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Please cite this article GENDER AND INTRA-FEMALE DIFFERENCES IN AUTONOMIC HEART RATE MODULATION DURING REST AND AFTER A SUPRAMAXIMAL ANAEROBIC TEST WITH REFERENCE TO MENSTRUAL CYCLE

RAZLIKE U AUTONOMNOJ MODULACIJI SRČANOG RITMA U MIRU I NAKON SUPRAMAKSIMALNOG ANEROBNOG TESTA PREMA POLU I MEĐU ŽENAMA U ODNOŠU NA MENSTRUALNI CIKLUS

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UDC:

DOI: https://doi.org/10.2298/VSP190129084A

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be carried over.
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Differences in autonomic control
Abstract

**Background / Aim.** Heart rate variability (HRV) and heart rate recovery (HRR) show differences between genders and dissimilarities were, as well reported in women in various menstrual cycle (MC) phases. The goal of this research was to analyze cardiac autonomic indices during rest and in recovery after a Wingate test between genders in young, sedentary population and to investigate whether a MC phase can influence these indices. **Methods.** Twenty-five females (20.5±0.7 years) and sixteen males (20.4±0.7 years) performed a Wingate anaerobic test on a cycle ergometer with their HRR and resting and recovery HRV indices obtained. In females data were collected during three distinctive MC phases. **Results.** Natural logarithm of low frequency HRV marker (lnLF) and natural logarithm of high frequency HRV marker (lnHF) were higher in males during rest in regard to women in all MC phases, except in late follicular phase, where no differences in lnHF between genders were observed. Markedly higher lnLF and lnHF were recorded in males after the Wingate test. There were no differences in HRV between women in various MC phases during rest. Surprisingly, parasympathetic time domain marker (the square root of the mean squared differences of successive NN intervals, RMSSD) and lnLF were both higher in early follicular in compare to luteal phase during the recovery. HRR was faster in men in compare to women in all MC phases. **Conclusion.** In conclusion, males show greater HRR and total variability during rest and recovery, but it appears that resting parasympathetic activity is similar when females are in late follicular phase. Intra-female resting autonomic variability is not affected by sex hormonal cycle. Post-exercise HRV in early follicular phase reflects a significantly favourable autonomic profile in compare to luteal phase.

**Key words:**

heart rate variability, heart rate recovery, Wingate test, sex, hormonal cycle.

Abstrakt
Uvod / Cilj. Razlike u varijabilnosti srčane frekvencije (VSF) i oporavku srčanog ritma (OSR) postoje kako između polova, tako i unutar žena u različitim fazama menstrualnog ciklusa (MC). Svrha ovog istraživanja bila je da ispita autonomne indekse u miru i u oporavku nakon Vingejt testa između polova u mladoj, sedentornoj populaciji i da li faza MC može imati uticaj na ove indekse. Metode. Dvadeset i pet ispitanica (20.5±0.7 godina) i šesnaest ispitanika (20.4±0.7 godina) izvodili su Vingejt anaerobni test na bicikl ergometru pri čemu su im registrovani OSR i VSF u miru i oporavku. Kod ispitanica podaci su prikupljani tokom tri faze MC. Rezultati. REZULTATI: Prirodni logaritam markera niskih frekvencija VSF (lnLF) i prirodni logaritam markera visokih frekvencija VSF (lnHF) bili su u miru veći kod muškog pola u odnosu na žene u različitim fazama MC, osim u slučaju kasne folikularne faze gde nije bilo razlike u lnHF među polovima. Značajno veći lnLF i lnHF uočeni su kod muškaraca tokom oporavka od Vingejt testa. Nije bilo razlike u parametrima VSF u miru među ženama u različitim fazama MC. Iznenadjujuće, parasimpatički marker vremenskog domena – kvadratni koren srednje vrednosti sume kvadrata razlika između sukcesivnih NN intervala (RMSSD) i lnLF su bili veći u ranoj folikularnoj u odnosu na lutealnu fazu ciklusa tokom oporavka. OSR bio je brži kod muškaraca u odnosu na žene u svim fazama MC. Zaključak. Muškarci pokazuju brži OSR i veću ukupnu varijabilnost tokom mira i u oporavku, ali čini se da je parasimpatička aktivnost u miru slična između polova kada su žene u kasnoj folikularnoj fazi. Hormonski ciklus kod žena nema uticaj na autonomnu varijabilnost u miru. Rana folikularna faza pokazuje poželjniji autonomni profil u oporavku u poređenju sa lutealnom fazom.

Introduction

Besides autonomic regulation, the rhythmicity of cardiac beats is finely directed by humoral factors, hence the influence of hormonal fluctuations on heart rate variability (HRV) throughout the menstrual cycle (MC) (1,2). Female monthly sexual cycle is dominantly regulated through influences of hypothalamic releasing hormone – gonadotropin releasing hormone (GnRH), anterior pituitary sex hormones – follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and the ovarian hormones – estrogen (mainly in a form of β-estradiol) and progestogens (almost exceptionally progesterone). At the very beginning of the proliferative phase there is a rise in FSH and LH levels, where FSH increases estrogen production in primary follicles leading to a peak
secretion just before the ovulation. Two days prior to ovulation the LH surge happens, rising by 6-10 fold, with the about 2-3 fold increase in FSH production at the same time. After the ovulation, concentrations of progesterone and estrogen start to increase until the late luteal phase when involution of corpus luteum and cessation of progesterone and estrogen secretion removes the feedback inhibition and levels of FSH and LH start to rise again (3). The influence of the MC phases on HRV is not yet clear. Higher sympathetic activity in luteal phase has been reported proposing the effect of progesterone increase on parasympathetic withdrawal (4–7), while others have reported the opposite, or did not find any significant phase differences (8,9). On the other side, estrogen, the leading hormone of the follicular phase, has a positive relationship with vagal activity (9). It acts on presynaptic alpha 2 adrenceptors leading to a decrease in nor epinephrine secretion and is also associated with an increase in acetylcholine production (10), thus, it may be that the rise in FSH, LH and progesterone levels accounts for the inhibition of estrogen-related vagal control (9). Some studies show marked sympathetic tone in male athletes, while the parasympathetic nervous system dominates in female athletes (11,12). A research conducted on non-athletes showed diminished parasympathetic influence in younger and middle aged women (13). In another study parasympathetic influence prevailed among adolescent female non-athletes, as opposed to their age matched male counterparts (14). Women have a faster vagal post exercise recovery after a maximal aerobic capacity test (15), but supra-maximal anaerobic test has a greater impact on autonomic reactivation in women (16,17). Women are in general underrepresented in exercise studies and majority of those that include them do not hold MC phases into account.

The goal of this paper was to investigate the influence of different MC phases (especially early and late follicular phase) on resting and post-exercise autonomic modulation between genders, as well as in females solely. It was postulated that females would have higher parasympathetic indices when in early and/or late follicular phase in compare to males and in intra subject relations. Secondly, we wanted to examine in what way MC phases possibly influence the results of a supra-maximal (Wingate) test in females.

Methods

Participants
Forty-one participant (25 females), aged between 18 to 24 years (age 20.4±0.7, 20.5±0.7; height 184±5cm, 168±5cm; body mass 79.38±9.42kg, 60.96±6.93kg; body mass index 23.53±2.83, 21.57±2.23 for males and females respectively) voluntary entered the study. All participants were regularly enrolled in studies of Medicine, at the University of Novi Sad’s Faculty of Medicine. Subjects were in self reported good health, without the use of medications and with no medical history of cardiovascular and neuromuscular diseases, including neuro-vegetative dystonia. Inclusion criterion for female participants was regular MC. Additional criteria implied that leisure time physical activity in the past six months has not exceeded an hour of sports activity per day for not more than three days a week.

The research was approved by the Ethics Committee of the University of Novi Sad’s Faculty of Medicine and it was conducted according to the Declaration of Helsinki. Participants were thoroughly introduced with the study procedure and its goal, and they all gave written informed consent.

All measurements were conducted at the Department of Physiology’s Laboratory for functional diagnostics, between 10 and 12 am, at room temperature around 22-24°C. Participants were strongly advised to restrain from intensive training and from consuming caffeinated and alcoholic beverages, including stimulant substances, 24h before the test. Female subjects were required to come at three phases of their MC. MC phase calculation was performed via recommendations provided by Stricker et al. (18) where the 14th day of the cycle is marked as day zero. The measurements were undertaken during the phase of menstrual bleeding (from day -15 until -6) – the early follicular phase, when levels of both estrogen and progesterone are low; in the middle of late follicular phase (from day -5 until -1), when estrogen reaches its peak and in mid-luteal phase (from day +5 until +9), when progesterone peak is expected.

Study protocol and data acquisition

The protocol consisted of two modes of heart rate acquisition – at rest and during recovery, using a telemetric pulsmeter (Polar RS800CX, Finland). Firstly, participants were required to sit quietly and breathe spontaneously for 5 minutes on a cycle-ergometer (Wattbike, Wattbike Ltd, Nottingham, UK), having their feet placed on a platform in front of the pedals, with knees flexed in a 90 degree angle and arms resting on thighs, while heart
rate recordings were obtained. The Wingate anaerobic test was preceded with 3 minute warm-up where resistance was set at 50 W. Throughout this period, they performed 2-3 bouts of sprint, in order to get adjusted to the level of speed and exertion they had to engage for the test. After the warm-up period, subjects were instructed to pedal at full speed in a standing position against the constant breaking force (7.5% of body weight). Upon cessation of the exercise test, heart rate recording was started again for a duration of 5 minutes. During the first minute of the recovery period participants continued pedalling without any resistance, and afterwards, they were required to stay in the same body position as before the exercise for additional 4 minutes.

Data analysis

Ergometric parameters

Peak power (PP) was a value of the highest power achieved at any 5 second stage. Mean power (MP) was defined as an average of all obtained power values.

Heart rate variability

A sampling rate of 1000 Hz was chosen and data from the pulsmeter were transferred to a laptop computer via a USB interface where they were analyzed in Polar ProTrainer 5 (Polar, Finland) software. Ectopic beats and artefacts were identified with visual inspection and removed. They were deleted with the post extra systolic beat and replaced automatically with interpolated adjacent R-R interval values. HRV indices (the square root of the mean squared differences of successive NN intervals – RMSSD, low frequency spectral power – LF (0.04-0.15 Hz) and high frequency spectral power – HF (0.15-0.40 Hz)) were calculated for all 5 minutes of resting and for the 3-minute recovery period (minutes 3.-5.). In order to ensure the stability of the data and reduce bias arising from non-uniformity of error, natural log-transformations (ln) of spectral HRV indices were performed.

Heart rate recovery

HRR was assessed via indexes which were extracted from the 5-minute recovery recordings. HRR60 represents the absolute difference between heart rate values at 60 seconds after exercise termination (HR60) and peak heart rate values registered immediately after termination of the test (HRmax). Resting heart rate (HRrest) was
presented as a mean heart rate value acquired from pre-exercise 5 minute recordings. Heart rate readings at the end of post-exercise period were also obtained (HREnd). T30 is a time constant of the rapid heart rate decay during the first 30 seconds of recovery and it represents the negative reciprocity of regression line slope. T is the time constant decay obtained by fitting the 5 minute post exercise HRR into a first-order exponential curve (16,19,20), where heart rates were modelled with an iterative technique using MatLab software (The Math Works Inc, Natick, MA, USA) to fit the following equation:

\[ HR = HR_o + HR\Delta e^{(-t+T)} \]

Where: HR= heart rate, HR_o= stabilized heart rate following exercise, HR\Delta= maximal heart rate - HR_o, t= time (s), T= time constant of exponential heart rate decay

**Statistical analysis**

The normality of the distribution was assessed with the Lilliefors normality test. Microsoft excel data analysis tool was used for statistical inspection. F-test was performed to assess the equality of variances between groups, after which we did the two-sample T test. The data are presented as means ± SD with respect to confidence interval. Statistical significance was indicated at \( p<0.05 \).

**Results**

There were significant differences in mean values of PP comparing the results in men with the results in women in early follicular (\( p=0.0000117 \)), late follicular (\( p=0.000016 \)) and luteal phase (\( p=0.0000157 \)). There were also significant differences in mean values for MP comparing the results in men with the ones in women in early follicular (\( p=0.00000213 \)), late follicular (\( p=0.00000871 \)) and luteal phase (\( p=0.00000209 \)) (Fig. 1). There were no statistical significances in these parameters between menstrual cycle phases in women.

By analysing HRV indices during rest in reference to sex, RMSSD has not showed valuable differences, lnLF was significantly higher in men in compare to women throughout all three phases and lnHF was significantly higher in men in contrast to women in early follicular and luteal phase (Table 1, 2, 3).
After analysing the HRV recovery parameters it was noticed that RMSSD did not show statistical significance when comparing any of the female cycle phases with males. On the other side, lnLF and lnHF values markedly differed between men and women in all MC phases (Fig. 2).

When comparing females during rest in various MC phases no differences were observed in HRV. However, during the recovery from Wingate anaerobic test, RMSSD was noticeably higher while females were in early follicular phase vs. luteal phase (6.29±1.06, 5.20±0.83; p=0.011415). Also, in early follicular phase female participants had greater values of lnLF in compare to luteal phase (3.62±0.20, 3.39±0.21; p=0.008511).

Mean values of HRrest, HRmax, HR60, HRR60, HRend and T did not significantly differ between men and women in examined MC phases (p>0.05), but heart rate recovery perceived through T30 was faster in men in compare to women in all MC phases (Table 4). Not one parameter showed differences in various phases intra females (p>0.05).

**Discussion**

Opposed to what we have expected, males had a more favourable autonomic profile than females. Our study shows that males have greater resting and post-exercise overall HRV, as well as faster HRR no matter in which phase of menstrual cycle women are in. Contrary to findings of some authors (21,22), we did not find intra-subject HRV differences in regard to cycle phase during rest. Surprisingly, both RMSSD and lnLF were augmented in early follicular in compare to luteal phase.

The anaerobic capacity results (PP,MP) after the Wingate test were in accordance with the existing literature (16,23,24). Muscle hypertrophy and variations in muscle fibre type are, allegedly, main causes for higher values of PP and MP in men. There is a prevalence of slow twitching fibres in skeletal muscle sections (25,26) in women. Also, some authors suspect that differences in sarcomeral metabolism might have an influence on divergence in muscle power between sexes (27). Similarly to recently published results (28,29), the difference in anaerobic power parameters in women concerning menstrual cycle phases was not observed in our paper.
In general population, sympathetic nervous system activity is higher in men and parasympathetic in premenopausal women (12,14,30–32). Time and frequency domain differences between genders gradually fade out with growing age and more progressively after a third decade (33). Numerous papers point out that the differences disappear after the age of 50 years (12,34), which is addressed to postmenopause and a lowered protective effect of endogenous sex hormones in women. In our study, resting values of lnLF were higher in men, which is in agreement with above mentioned, if we consider LF as a marker of solely sympathetic activity. But LF portrays joined actions of both autonomic branches with a slight predominance in sympathetic activity, especially after workout (35). LF also represents oscillations in baroreceptors system (36,37) and baroreflex sensitivity (BRS) is said to be higher in men while in rest (38). Our participants had their HRV registered in a sitting position, which provokes the sympathetic response, but we saw no change in lnLF while comparing females in different phases, although the baroreflex response of a sympathetic component in women is found to be more pronounced in menstrual and/or luteal phase (21). In fact, significant number of papers imply that sympathetic nervous system is more active during luteal phase (5,7,10), but there are also ones where no differences between phases were observed (6,9). On the other hand, our results also show that parasympathetic influence (lnHF) during rest is more prominent in male sex in comparison to women in early follicular and luteal menstrual cycle phase. The lack of differences between genders when females were in late follicular phase might express the evolving vagal tone while approaching peak levels of estrogen. Despite a much greater number of opposing results (39–41) that did not take MC into account, it appears that shift work can indeed influence female HRV depending on MC phase (8). In this case follicular phase shows a fall in vagal and an increase in sympathetic activity. It is possible that the results we got might have an explanation in stress and lack of sleep that medical students deal with, which may have heightened sympathetic tone in male and lessened parasympathetic tone in female participants. On the other side, our study lacks information about physical activity level. Greater participation in recreational sport could explain prevailed vagal indices in men.

Markedly higher lnLF and lnHF values were obtained in males after the Wingate test in compare to women in all menstrual cycle phases. These findings contrast the ones found by authors who reported higher values of HF in women during recovery from the test.
for maximal oxygen consumption and concluded that women have faster vagal post-exercise reactivation (15). In general, HRR is faster after a Wingate test and recovery after an incremental VO2max test sometimes takes several days (42,43), but supra-maximal exercise has a greater impact on autonomic modulation in women. Significantly decreased HF power after a Wingate test in females in contrast to males was reported in one study with upright sitting position where only vagal indices were analyzed (16), and significant increase in LF power was reported in another where recovery took place in a supine position (17). Despite that men have accentuated resting BRS, women might possess a higher diapason of its effect during post-exercise recovery. This was supported by a persistent reduction in heart rate in women while seated, but not in men (38). Contrary to this, another study found that seated position provokes less favourable recovery than supine (19). In our study women had a slower HRR, and a lesser lnLF after exercise. Although stress can be addressed for suppressed BRS (44), our participants were subjected to same levels of it. Maybe poor engagement in sport in our female participants can be hold responsible for such results, but we do not have evidence to support that.

Intra-subject differences in HRV during recovery were observed in females. A marker of vagal activity, RMSSD, was higher in early follicular phase in compare to luteal. Among eumenorrheic women, even in those who report premenstrual symptoms, resting RMSSD is mostly higher in follicular in compare to luteal phase (10,45). However, same authors consider follicular phase as the one that follows menstrual phase, starting at day 8 or 9 of the MC, which is by our classification addressed as the late follicular phase. We also found that post-exercise lnLF was higher in early follicular in relation to luteal phase. Whether dysmenorrhoea can be the cause of disrupted autonomic modulation was a matter of subject in various papers (46,47), which stated that slight increase in LF/HF can exist during menstruation pointing out to a fall in parasympathetic activity. But in our female population vagal index – RMSSD, was marked in early follicular phase. It is possible that BRS during post-exercise recovery is more pronounced in early follicular in compare to other phases, or that the parasympathetic component of low frequency domain is augmented.

Resting heart rate did not differ between sexes in our study. Literature shows favourably lower resting heart rates in men (16,48,49). The lack of this difference in our results might be because of the small sample size, but also because of the specificity of
medical student population. There are proofs that stress and work in shifts can significantly lower HRV indices (SDNN, TP, HF) among male health workers, without greater interfering with these indices in females (39–41). It is possible that similarly stressful life milieu of our participants influenced diminished differences in genders. Maximally achieved heart frequencies did not stand in contrast between men and women in our study, which is consistent to previous results (15,17,23).

Our results show, in accordance to previous findings (16), faster vagal reactivation in males, perceived by T30 which is an immediate post-exercise index of vagally mediated cardiac rate decay (50,51). HRR was found to be in a strong correlation with the level of physical activity (52). It was also found to negatively correlate with resting supine parasympathetic markers of HRV when in standing position during the first minute of recovery, but the higher the indices of combined autonomic modulation were (LF, lnLF), greater was the HRR in third and fifth minute post-exercise (53). Existing data report no correlation of estradiol levels with initial HRR dynamics (38). The same as in a study of Pestana et al (29), we did not find statistical differences between menstrual cycle phases and HRR.

To conclude, men have a greater total variability and a more favourable autonomic profile during rest and in a seated recovery after a Wingate test. Our study supports the notions that supra-maximal exercises present a heavier load to female autonomic nervous system. It is possible that products of anaerobic metabolism and muscle metaboreflexes are in a way responsible for this. We would also like to instigate more research towards understanding HRV dynamics in respect to early and late follicular phases. Our guesses are that in a resting state, vagal influence could be expected in late follicular phase coinciding with the peak levels of estrogen. On the other hand, in a recovery state, vagal reactivation might preferably be recorded in early follicular phase, before the preovulatory FSH and LH surge happen.

REFERENCES


Table 1

Resting heart rate variability indices in men and women in early follicular menstrual cycle phase, Mean ± SD [95% CI]

<table>
<thead>
<tr>
<th>Indices</th>
<th>Men</th>
<th>Women</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMSSD</td>
<td>27.25±8.27</td>
<td>29.25±15.82</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>[22.84; 31.66]</td>
<td>[22.24; 36.27]</td>
<td></td>
</tr>
<tr>
<td>lnLF</td>
<td>7.49±0.51</td>
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<td></td>
<td>[7.21; 7.76]</td>
<td>[6.24; 6.99]</td>
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<tr>
<td>lnHF</td>
<td>6.32±0.58</td>
<td>5.73±0.83</td>
<td>&lt;0.019372</td>
</tr>
<tr>
<td></td>
<td>[2.82; 3.81]</td>
<td>[5.36; 6.09]</td>
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</table>

Table 2

Resting heart rate variability indices in men and women in late follicular menstrual cycle phase, Mean ± SD [95% CI]

<table>
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<tr>
<td>RMSSD</td>
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<td>31.87±14.92</td>
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<td></td>
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<td>[25.41; 38.32]</td>
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<td>[6.29; 6.93]</td>
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<tr>
<td>lnHF</td>
<td>6.32±0.58</td>
<td>5.93±1.10</td>
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<tr>
<td></td>
<td>[2.82; 3.81]</td>
<td>[5.45; 6.40]</td>
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</table>
Table 3

Resting heart rate variability indices in men and women in luteal menstrual cycle phase, Mean ± SD [95% CI]

<table>
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<th>Luteal phase</th>
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<td></td>
<td>Men</td>
<td>Women</td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td>RMSSD</td>
<td>27.25±8.27 [22.84; 31.66]</td>
<td>28.66±12.92 [23.20; 34.11]</td>
<td>p&gt;0.05</td>
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<tr>
<td>lnLF</td>
<td>7.49±0.51 [7.21; 7.76]</td>
<td>6.47±0.82 [6.12; 6.81]</td>
<td>p=0.0000232</td>
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<tr>
<td>lnHF</td>
<td>6.32±0.58 [2.82; 3.81]</td>
<td>5.67±0.89 [5.29; 6.05]</td>
<td>p=8.79E-10</td>
<td></td>
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</table>
Table 4

Resting heart rate and heart rate recovery indices after a Wingate anaerobic test in men and women in different menstrual cycle phases, Mean ± SD [95% CI]

<table>
<thead>
<tr>
<th></th>
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<th>Women – late follicular phase</th>
<th>Women – luteal phase</th>
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<td><strong>HRrest</strong></td>
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<td>89±12</td>
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<td>90±13</td>
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<tr>
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<td>[84; 95]</td>
<td>[70; 95]</td>
<td>[81; 93]</td>
<td>[84; 95]</td>
</tr>
<tr>
<td><strong>HRmax</strong></td>
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<td>186±7</td>
<td>185±9</td>
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<td>[182; 191]</td>
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<td><strong>HR60</strong></td>
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<td>[27; 33]</td>
<td>[25; 33]</td>
<td>[27; 37]</td>
</tr>
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<td><strong>HRend</strong></td>
<td>116±6</td>
<td>113±13</td>
<td>110±15</td>
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<td></td>
<td>[112; 119]</td>
<td>[107; 119]</td>
<td>[104; 117]</td>
<td>[108; 122]</td>
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<td><strong>T30</strong></td>
<td>262.1±91.8</td>
<td>621.4±161.1*</td>
<td>607.04±150.6†</td>
<td>661.34±206.9‡</td>
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<td>[213; 311]</td>
<td>[548; 695]</td>
<td>[538; 675]</td>
<td>[570; 753]</td>
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<td><strong>T</strong></td>
<td>135.7±53.1</td>
<td>134.2±47.1</td>
<td>123.7±44.2</td>
<td>117.8±43.4</td>
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<td>[107; 164]</td>
<td>[112; 156]</td>
<td>[102; 145]</td>
<td>[98; 138]</td>
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* *p=6.88E-10; † p=4.72E-10; ‡ p=4.56E-09
Received on January 29, 2019.
Revised on April 7, 2019.

Accepted June 21, 2019.
Online First September, 2019.