Determination of minimal recording period to assess resting heart rate variability during pregnancy

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| Novelty bullets: points that summarize the key findings in the work: | Ln rMSSD can be measured for 5-min in pregnant women, with the last 1-min segment analyzed., The last 1-min segment from 3-min can be used for rHRV measurement in non-pregnant women., The shortened rHRV assessment can facilitate its applicability in clinical/exercise-training settings. |
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Determination of minimal recording period to assess resting heart rate variability during pregnancy

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Abstract

Traditionally, resting heart rate variability (rHRV) is measured for 10-min using the last 5-min for analyses (e.g., criterion period). It is unknown whether the measurement period can be shortened in pregnant women as there are currently no established standards. We aimed to compare shorter time segments (e.g., from the 1st-to-10th minute) of the parasympathetic index Ln rMSSD with the criterion period in pregnant and non-pregnant women. Twelve pregnant (age: 30.8 ± 3.4 years; gestational age: 20.1 ± 5.0 weeks) and 15 non-pregnant women (age: 29.8 ± 4.0 years) were included. rHRV was measured using a portable heart rate monitor for 10-min while sitting. Ln rMSSD difference/agreement between shorter time segments and criterion period was analyzed. The result observed between the 4th-5th minute was the shortest time segment not different from/highly agreed with the criterion period in pregnant women (Difference 95%CI: -0.10[-0.22–0.02]/Bias±1.96*SD: -0.06[-0.38–0.25]). In non-pregnant women, the 2nd-3rd minute segment was the shortest with similar results (Difference 95% CI: -0.04[-0.15–0.07]/Bias±1.96*SD: -0.03[-0.39–0.32]). The Ln rMSSD was found to be stable from the 5th-10th minute and the 3rd-10th minute in pregnant and non-pregnant women, respectively. A shortened rHRV assessment can increase its applicability in clinical/exercise-training settings.

Novelty:

- Ln rMSSD can be measured for 5-min in pregnant women, with the last 1-min segment analyzed.
- The last 1-min segment from 3-min can be used for rHRV measurement in non-pregnant women.
- The shortened rHRV assessment can facilitate its applicability in clinical/exercise-training settings.

Keywords: parasympathetic activity; autonomic nervous system; pregnant women; heart rate; heart rate variability; time domain; pregnancy.
Introduction

Pregnancy is known to increase physiological stress and consequently leads to important changes in cardiac autonomic function (Davenport et al. 2016), such as increased sympathetic nervous system activity and thus blunted parasympathetic activity as pregnancy advances (Purdy et al. 2019). Acute bouts of exercise are known to similarly increase sympathovagal balance (Purdy et al. 2019), although chronically, findings are conflicting regarding the effects of exercise on maternal parasympathetic activity (May et al. 2016; Carpenter et al., 2017). May et al. (2016) found greater HRV in pregnant women engaging in 30 minutes or more of moderate-intensity aerobic exercise three times per week compared to the control group. On the other hand, Carpenter et al. (2017) demonstrated lower HRV in pregnant women who were exercising at light-to-moderate intensity once a week for 43 minutes (only 15 min of aerobic exercise) in their 3rd trimester. These differences might be due to different factors, such as exercise intensity, frequency, volume, and type (Dietz et al., 2016). Although not focused on HRV-related benefits, the most recent Canadian physical activity guideline throughout pregnancy recommends at least 150 minutes per week of moderate-intensity physical exercise for health benefits (Mottola et al., 2018) and, evidence suggests that maternal exercise effects reflect positively on the baby’s cardiac health (May et al. 2014).

Resting heart rate variability (rHRV), a potential marker of autonomic cardiac control/function (Task Force 1996), provides information on parasympathetic alterations related to pregnancy and exercise. This variable can be characterized using various indices (e.g., time, frequency and non-linear domain indices) and may be easily measured through non-invasive techniques, such as heart rate monitors that show consistent agreement with ECG measurements (Giles et al. 2016). One of the time domain indices, called Root Mean Squared of Successive Differences (rMSSD), is a short-term HRV index (May et al. 2014) considered the ‘gold-standard’ measurement. This rMSSD measure, regarded as the index of greatest practical applicability, especially in exercise settings (Buchheit 2014; Da Silva et al. 2019), is due to its lesser sensitivity to variations in breathing patterns (Penttila et al. 2001), no influence of shorter recording duration (Buchheit 2014), and greater reliability (Al Haddad et al. 2011).

High rHRV is associated with better cardiorespiratory fitness in both healthy (Buchheit and Gindre 2006; Hautala et al. 2009) and in clinical (Da Silva et al. 2014) populations. Additionally, the rHRV is thought to respond to exercise training load changes (Buchheit 2014) and can be used by exercise professionals to guide decisions regarding training intensity (i.e., aerobic exercise) in both trained (Vesterinen et al. 2016) and untrained individuals (Da Silva et al. 2019). The particular
cardiac autonomic characteristics related to pregnancy suggest rHRV could be a valuable tool for guiding exercise prescription in this population as well (Purdy et al. 2019). Since guidelines provide specific exercise recommendations for this population, mainly for aerobic exercise (ACSM 2017; Mottola et al. 2018), rHRV could contribute to achieving a more personalized and controlled training program.

However, the traditional period of 10 minutes (min) for rHRV data acquisition (Task Force 1996; Aubert et al. 2003) is lengthy and can make frequent data collection difficult in practical settings. It has been recommended that the first 5-min typically serve as a stabilization period, and the last 5-min represents the criterion period to be analyzed (Task Force 1996; Pereira et al. 2016). Although rHRV is used in pregnancy research as a marker for stress, anxiety, and cardiovascular health, the measurement period varies greatly ranging from 5- to 18-min (Purdy et al. 2019; May et al. 2014; Kudo et al. 2014; Stutzman et al. 2010; Satyapriya et al. 2009). Therefore, a standard assessment period of rHRV measurement in pregnancy is currently lacking.

In hopes of providing recommendations to reduce data acquisition time, some investigations have demonstrated that shorter criterion periods (e.g., 1-min) agree with the traditional criterion period time in athletes (Esco and Flatt 2014) and non-athletes (i.e., diabetes mellitus patients) (Nussinovitch et al. 2012). These shorter criterion periods were analyzed for the parasympathetic HRV index rMSSD with (Esco and Flatt 2014) and without (Nussinovitch et al. 2012) natural logarithm transformation (Ln rMSSD). Reducing the stabilization period from 5 to 1-min (Flatt and Esco 2016) and the criterion to 1-min as well (i.e., a segment from the 1st to 2nd minute) (Pereira et al. 2016), displayed similar results. However, these data were restricted to a population of athletes.

The possibility of reducing the measurement time for rHRV in pregnant women has yet to be studied. It is known that as pregnancy advances, resting heart rate increases and parasympathetic activity decreases, especially in the third trimester (i.e., blunted rHRV) (Davenport et al. 2016; Carpenter et al., 2017; Purdy et al. 2019). What is not known is the extent to which these unique characteristics of cardiac autonomic function during pregnancy might influence the stabilization period of rHRV measurement. Extrapolating findings from athletic or healthy non-pregnancy-related populations suggesting a reduction in the rHRV measurement time could be premature. Thus, we aimed to compare shorter time segments (e.g., from the 1st up to the 10th minute) of the Ln rMSSD with the last 5-min (i.e., criterion period) in pregnant women and non-pregnant women. We hypothesized that a 1-min time segment within the criterion period (e.g., 6th minute) is representative of the entire 5-min segment, allowing for a reduced rHRV measurement timeframe in both pregnant and non-pregnant women.
Methods

Ethical approval

All experimental procedures were approved by the University of Ottawa Research Ethics Board (H-06-18-634), in agreement with the Declaration of Helsinki. Informed written consent was obtained from all study participants after explanation of all study objectives and procedures.

Participants

Pregnant and non-pregnant women were recruited from the greater Ottawa-region for participation and eligibility was confirmed by investigators. Inclusion criteria were as follows: age between 18-40 years, self-reported pre/non-pregnant body mass index (BMI) between 18.5 – 29.9 kg/m², weight-stable (± 5kg) for six months before the study (or before pregnancy), and for pregnant women, between 13-28 weeks of pregnancy carrying a singleton fetus. Exclusion criteria included: diabetes, untreated thyroid disease, hypertension, or frequent use of tobacco, drugs or alcohol. Twelve pregnant and fifteen non-pregnant participants were included. Height was determined using a Tanita HR-200 wall-mounted stadiometer (accuracy: 0.1cm; Lachine, QC) and body weight was assessed using a Tanita BWB-800 scale. To determine gestational weight gain (GWG) in pregnant women, self-reported pre-pregnancy weight was subtracted from the weight measured at the study visit.

Resting heart rate variability analysis

Resting heart rate variability (rHRV) was measured using a heart rate monitor (Polar V800, Lachine, QB) validated for this purpose (Giles et al. 2016). The measurements occurred while participants were seated comfortably with both feet flat on the ground and arms on armrests, with eyes opened and spontaneous breathing over data acquisition (Pereira et al. 2016; Bloomfield et al. 2001). The sitting position was chosen to avoid possible saturation of rHRV. This specific phenomenon might occur because the relationship between parasympathetic-related rHRV indices and the parasympathetic modulation of heart rate is not linear, but quadratic. Meaning that at both low parasympathetic tone (i.e., high heart rate) and high parasympathetic tone (i.e., low heart rate), vagal-related indices of rHRV could be attenuated (Plewes et al., 2013). The physiological mechanism
behind the saturation of rHRV, commonly observed in athletes, is likely the saturation of acetylcholine receptors at the myocyte level (Plews et al., 2013). As many of our participants self-reported being quite active (unpublished data), we opted for the seated position, as Buchheit (2014) recommended this position as a strategy to avoid low heart rate levels and consequently rHRV saturation. R–R intervals were continuously recorded for 10-min, at a sampling rate of 1,000 Hz, in a quiet room at ambient temperature. The data for R–R intervals were downloaded using Polar FlowSync Software. Data were analyzed by Kubios HRV analysis software (Biosignal Analysis and Medical Imaging Group at the Department of Applied Physics, University of Eastern Finland, Kuopio, Finland) (Tarvainen et al. 2014). Occasional artifact noise was automatically replaced with interpolated adjacent RR interval values (filter power <low) (Nakamura et al. 2017). The R-R intervals were analyzed using the time-domain index square root of the mean of the squares of successive R-R intervals differences (rMSSD) (Buchheit 2014; Vesterinen et al. 2016), which was determined in the segment representing the last 5-min as well as in each minute of the 10-min range separately. As rMSSD was not normally distributed, all rMSSD data were transformed using the natural logarithm. Although transformed, the variable is expressed in milliseconds (Nakamura et al. 2017).

**Statistical analysis**

All data are presented as mean ± standard deviation, and the Shapiro-Wilk test was used to assess the normality of data distribution. To analyze the differences between the last 5-min (i.e., criterion period) with each 1-min segment of the entire 10-min range for Ln rMSSD, and to account for group differences (i.e., pregnant vs. non-pregnant women) at the different time points, we used a mixed (within-between) ANOVA for repeated measures. Sphericity was tested according to Mauchly’s test, and the degrees of freedom were corrected based on Greenhouse-Geisser Epsilon. Multiple comparisons were performed by Bonferroni adjustment. By analyzing the results of the multiple comparison, we observed when a 1-min segment presented no significant difference from the criterion period, and we subsequently compared this segment with the following 1-min segments to determine if stabilization had occurred (i.e., no significant differences). Significant p-values were set at p < 0.05. Bonferroni post-hoc adjusts the analysis according to the number of time points (e.g., 11 – criterion period and every 1-min segment). The p-values described in our tables and within the text are already adjusted and, due to the conservative nature of the test, may be equal to 1.000. The agreement analysis was performed using an intra-class correlation, and the reliability was interpreted as follows: <0.8 (questionable), 0.8 to 0.89 (moderate), ≥0.9 (high) (Vincent 2005). We also used the
Bland-Altman analysis with upper and lower limits of agreement to examine agreement between different times (Bland and Altman 1986) to confirm if stabilization had occurred (e.g., similar biases from the earliest non-significant 1-min time segment to the 10th minute).

**Results**

Pregnant and non-pregnant women were of similar age and pre-pregnancy (or non-pregnant) body mass index (BMI) (Table 1). Based on the mixed ANOVA for repeated measures, we found an interaction (F=4.903; p=0.001) between group (i.e., pregnant and non-pregnant women) and time (criterion period and every 1-min time segment). The same statistical test also showed a time effect (F=18.886; p<0.001) and no group effect on rHRV (F=0.622; p=0.438).

The multiple comparison analyses showed that Ln rMSSD values were lower during the last 5-min segment compared to the 1st (p=0.010), 2nd (p=0.002), 3rd (p=0.006), and 4th (p=0.028) minutes in the pregnant group and the other 1-min segments were not different from the criterion period (Table 2). In the non-pregnant group, the 1st minute, although nearing significance (p=0.062), was not different from the last 5-min, and none of the other 1-min time segments yielded significant differences from the criterion period (Table 3).

The first 1-min segment that was not significantly different from the last 5-min segment was from the 4th-5th min in the pregnant women (Table 2). The multiple comparison analyses also showed that this 1-min segment was not different from the 6th, 7th, 8th, 9th, and 10th min, indicating rHRV stabilization (Supplementary Table S1).

In the non-pregnant group, rHRV in the 1st min, although not statistically different from the criterion period (p=0.062), was higher than 4th (p=0.013), 5th (p=0.040), 6th (p=0.017), and 8th minutes (p=0.001), which did not demonstrate stability. On the other hand, the 2nd min was not different from the 3rd to the 10th minutes, indicating rHRV stabilization (Supplementary Table S1).

The earliest non-significant and stable 1-min segment for pregnant women (5th minute) and non-pregnant women (2nd minute) also highly correlated with the criterion period in the intra-class correlation analysis (ICC=0.96 for pregnant women and 0.93 for non-pregnant women).

For pregnant women, the 5th minute also presented a narrow limit of agreement from Bland-Altman analysis (Table 2 and 3; Supplementary Figure S1 and Supplementary Figure S2) with close to zero bias (0.09) and consistent with the range of bias observed up to the 10th minute (from 0.00 to 0.09), confirming rHRV stability (Table 2; Figure 1). In the non-pregnant women, the 2nd minute presented a bias of 0.15, while we noticed in the 3rd minute a bias of 0.04. Considering the range of
bias observed up to the 10th minute in non-pregnant women (from 0.00 to 0.04), it appears that rHRV began to stabilize at the 3rd minute (Table 3; Figure 1).

Discussion

The novel finding of our study was that Ln rMSSD observed between the 4th-5th min segment of the 10-min rHRV measurement was not different from and highly agreed with the last 5-min (i.e., criterion period) in pregnant women. Whereas in non-pregnant women, the 2nd-3rd min segment was not different compared to the criterion period. The Ln rMSSD was found to be stable from the 5th-10th min and the 3rd-10th min in pregnant and non-pregnant women, respectively.

While rHRV has been measured in pregnant women over different durations, including 5- (Purdy et al. 2019; Satyapriya et al. 2009; Narita et al. 2018), 6- (Carpenter et al. 2017), 10- (Stutzman et al. 2010) or 18-min (Van Leeuwen et al. 2014), with differing periods of rest (or stabilization) or assessment positions (i.e. supine, standing), our data indicate that rHRV can be measured for a period as short as 5-min. This condensed timeframe is particularly beneficial in the pregnant population who generally experience heightened discomfort.

As rHRV has become a practical tool for the non-invasive assessment of cardiac autonomic function, there is a need for more suitable recording approaches to improve its practical application in different settings, and populations (Buchheit 2014; Nussinovitch et al. 2012). The traditional rHRV measurement is based on a 10-min data acquisition period with the focus being the last 5-min (Task Force 1996; Aubert et al. 2003). This recommendation suggests using the first 5-min as a stabilization period to allow for a steady measurement of parasympathetic indexes to be captured in the last 5-min (also called the criterion period) (Pereira et al. 2016).

Predominantly, the rMSSD index is considered a preferred measurement over the other indices because it i) is not influenced by shorter recording duration (Buchheit 2014), ii) is less sensitive to alterations related to breathing patterns compared to spectral indices (Penttila et al. 2001), and iii) has greater reliability (Al Haddad et al. 2011). Although we performed the rMSSD analysis using specific HRV software, Buchheit (2014) suggests that it can be easily calculated using an Excel spreadsheet. The most important consideration is that the day-to-day variation of HRV in time domain indices, such as rMSSD, is lower than frequency domain indices (Buchheit 2014; Al Haddad et al. 2011), which accounts for application in real-world settings.

The ease and convenience of the rMSSD index make this a practical tool that can be applied to guide exercise training intensity prescription. For athletes, Vesterinen et al. (2016) identified that

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guiding exercise training by using longer trends of rMSSD (e.g., week rolling average) is most suitable. Essentially, when rMSSD increases as compared to previous values, it may be a sign of insufficient recovery (Vesterinen et al. 2016). A similar approach was tested in untrained non-pregnant women by measuring rHRV and comparing it to the mean ± one standard deviation of previous measurements thereby guiding training intensity throughout each training session (Da Silva et al. 2019). In pregnant women, rHRV could also be used to manage training intensities as well as increase safety during exercise. While there exists a clear consensus that women should engage in 150+ min/wk of moderate-intensity physical activity throughout pregnancy (Mottola et al., 2018), the results of the few studies on the topic of rHRV and exercise in pregnancy are not consistent (May et al., 2016; Carpenter et al., 2017). Factors that might contribute to this inconsistency are exercise intensity, frequency, duration and type (Dietz et al., 2016; May et al., 2016; Carpenter et al., 2017). These discrepant findings may correspond with the steady decline in parasympathetic activity shown across pregnancy, and its exacerbation in the last trimester (Purdy et al., 2019; Carpenter et al., 2017). As time constraints might be an issue when monitoring exercise training (Nakamura et al., 2017; Pereira et al., 2016), the quicker the measurement, the more practical it becomes. Thus, the decline in rHRV measurement time supported by our findings may increase the practical application of this tool by helping exercise professionals assess changes in cardiac autonomic function across pregnancy more efficiently. Additionally, the reduced measurement time will improve use in research settings evaluating the effects of different exercise programs on maternal rHRV.

Furthermore, standardizing and reducing the period of rHRV measurement could improve the generalizability and applicability of this tool in other real-world scenarios related to pregnant women. Psychologists and other primary health care providers could include shortened rHRV measurement in their routines, given that many studies have used rHRV as an objective surrogate marker for stress (Satyapriya et al. 2009) and anxiety (Kudo et al. 2014; Narita et al. 2018) in pregnant women. Gestation has been equated to a metabolic stress-test, exposing one’s physiological susceptibility (Williams, 2003), and monitoring rHRV may prove useful in identifying whether pregnant women are entering the final stages of gestation at increased cardiovascular risk. Finally, rHRV may also be used as a barometer for lifestyle and thus motivate those considering a lifestyle change. It is possible to influence rHRV by adopting behaviours related to mindfulness, meditation, and healthful sleep patterns (Stutzman et al. 2010; Satyapriya et al. 2009).

Pereira et al. (2016) showed that the 2nd 1-min segment of Ln rMSSD 10-min data acquisition was not different from the last 5-min in athletes, suggesting that the traditional period was too long and that athletes tend to stabilize very quickly during the resting measurement. In our study, we found that non-pregnant women needed two minutes to stabilize, and from the 3rd-10th min, they
remained stable. However, pregnant women only stabilized after four minutes and remained consistent from the 5th-10th min. Although pregnant versus non-pregnant women presented different trends of Ln rMSSD stabilization, no differences between them in the absolute values of this index were found, a finding consistent with the current literature. Purdy et al. (2019) reported a reduction in rMSSD only when comparing pregnant women in their third trimester versus non-pregnant controls, with no differences between women in the first or second trimester of pregnancy. As we had a broad range of gestational ages included in the study, we tested if gestational age was correlated with Ln rMSSD (criterion period and the 5th minute), but no association was found (data not shown).

We hypothesize that physical-related stress factors, emotional factors and/or physiological factors may be taken into account to explain why pregnant women would take longer to become stable during the measurement of rHRV. Factors that could affect the autonomic nervous system activity include: i) extra pregnancy weight, and pressure on the diaphragm due to uterine growth (Biringer 1968), ii) abdominal pain in the lower region commonly observed in the second trimester (Gregory et al. 2017), iii) an elevation in daily hassles associated with pregnancy (perceived or real) and pregnancy-related anxieties (Huizink et al. 2003), iv) a blunting of parasympathetic activity as pregnancy progresses (Purdy et al. 2019), v) changes in reproductive hormone production, metabolism and thermoregulation, as well as hemoglobin concentrations (Kumar and Magon 2012; Byrne et al. 2011; Chandra et al. 2012; Kiviniemi et al. 2010).

Our study is not without limitations. Although rHRV could be influenced by training status (Buchheit 2014; Pereira et al. 2016), we were unable to assess this variable objectively. Though, many of these women self-reported being quite active (unpublished data), none were engaged in exercise training (i.e., systematic and prescribed training). As this study included a relatively small sample size, we opted not to include this self-reported data in the analysis since subjective data have been found to overestimate physical activity levels (Brett et al. 2015). In view of studies in athletes (Pereira et al. 2016; Esco and Flatt 2014; Flatt and Esco 2016; Nakamura et al. 2017) not finding a difference between shorter rHRV recording periods (ultra-short-term measurement periods [~2min]) and the criterion period (i.e., last 5-min from a 10-min time range), we recommend that future studies consider examining physically active and non-active pregnant women.

In pregnant women, Ln rMSSD can be sufficiently measured in five minutes, analyzing last 1-min segment, allowing clinicians, exercise specialists, and pregnant women to conduct more time-efficient assessments. This shorter measurement period can significantly increase the application of rHRV in the guidance of exercise training, stress/anxiety management, and in research settings by improving convenience and increasing compliance.
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Conflict of interest statement
The authors declare no conflicts of interest.

References


Figure Caption

**Figure 1.** Analysis of variance between the 5th-10th minutes for pregnant women and between the 3rd-10th minutes for non-pregnant women. Dotted rectangles represent the stable period.
### Table 1. Study population demographics.

<table>
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<tr>
<th></th>
<th>Non-pregnant (n=15)</th>
<th>Pregnant (n=12)</th>
<th>p-value</th>
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<tr>
<td>Age (years)</td>
<td>29.8 ± 4.0</td>
<td>30.8 ± 3.4</td>
<td>0.484</td>
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<tr>
<td>Gestational age (weeks)</td>
<td>N/A</td>
<td>20.1 ± 5.0</td>
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<tr>
<td>Height (cm)</td>
<td>164.1 ± 5.7</td>
<td>167.1 ± 5.3</td>
<td>0.174</td>
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<tr>
<td>Non/pre-pregnant body weight (kg)</td>
<td>63.5 ± 10.0</td>
<td>58.6 ± 7.5</td>
<td>0.140</td>
</tr>
<tr>
<td>Non/pre-pregnant BMI (kg·m⁻²)</td>
<td>23.2 ± 3.8</td>
<td>21.8 ± 2.4</td>
<td>0.132</td>
</tr>
<tr>
<td>Weight gain (kg)</td>
<td>N/A</td>
<td>5.3 ± 3.4</td>
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Values are presented as mean ± SD. BMI = body mass index; SD = standard deviation.
Table 2. Comparison between the last 5-min of Ln rMSSD with each 1-min segment of the 10-min measurement period for pregnant women (n=12).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD (ms)</th>
<th>Difference (95% CI)</th>
<th>p-value*</th>
<th>ICC (95% CI)</th>
<th>Bias (± 1.96*SD)</th>
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<tbody>
<tr>
<td>Last 5min</td>
<td>3.05 ± 0.67</td>
<td>---</td>
<td>---</td>
<td>---</td>
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</tr>
<tr>
<td>0-1min</td>
<td>3.63 ± 0.48</td>
<td>-0.58 (-1.06 - -0.11)</td>
<td>0.010</td>
<td>0.80 (0.44 - 0.94)</td>
<td>-0.58 (-1.30 - 0.13)</td>
</tr>
<tr>
<td>1-2min</td>
<td>3.47 ± 0.62</td>
<td>-0.42 (-0.71 - -0.13)</td>
<td>0.002</td>
<td>0.94 (0.81 - 0.98)</td>
<td>-0.42 (-0.86 - 0.02)</td>
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<tr>
<td>2-3min</td>
<td>3.39 ± 0.67</td>
<td>-0.34 (-0.60 - -0.08)</td>
<td>0.006</td>
<td>0.96 (0.85 - 0.99)</td>
<td>-0.34 (-0.73 - 0.05)</td>
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<tr>
<td>3-4min</td>
<td>3.29 ± 0.59</td>
<td>-0.24 (-0.46 - -0.02)</td>
<td>0.028</td>
<td>0.96 (0.88 - 0.99)</td>
<td>-0.24 (-0.57 - 0.10)</td>
</tr>
<tr>
<td>4-5min</td>
<td>3.15 ± 0.63</td>
<td>-0.10 (-0.36 - 0.15)</td>
<td>1.000</td>
<td>0.96 (0.85 - 0.99)</td>
<td>-0.09 (-0.48 - 0.28)</td>
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<tr>
<td>5-6min</td>
<td>3.05 ± 0.78</td>
<td>0.00 (-0.29 - 0.30)</td>
<td>1.000</td>
<td>0.95 (0.84 - 0.99)</td>
<td>0.00 (-0.44 - 0.45)</td>
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<tr>
<td>6-7min</td>
<td>3.07 ± 0.73</td>
<td>-0.02 (-0.25 - 0.20)</td>
<td>1.000</td>
<td>0.97 (0.90 - 0.99)</td>
<td>-0.02 (-0.36 - 0.32)</td>
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<td>7-8min</td>
<td>3.02 ± 0.63</td>
<td>0.03 (-0.12 - 0.18)</td>
<td>1.000</td>
<td>0.98 (0.95 - 0.99)</td>
<td>0.03 (-0.20 - 0.25)</td>
</tr>
<tr>
<td>8-9min</td>
<td>3.00 ± 0.64</td>
<td>0.05 (-0.17 - 0.28)</td>
<td>1.000</td>
<td>0.97 (0.88 - 0.99)</td>
<td>0.06 (-0.28 - 0.40)</td>
</tr>
<tr>
<td>9-10min</td>
<td>2.95 ± 0.70</td>
<td>0.10 (-0.27 - 0.46)</td>
<td>1.000</td>
<td>0.92 (0.73 - 0.98)</td>
<td>0.09 (-0.46 - 0.65)</td>
</tr>
</tbody>
</table>

*p-values are adjusted for multiple comparisons by Bonferroni post-hoc and are related to the comparison with the last 5-min (criterion period). SD = standard deviation; CI = confidence interval; ICC = intra-class correlation.
Table 3. Comparison between the last 5-min of Ln rMSSD with each 1-min segment of the 10-min measurement period for non-pregnant women (n=15).

<table>
<thead>
<tr>
<th>Variable (time segment)</th>
<th>Mean ± SD (ms)</th>
<th>Difference (95% CI)</th>
<th>p-value*</th>
<th>ICC (95% CI)</th>
<th>Bias (± 1.96*SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Last 5min</td>
<td>3.36 ± 0.71</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>0-1min</td>
<td>3.61 ± 0.72</td>
<td>-0.25 (-0.50 - 0.01)</td>
<td>0.062</td>
<td>0.95 (0.85 - 0.98)</td>
<td>-0.25 (-0.71 - 0.22)</td>
</tr>
<tr>
<td>1-2min</td>
<td>3.51 ± 0.78</td>
<td>-0.15 (-0.46 - 0.15)</td>
<td>1.000</td>
<td>0.93 (0.80 - 0.98)</td>
<td>-0.15 (-0.71 - 0.41)</td>
</tr>
<tr>
<td>2-3min</td>
<td>3.41 ± 0.77</td>
<td>-0.04 (-0.26 - 0.17)</td>
<td>1.000</td>
<td>0.96 (0.90 - 0.99)</td>
<td>-0.04 (-0.44 - 0.36)</td>
</tr>
<tr>
<td>3-4min</td>
<td>3.37 ± 0.73</td>
<td>0.00 (-0.18 - 0.17)</td>
<td>1.000</td>
<td>0.98 (0.93 - 0.99)</td>
<td>0.00 (-0.31 - 0.31)</td>
</tr>
<tr>
<td>4-5min</td>
<td>3.37 ± 0.81</td>
<td>0.00 (-0.22 - 0.21)</td>
<td>1.000</td>
<td>0.97 (0.90 - 0.99)</td>
<td>0.00 (-0.39 - 0.39)</td>
</tr>
<tr>
<td>5-6min</td>
<td>3.33 ± 0.71</td>
<td>0.04 (-0.12 - 0.19)</td>
<td>1.000</td>
<td>0.98 (0.94 - 0.99)</td>
<td>0.03 (-0.24 - 0.31)</td>
</tr>
<tr>
<td>6-7min</td>
<td>3.36 ± 0.81</td>
<td>0.00 (-0.18 - 0.17)</td>
<td>1.000</td>
<td>0.97 (0.93 - 0.99)</td>
<td>0.00 (-0.31 - 0.31)</td>
</tr>
<tr>
<td>7-8min</td>
<td>3.31 ± 0.72</td>
<td>0.05 (-0.07 - 0.16)</td>
<td>1.000</td>
<td>0.99 (0.97 - 0.99)</td>
<td>0.04 (-0.17 - 0.26)</td>
</tr>
<tr>
<td>8-9min</td>
<td>3.36 ± 0.81</td>
<td>0.00 (-0.12 - 0.13)</td>
<td>1.000</td>
<td>0.99 (0.96 - 0.99)</td>
<td>0.00 (-0.23 - 0.24)</td>
</tr>
<tr>
<td>9-10min</td>
<td>3.38 ± 0.71</td>
<td>-0.02 (-0.15 - 0.12)</td>
<td>1.000</td>
<td>0.99 (0.96 - 0.99)</td>
<td>-0.01 (-0.26 - 0.23)</td>
</tr>
</tbody>
</table>

*p-values are adjusted for multiple comparisons by Bonferroni post-hoc and are related to the comparison with the last 5-min (criterion period). SD = standard deviation; CI = confidence interval; ICC = intra-class correlation.
Figure 1. Analysis of variance between the 5th-10th minutes for pregnant women and between the 3rd-10th minutes for non-pregnant women. Dotted rectangles represent the stable period.

P > 0.05 for within-time comparisons (3rd to 10th)
P > 0.05 for within-time comparisons (5th to 10th)

Time segment (minutes)