although this was adjusted if an insufficient BP perturbation was achieved (systolic BP change <15 mm Hg). Therefore, the total number of injections ranged from 2 to 4 per trial. To account for known baroreflex delays, systolic BP values were matched to either the concurrent heartbeat for R-R intervals >800 ms or a 1 beat delay for shorter heart periods (typically between 500 and 800 ms).[21] Baroreflex sensitivities were calculated separately for SNP and PE injections to identify the sensitivity against falling (G_{down}) and rising (G_{up}) BPs.

Integrated sensitivity was determined by plotting the R-R interval–systolic BP relationship, which for G_{down} began at the onset of the systolic BP decrease after the SNP bolus injection and ended when systolic BP reached its nadir. For G_{up} the section of data selected began at the nadir in systolic BP and ended when pressure peaked after the bolus injection of PE. To identify and remove the saturation and threshold regions, a piecewise linear regression algorithm was applied to the raw data points to statistically identify breakpoints that occur at the upper and lower ends of the data set (Figure 1).[22] After this, respiratory-related fluctuations in R-R interval and systolic BP were accounted for by averaging R-R intervals across 2-mm Hg bins.[20,23] Although there is evidence for no effect of binning on the estimation of baroreflex sensitivity,[24] it is the standard treatment to bin data in this manner, and, therefore, our data are comparable to previous studies.

The mechanical and neural components of the baroreflex sensitivity were calculated for both G_{up} and G_{down}, with exclusion of the same threshold and saturation regions removed for integrated sensitivity. For the mechanical component, carotid diameter measurements were plotted against systolic BP, and for the neural component, R-R intervals were plotted against carotid diameter.[9]

Statistics

The researcher who calculated the baroreflex sensitivities was not blinded to the trial within which each data set was collected.

Table 1. Summary of Baseline Cardiovascular Variables at Both Times of Day (n=12)

Variable	Morning	Afternoon
Heart rate, bpm	68.4±12.1	67.2±13.7
Systolic blood pressure, mm Hg	126.3±16.7	130.3±22.3
Diastolic blood pressure, mm Hg	61.0±7.0	61.7±9.6
Mean arterial pressure, mm Hg	79.8±8.5	80.8±12.3
Carotid diameter, mm	5.71±0.63	5.97±0.58*

*$P<0.05$ vs morning.

Nevertheless, the researcher who undertook the statistical analysis was blinded to the time of day for each data set. Linear mixed models were used to compare integrated, mechanical, and neural sensitivities between the 2 times of day.[18] This method controls for the fact that systolic BP, R-R interval, and carotid diameter data are collected using a within-subjects design over time and are, therefore, correlated in nature. Using the integrated sensitivity as an example, the "participants" factor is entered into the linear mixed model as a random effect, "R-R interval" is the dependent variable, and "systolic BP" is entered as a covariate. To investigate diurnal variation, a "time-of-day" factor was added to the linear mixed model as a fixed effect, and a time-of-day × systolic BP interaction term was added as another covariate to allow integrated baroreflex sensitivities to be compared between morning and afternoon. Linear mixed models were also used to analyze the data for the mechanical component, with carotid diameter as the dependent variable and systolic BP as a covariate, and for the neural data, with R-R interval as the dependent variable and carotid diameter as the covariate. Values are mean±SE unless otherwise stated. All of the data were analyzed using SPSS 17 (SPSS Inc, Chicago, IL).

Results

Participants
All of the participants completed ≥1 morning and 1 afternoon Oxford trial. After an assessment of the quality of the recordings, the G_{down} data for 2 women were excluded. There were no significant effects of sex on baroreflex sensitivity, so the data for men and women were pooled; therefore, analyses for G_{up} and G_{down} were based on n=12 and n=10, respectively.

Baseline Cardiovascular and Respiratory Variables
Table 1 shows average resting values over a 5-minute baseline period for morning and afternoon trials. There was no significant diurnal variation in baseline heart rate, systolic BP, diastolic BP, or mean arterial pressure. However, baseline carotid diameter (mean±SD) was significantly greater in the afternoon (5.97±0.58 mm) compared with the morning (5.71±0.63 mm; $P=0.02$).

Diurnal Variation in Baroreflex Sensitivity
Table 2 shows the integrated, mechanical, and neural G_{up} and G_{down} for both times of day. There was diurnal variation in integrated G_{up}, which was lower in the morning (13.0±0.6 ms/mm Hg; $P=0.01$) compared with the afternoon (15.1±0.6 ms/mm Hg). Integrated G_{down} was also reduced in the morning (6.3±0.4 ms/mm Hg; $P<0.01$) compared with the afternoon (12.6±0.4 ms/mm Hg). There was diurnal variation in mechanical G_{up}, which was lower in the morning (0.015±0.001 mm/mm Hg) compared with the afternoon (0.018±0.001 mm/mm Hg; $P<0.01$) but no diurnal variation

Table 2. Integrated, Mechanical, and Neural Baroreflex Sensitivity and Correlation Coefficients for Morning and Afternoon

Baroreflex Component	Variable	Morning	Afternoon
Integrated, ms/mm Hg	G_{up}	13.0±0.6	15.1±0.6*
(R±SE)		(0.91±0.019)	(0.92±0.021)
Integrated, ms/mm Hg	G_{down}	6.3±0.4†	12.6±0.4*†
(R±SE)		(0.92±0.030)	(0.98±0.005)
Mechanical, mm/mm Hg	G_{up}	0.015±0.001	0.018±0.001*
(R±SE)		(0.85±0.017)	(0.92±0.022)
Mechanical, mm/mm Hg	G_{down}	0.013±0.001	0.013±0.001†
(R±SE)		(0.72±0.041)	(0.78±0.047)
Neural, ms/mm	G_{up}	589.1±39.7	682.0±37.5
(R±SE)		(0.83±0.018)	(0.86±0.033)
Neural, ms/mm	G_{down}	256.0±30.6†	494.9±48.8*†
(R±SE)		(0.73±0.050)	(0.80±0.043)

Values are mean±SE. n=12 (G_{up}); n=10 (G_{down}).
*Data were significantly different from morning (diurnal variation) ($P<0.05$).
†Data were significantly different from G_{up} (hysteresis) ($P<0.05$).

for the neural G_{up} ($P=0.09$; Figure 2). However, there was a difference between neural G_{down} for morning (256.0±30.6 ms/mm) and afternoon (494.9±48.8 ms/mm; $P<0.01$) but no difference between mechanical G_{down} ($P=0.97$).

Baroreflex Hysteresis
Table 2 shows that the integrated sensitivity was greater for G_{up} than G_{down} for both the morning ($P<0.01$) and afternoon trials ($P=0.03$). There was also neural hysteresis in the morning ($P<0.01$) and afternoon ($P=0.01$). However, in the morning there was no hysteresis in the mechanical component ($P=0.36$), despite mechanical hysteresis in the afternoon ($P<0.01$).

Discussion
A key finding of the present study was the diurnal variation in integrated baroreflex sensitivity, which was attenuated in the morning compared with the afternoon. For falling pressures, the lower integrated sensitivity in the morning was caused by reduced neural sensitivity compared with the afternoon. In contrast, for rising pressures, the lower integrated sensitivity in the morning was caused by reduced mechanical sensitivity compared with the afternoon. These findings provide new insights into the mechanisms underlying the attenuated baroreflex control during the morning hours and may explain in part why this time of day is associated with greater risk of cerebrovascular and cardiovascular events.

Diurnal Variation in the Mechanical and Neural Components of the Baroreflex
A novel aspect of the study was the measurement of carotid artery diameter to delineate the mechanical and neural contributions underlying the diurnal variation in the integrated baroreflex response. For rising pressures, the mechanical sensitivity was lower in the morning than in the afternoon. In the absence of differences in the neural component, this
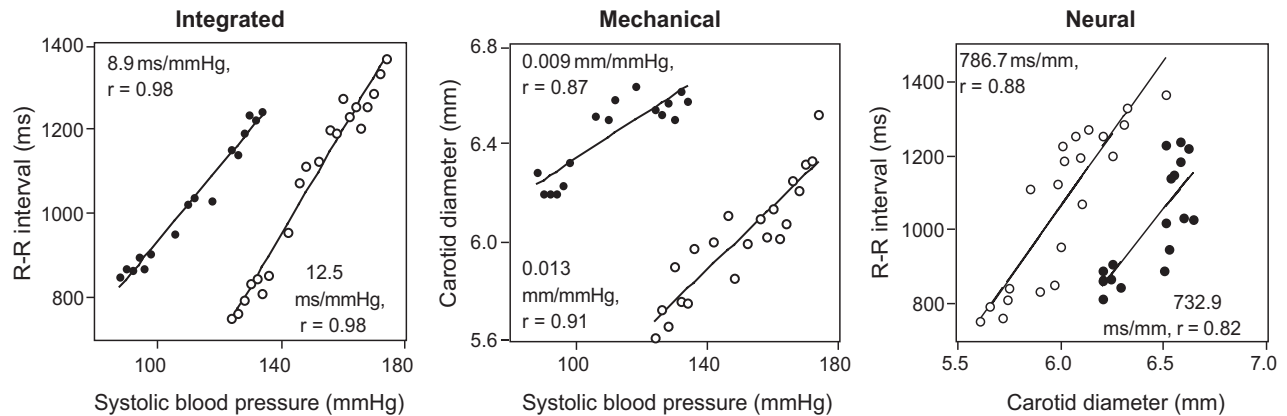
Figure 2. Integrated, mechanical, and neural baroreflex sensitivities for rising pressures in the morning (●) and afternoon (○) for one subject.

suggests that changes in the mechanical component cause diurnal variation in integrated sensitivity. Interestingly, in keeping with previous reports of diurnal variation in carotid artery caliber,[15,16] baseline carotid artery diameter was lower in the morning compared with the afternoon. Although the underlying mechanisms are unclear, vessel dilation has been shown to enhance vascular compliance,[25] and it has been found previously that carotid distensibility is reduced at night.[15] Given that baroreflex sensitivity is significantly related to carotid artery compliance,[26] we speculate that diurnal variation in central arterial compliance and or distensibility may account for the variation in mechanical sensitivity.

For falling pressures, the neural sensitivity was greater in the afternoon than the morning, accounting for the greater integrated sensitivity in the afternoon. The neural component of the baroreflex response encompasses the transduction of barosensory vessel stretch into afferent baroreceptor feedback, central integration, efferent autonomic outflow, and, consequently, changes in R-R interval. Although we could not determine the stage in this pathway responsible for the attenuated morning response, one possibility is that diurnal variation in sympathetic activity modulates sinoatrial node responsiveness to vagus nerve stimulation.[27] Although we could not verify this possibility, there is indirect evidence that sympathetic activity is greater in the morning[7,8,28] and that sympathetic excitation may accompany diminished baroreflex sensitivity.[29] In contrast, however, Middlekauff and Sontz[30] found that muscle sympathetic nerve activity (which correlates strongly with cardiac sympathetic nerve activity[31]) was not elevated in the morning (6:30 AM to 8:30 AM) when compared with the afternoon (2:00 PM to 4:00 PM). This would suggest that changes in neural G_{down} might be attributed to diurnal variation in the integration processes within the central nervous system. Another possibility is that sympathetic activity may have an effect on the mechanical component via changes in vascular compliance. For example, Grassi et al[32] demonstrated that radial artery compliance could be modulated in either direction by reflex or central changes in sympathetic drive. It remains possible that such tonic adrenergic influences on the mechanical properties of the radial artery may apply to other major arteries such as the carotid.

Comparison With Previous Studies

In contrast to this study, previous research has focused on sleep/wakefulness differences[10,11] or applied spontaneous methods of determining baroreflex sensitivity.[10,12,33,34] Hossmann et al[14] were the first to investigate baroreflex sensitivity changes over a full 24-hour period, which was examined at 3-hour intervals using l-noradrenaline infusions to perturb BP. Although the times of the trials do not coincide precisely with those of this study, our 7:00 AM trial is broadly comparable with their 9:00 AM trial, a time in which they also reported low baroreflex sensitivity. Both trials represent a time point 2 hours after waking in their respective studies, thus it is possible that wake time may be an influential factor. Hossmann et al[14] reported a second trough at 3:00 PM, which is the closest time point to the afternoon trial in the present study (4:00 PM). Our findings indicate greater baroreflex sensitivity at this time compared with the morning and so are somewhat contradictory; however, it is important to acknowledge that the use of steady-state l-noradrenaline infusions as a means to assess dynamic baroreflex sensitivity has been questioned.[35]

Studies using spontaneous baroreflex indices have consistently shown that the highest sensitivities occur during the night, with reduced sensitivities in the morning after waking.[10,12,13,33] However, the variations during the daytime have not been consistent between studies. For example, Tochikubo et al[12] found the circadian variation to be bimodal with a secondary peak in baroreflex sensitivity at 7:00 PM and trough at 11:00 PM. Although these findings support those of Nakazato et al,[33] who reported no difference in sensitivity between morning (7:00 AM) and evening (11:00 PM), earlier research reported a peak at 11:00 PM.[10] Although baroreflex sensitivity was not investigated in the evening in the present study, it is possible that the greater afternoon sensitivity that we have reported may continue to rise later into the day. Future studies are needed to explore the endogenous rhythm in baroreflex verses the influence by the sleep-wake cycle.

Methodological Considerations

This study is the first to apply the modified Oxford method to assess diurnal variation in baroreflex function. Previous studies of baroreflex function assessed via the modified

Oxford method have found estimates of integrated, mechanical, and neural sensitivity to be highly reproducible.[9] In contrast to previous applications,[22,24,36] we applied a linear mixed model to compare baroreflex sensitivities between the 2 times of day.[18] This approach improves precision by ensuring that all of the data points are entered into the analysis in a single step.[37] The linear regression method commonly used in the determination of baroreflex sensitivities is based on the assumption that x-y cases are mutually exclusive, that is, data pairs have been collected from independent participants. However, the slope of the baroreflex response is made up of data pairs from one individual, which clearly violates the assumption of case independence. By controlling for the correlated nature of the data, the linear mixed model not only reduces the susceptibility to outliers in small samples, but the statistical power is greatly improved compared with conventional summary measure analyses.[18] However, we acknowledge that by focusing only on cardiac baroreflex control, the question of whether there is diurnal variation in integrated and neural control of peripheral resistance is unanswered. Given the importance of vascular sympathetic regulation in cardiovascular homeostasis, further research is clearly warranted.

Clinical Perspectives

Low baroreflex sensitivity is a marker of cardiovascular disease,[17] and it has been speculated that diminished baroreflex functioning in the morning may explain the elevated incidence of myocardial infarction, stroke (all types), and syncope at this time.[3–6] Our findings represent an integral step in unraveling the mechanisms involved in baroreflex function and, importantly, how diurnal variation in the separate components may potentially contribute to greater risk of cardiovascular and cerebrovascular events. We felt it important to clearly delineate these mechanisms in otherwise healthy individuals to document the "normal" physiological response in the absence of any cofounding pathology and/or medications. Although the study of young healthy individuals is limited as far as generalizing the results to clinical populations, diurnal variation in baroreflex sensitivity is robust in hypertensive patients,[12,13] and our findings, therefore, bring to question whether better management of cardiovascular events may be achieved through interventions targeted specifically at the neural and/or mechanical components. The potential for the use of targeted therapy has been suggested in relation to sympathetic baroreflex function.[38] Although the concept of targeted therapy for both cardiovagal and sympathetic baroreflex sensitivity clearly requires further clinical validation, it is relevant to note that the enhancement of cardiovagal baroreflex function with regular exercise has been shown recently to occur through selective changes in the neural component.[39] Therefore, as an example, exercise prescription may be more effective in patients who experience morning orthostatic intolerance presenting with low neural sensitivity. Future work should be directed at understanding the changes in the mechanical and neural components of the baroreflex under different disease states and determining how baroreflex-enhancing effects of medications, such as angiotensin-converting enzyme inhibitors and β-blockers,[40] or lifestyle factors, such as a low-salt diet[41] and physical activity,[34] are ultimately achieved.

Disclosures
None.

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