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ΥΠΕΥΘΎΝΟΣ ΚΑΘΗΓΉΤΗΣ: Δρ ΓΙΑΝΝΗΣ ΚΟΥΤΕΝΤΑΚΉΣ ΑΚΑΔΗΜΑΪΚΟΣ ΣΥΜΒΟΎΛΟΣ: Δρ ΑΝΔΡΕΑΣ ΦΛΟΎΡΗΣ

Abstract

Previous studies have demonstrated that exercise training results in adaptations of the autonomic nervous system by increasing the parasympathetic nervous system (PNS) activity at rest, which is an indicator of a good health profile. On the other hand, physical inactivity has been associated with decreased PNS activity and a risk factor for cardiovascular diseases. Heart rate variability (HRV) is a non-invasive valid method and has been widely used to assess the activity of the autonomic nervous system and as well as an indicator of cardiovascular health problems. The aim of the present study was to examine the differences that might appear in HRV among runners as well as non-athletic subjects prior to, during, and after a submaximal test of aerobic capacity. Methods: All participants underwent the same experimental protocol which consisted of questionnaires concerning their health history and physical activity, anthropometric measurements and the 6 minute walk test (6MWT). HRV measurements were recorded prior to the test at rest for five minutes, during the 6MWT and throughout the recovery time, which was divided into two 5-min intervals, using a Polar PS800CX (Polar Electro Oy, Kempele, Finland). The HRV indices studied were the root mean square of differences of successive R-R intervals (rMSSD) for the time domain, as well as the ratio (LF/HF) of the low (LF) and high frequency (HF) for the frequency domain. Results: The main results of the present study were the statistically significant differences found between the two groups in the rMSSD (p<0.05) in the 5-min interval before the 6mwt at rest and the first 5-min interval during recovery, with runners demonstrating higher values than non-runners. Conclusions: Our study suggests that runners have a cardiovascular autonomic adaptation with higher HRV and PNS activation than non-runners. This may indicate

that endurance training is associated with a lower risk of cardiovascular diseases in the future.

Key words: runners, athletes, non-athletes, exercise, endurance training, physical activity, heart rate variability,

Περίληψη

Προηγούμενες μελέτες έχουν δείξει ότι η άσκηση προκαλεί προσαρμογές στο αυτόνομο νευρικό σύστημα αυξάνοντας την δράση του παρασυμπαθητικού νευρικού συστήματος (ΠΝΣ) στην ηρεμία, το οποίο είναι επιδεικνύει καλό προφίλ υγείας. Από την άλλη πλευρά, η έλλειψη φυσικής δραστηριότητας έχει συσχετιστεί με μειωμένη δραστηριότητα του ΠΝΣ και επίσης προδιάθεση για καρδιαγγειακά νοσήματα. Η διακύμανση καρδιακής συχνότητας (ΔΚΣ) είναι μία μη παρεμβατική και έγκυρη μέθοδος και έχει χρησιμοποιηθεί ευρέως για την αξιολόγηση της δραστηριότητας του αυτόνομου νευρικού συστήματος καθώς και ως δείκτης για την διάγνωση καρδιαγγειακών προβλημάτων υγείας. Ο σκοπός της παρούσας μελέτης ήταν να εξεταστούν οι διαφορές που μπορεί να προκύψουν στην ΔΚΣ μεταξύ δρομέων καθώς και μη αθλητών πριν, κατά τη διάρκεια και έπειτα από ένα υπομέγιστο τεστ αερόβιας ικανότητας. Μεθοδολογία: Όλοι οι συμμετέχοντες υποβλήθηκαν στο ίδιο πειραματικό πρωτόκολλο το οποίο αποτελούνταν από ερωτηματολόγια που αφορούσε το ιατρικό ιστορικό τους και τα επίπεδα φυσικής τους δραστηριότητας, την καταγραφή των ανθρωπομετρικών τους χαρακτηριστικών και την εξάλεπτη δοκιμασία βάδισης (6MWT). Η καταγραφή της ΔΚΣ πραγματοποιήθηκε πριν το τεστ σε ηρεμία, διάρκειας πέντε λεπτών, κατά τη διάρκεια του 6ΜWT και κατά τη φάση της αποκατάστασης έπειτα από το τεστ, η οποία καταγραφή χωρίστηκε σε δύο χρονικά διαστήματα των πέντε λεπτών, χρησιμοποιώντας το καρδιοσυχνόμετρο Polar PS800CX (Polar Electro Oy, Kempele, Finland). Οι δείκτες της ΔΚΣ που μελετήθηκαν ήταν η μέση τετραγωνική ρίζα των διαφορών μεταξύ διαδοχικών διαστημάτων R-R (rMSSD), και ο λόγος (LF/HF) της χαμηλής (LF) και υψηλής συχνότητας (ΗΕ). Αποτελέσματα: Τα κύρια αποτελέσματα της παρούσας μελέτης ήταν οι στατιστικά σημαντικές διαφορές που βρέθηκαν μεταξύ των δύο ομάδων (δρομείς, μη-δρομείς). Οι διαφορές αυτές αντιστοιχούν στον δείκτη rMSSD (p<0.05) στην καταγραφή ΔΚΣ σε ηρεμία πριν την εφαρμογή του 6MWT και στο πρώτο χρονικό διάστημα της αποθεραπείας, επιδεικνύοντας ότι οι δρομείς είχαν υψηλότερες τιμές του προαναφερόμενου δείκτη σε σχέση με τους μη-δρομείς. Συμπεράσματα: Η συγκεκριμένη έρευνα υποδηλώνει ότι οι δρομείς έχουν προσαρμογές του αυτόνομου νευρικού συστήματος και της καρδιακής λειτουργίας με υψηλότερη ΔΚΣ και δραστηριοποίηση του ΠΝΣ σε σχέση με τους μη δρομείς. Αυτό μπορεί να υποδεικνύει ότι η προπόνηση αντοχής σχετίζεται με χαμηλότερη προδιάθεση για καρδιαγγειακά νοσήματα στο μέλλον.

Λέζεις κλειδιά: δρομείς, αθλητές, μη-αθλητές, άσκηση, φυσική δραστηριότητα, προπόνηση αντοχής, διακύμανση καρδιακής συχνότητας

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Introduction

It is widely known that half marathon runners (21,100 meters) and marathon runners (42,195 meters) usually have a very good health status and aerobic fitness. This typically results in having high heart rate variability (HRV) (Kiss et al., 2016). Several studies have also shown that endurance training is found to cause increased vagal tone with a lowered heart rate compared to normal population (Verlinde, Beckers, Ramaekers, & Aubert, 2001) as long as increased parasympathetic activity compared with other type of training (Abad et al., 2014). It is also estimated that in their elderly life they will have less health problems than people who did not exercise regularly; ending up having a better quality of life than non-athletes (Sotiriou, Kouidi, Samaras, & Deligiannis, 2013).

On the other hand, scientific evidence indicates that physical inactivity and therefore sedentary lifestyle can be the lead cause of most chronic diseases (Booth, 2012). As a matter of fact, physical inactivity is associated with decreased vagal tone, which is a risk factor for cardiovascular disease (Rennie et al., 2003) and it is also associated generally with a more poor health profile than those who are training regularly. Moreover, it has been shown that overweight or obese individuals have reduced HRV (Yadav et al., 2017) along with a higher risk of developing health problems later in life, including cardiovascular disease (Kaikkonen et al., 2014). Given the fact that the negative outcomes of sedentary life and physical inactivity are well established up to day, it would be very important to develop a practical and non-invasive method to detect early-in-advance predisposition for cardiovascular pathologies.

The method of assessing HRV is a promising candidate for detecting early-in-advance predisposition for cardiovascular pathologies. HRV is a straightforward and cost

effective technique to foresee health issues of cardiovascular nature and can be used to predict in advance smoking-induced health effects that may arise in the future (Hayano et al., 1990). Indeed, HRV abnormalities are linked with many cardiovascular diseases, including ischemic disease (Camm et al., 2004) and heart failure (Bilchick et al., 2002), and provide prognostic information for adverse outcomes (Tapanainen et al., 2002). However, there is a lack in the literature concerning the indices of the HRV before and after a submaximal test, while measuring heart rate at rest and recovery between runners and non-runners. This information will be valuable to strengthen the knowledge of public health as well as to scientists and physicians.

Heart rate variability (HRV)

Heart rate variability describes the variations of the R-R intervals, which is the time elapsing of R waves (from the QRS complex) between two consecutive heart beats throughout various recordings of an electrocardiogram (Electrophysiology, 1996). It is a valid technique and has been widely used as a non-invasive method to assess the activity of the autonomic nervous system (Agnieszka Zygmunt & Jerzy Stanczyk, 2010). The autonomic nervous system is divided into the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) which they have generally opposing actions (Gordan, Gwathmey, & Xie, 2015). The SNS and the PNS (the autonomic nervous system) are regulating the heart rate, the rhythm and the contractility of the heart (Franciosi et al., 2017; Gordan et al., 2015). As a matter of fact, the sympathetic activation increases heart rate and myocardial contractility while the parasympathetic stimulation decreases heart rate (Gordan et al., 2015). The SNS is interceded primarily through the actions of the neurotransmitter norepinephrine (noradrenaline) while the PNS is mediated primarily through acetylcholine activation

(Gordan et al., 2015; Shivkumar et al., 2016). It has been shown that an increase of the sympathetic nervous system that maybe along with or without a reduction in the parasympathetic nervous system can end up to ventricular tachycardia, subsequent arrhythmias, and in some severe cases, cardiac death (Franciosi et al., 2017). Furthermore, increased sympathetic activity may have accordance with reduced HRV (La Rovere et al., 2003). As following, low HRV is considered as a marker of less good health profile (Dekker et al., 2000), indeed reduced HRV has been shown to be a predictor of mortality after an acute myocardial infraction (Bigger et al., 1992) and also of mortality from all causes (Dekker et al., 1997). Moreover, it has been associated with increased risk of coronary heart disease (Dekker et al., 2000) and with risk for cardiac event (Tsuji et al., 1996). While on the other hand higher HRV is associated with healthy longevity (Zulfiqar, Jurivich, Gao, & Singer, 2010) and PNS activation at rest an indicator of a good health profile.

Exercise and HRV

It is well established that exercise training results in adaptations of the autonomic nervous system by increasing the PNS activity and decreasing SNS activity (Yamamoto, Miyachi, Saitoh, Yoshioka, & Onodera, 2001). Numerous studies have evaluated the autonomic nervous activity by studying the effect of physical activity on the HRV which is a valid and non-invasive method that reflects the autonomic nervous system activity. Moderate to vigorous activity has been associated with an increase in the vagal tone and a decrease in the resting heart rate (Carter, Banister, & Blaber, 2003) especially in endurance-trained athletes compared with sedentary subjects (Verlinde et al., 2001) while the opposite happens with physical inactivity which is associated with decreased vagal tone and a risk factor for cardiovascular disease (Rennie et al., 2003). It has been also found that long term endurance training

prevents the natural decrease of the HRV with age (Antelmi et al., 2004; Zhang, 2007) in elderly people (Galetta et al., 2005). Moreover, there are some studies held that when a 24h electrocardiogram monitoring was performed they are suggesting that athletes had higher time domain parameters of HRV than sedentary people (Kiss et al., 2016; Sotiriou et al., 2013) which shows increased parasympathetic adaptation. Furthermore, the parameters of HRV have also been investigated during an exercise protocol showing that the time domain parameters and the frequency domain were higher in endurance athletes rather than non-athletes (Kaltsatou, Kouidi, Fotiou, & Deligiannis, 2011). Finally, it can be implied that the endurance exercise and aerobic physical training can lead to a cardiovascular health development throughout the population.

Purpose

The aim of this study was to investigate the differences, if any, that may exist in heart rate variability among runners as well as individuals with normal or high (i.e., overweight/obese) body mass index (BMI) prior to, during, and after a submaximal test of aerobic capacity (6-min walk test).

Null Hypothesis

Heart rate variability will not have statistically differences between the two groups.

Alternative Hypothesis

Heart rate variability will differ statistically important between runners and non-runners.

METHODS

Participants

Fifteen healthy adults consented to participate voluntarily in the study from which they were divided into two groups, runners (7 male: age 32.3±4.9 years, BMI 24.9±3.6 kg/m², total body fat percentage 22±5.7%) and non-runners (4 male: age 34.75±3.4 years, BMI 25.8±3.2 kg/m², total body fat percentage 30.3±10.9% and 4 female: age 31.5±4 years, BMI 20.8±3.6 kg/m², total body fat percentage 32.7±11.4%). The physical characteristics of the participants can be seen as well in *Table 1*.

Table 1 Physical characteristics of the study population (means ± SD)

Groups	n	Age ± SD (year)	BMI ± SD (kg/m²)	Total fat ± SD (%)
Runners	7	32.3 ± 4.9	24.9 ± 3.6	22.1 ± 5.7
Non-runners	8	33.1 ± 3.9	23.3 ± 4.2	31.5 ± 10.4

Runners were considered the ones that were training regularly (\geq 3 times/week) in long distance running and have completed a half marathon or marathon within the past 12 months.

Non-runners were considered the ones that did not train regularly (<3 times/week) and who had not completed a half marathon or marathon within the past 12 months. Two participants in this group had thyroid and were included since they had had their medication under control for the last past years before they perform the measurements of the study.

Inclusion criteria included: Males and females were able to participate in the present study of ages between 20 and 40 years old, from any ethnicity.

Exclusion criteria included: Smoking within the last 2 years, pregnancy, recent muscle/joint/bone problems or injuries, upper respiratory illness or gastroenteritis, diabetes or any other chronic disease, current medications or stimulants including antidepressants, diuretics, antihypertensives, antihistamines, Ma Huang, ephedra, or pseudoephedrine, neurological illness with known Autonomic Nervous system effects, unstable angina and myocardial infarction during the previous month, resting heart rate of more than 120, a systolic blood pressure of more than 180 mmHg, a diastolic blood pressure of more than 100 mmHg.

The study was conducted in the Laboratory of Environmental Physiology, in the department of Physical Education and Sport Science at the University of Thessaly, Trikala, Greece. All participants prior the initiation of the measurements for the purposes of the present investigation they were informed about the procedure and the purpose of it and signed an informed consent form which was approved as long as the experimental protocol by the ethical review board at the University of Thessaly.

Procedures

All measurements were performed within the arrival of the participants at the laboratory in a random order between 1:00 and 18:30 am. An initial discussion about the experiment was organized with each participant to ensure complete understanding of the protocols and the procedures, benefits and associated risk of the study prior to their participation. During this discussion, which was made orally over the phone by an investigator, each subject was also instructed to have a full eight-hour sleep before running the experimental protocol, moreover to not perform any training activities or

any form of exercise for 24 hours before each measurement, as well as abstain from alcohol, caffeine and passive smoking for at least 15 hours before their arrival at the laboratory, further avoid consumption of food for 6 hours before their measurements.

Participants in both groups were also permitted *ad libitum* water ingestion prior to the initiation of the experimental protocol to promote euhydration.

Last but not least concerning their instruction as far as concerning the clothing they were notified to use appropriate shoes for walking, additionally wear comfortable clothing without any metallic detail on them for the correct interpretation of the DEXA analysis.

During their visit, all participants completed three questionnaires that were about assessing their health history and physical activity which were completed in the form of an interview by the same researcher to eliminate the chance of unfulfilled answers or misinterpretation of any question. Those consisted of the Fame Lab questionnaire which is a form of medical history developed by the Fame laboratory itself and approved by the ethical committee of the University of Thessaly. Further they completed the Physical Activity Readiness Questionnaire for Everyone (Parq) (Thomas, Reading, & Shephard, 1992) to examine their health history and the International Physical Activity Questionnaire (IPAQ) (Papathanasiou et al., 2009) to assess the level of their physical activity throughout a typical week. The volunteers also underwent anthropometric measurements that included the assessment of weight, height and body composition analysis with DEXA. The same day they also performed the 6-min walk test (6MWT) and heart rate variability was recorded 10 minutes at rest prior to, during, as well as 10 minutes following the test. During the recordings of HRV participants were sitting on a chair and were instructed to remain silent and as

calm as possible succeeding to relax and breathe at a natural rate (Penttila et al., 2001) throughout the data collection. The resting HRV data collection took place in a quiet room while being observed by an investigator to reassure the obedience of the participants to the instructions that were given. Also, arterial blood pressure was recorded prior as well as following the test throughout the recovery phase. The experimental protocol was conducted by the same order from everyone like it was mentioned above and its duration was approximately 70 minutes.

Anthropometric measurements

Body height and weight data were collected using standardized techniques and instruments. In particular, the height was recorded to the nearest centimeter. The measurement of the weight was carried out (precision to the nearest of 0.001 kg) with precise weight scale (KernDE 150K2D, KERN&SOHNGmbH, Balingen, Germany). Percent of body fat, body composition was assessed with DEXA analysis.

6-Minute Walk Test (6MWT)

The 6MWT was performed indoors along a 20m long flat, straight, enclosed corridor with a hard surface that is seldom traveled. The length of the corridor was marked every 2 meters. The turnaround points were marked with a bright colored tape as long as the starting line which coincides with the beginning and the end of each 40m lap (Laboratories, 2002).

For the purposes of the study a 30m long corridor was not found necessary, even though it may affect the outcome of the total score of this test, hence the objective in this case is the comparison between two different population groups during a submaximal form of exercise-or test on their HRV and not the implementation of the total score-distance covered from each group. Also, other investigations using 6MWT

have had the execution of the test in a corridor with less than 30 meters (Lipkin, Scriven, Crake, & Poole-Wilson, 1986), (Troosters, Gosselink, & Decramer, 1999).

Hear Rate Variability (HRV) Measurements

Participants were outfitted with a heart rate chest strap, and HRV data were being sampled for 10 min prior to, during, as well as for 10 min following the 6MWT through short-range telemetry at 1,000 Hz with a Polar RS800CX (Polar Electro, Kempele, Finland). Data collected during each phase were split into three recordings of 5-min intervals and one recording of 6-min interval for the 6MWT. The heart rate monitor signal was being transferred after each session to the Polar Precision Performance Software (release 3.00; Polar Electro Oy), and R-R intervals (beat-tobeat interval) were to be analysed in order to assess the modulation of sympathetic and parasympathetic activities as previously described (Flouris & Cheung, 2009). A computer software Kubios version 1.1 (Finland: Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland 2002) was used to perform the HRV analysis in both the time domain and frequency domain. The indices studied for the time domain were the root mean square of successive differences (rMSSD) with a normative value of 27± 12 ms which describes short-term variation and thus reflects parasympathetic activity (A. Zygmunt & J. Stanczyk, 2010) and also because it can be used to investigate recordings of short durations and has better statistical properties than other HRV indices (Electrophysiology, 1996). Additionally, the frequency domain variables examined were as well the LF/HF ratio (low frequency range, high frequency range) expressed in normalised units, which is considered to mirror sympathovagal balance (Electrophysiology, 1996) with a normal power of 1.2 ± 1 (Freeman, Dewey, Hadley, Myers, & Froelicher, 2006) and according to the HRV standards of measurement 5 min recordings are suggested and enough for addressing the LF, HF component and as a result the ratio of LF/HF (Electrophysiology, 1996).

Body composition

A DEXA system (Lunar model DPX Madison, WI) was used to assess the participants' body composition indicatively to measure the participant's whole-body fat and lean body mass. The risk associated with DXA testing is diminutive. The radiation dose from Dual Energy Absorptiometry is very low 0.02 μ Gy (<1% CV) which the volunteers where exposed to, when comparing this exposure with a dental X-ray which is 1mRem and an X-ray of the thorax which is 6mRem. Participants were also placed in the supine position in order to be scanned from head to toe while lying on their back with their arms at their side. Standard scanning setup procedures (recommended by the manufacturer) were used. The scan time for each subject was approximately 15 minutes.

Statistical Analysis

For the analysis of HRV we took the values from the second 5-min interval recorded (5-min pre) at rest prior to the 6MWT and we split the 10-min recording of the recovery time after the 6MWT into two 5-min intervals (5-min post1, 5-min post2). We performed the test of homogeneity between the variables and the time intervals and it was found that the values are non-normally distributed. Taking that into consideration we made all the statistical analysis with non-parametric tests for examining the HRV variables throughout the time intervals for the entire sample and between the groups.

Primarily a Kruskal-Wallis analysis was conducted for rMSSD and LF/HF ratio to examine if any differences would appear throughout the entire sample between the time intervals, the same test was conducted individually per group to examine further where the differences that might appear from the previous Kruskal-Wallis test come from. In addition, a Wilcoxon test (W) was conducted in order to examine the differences that might appear in the whole sample or in the groups separately within each time interval in the values of RMSSD and LF/HF. Last but not least a Mann-Witney (U) test was conducted in order to compare the two variables rMSSD and LF/HF in each time interval individually between the two groups (runners, non-runners). All the statistical analysis were completed using SPSS 22.0 and the level of significance was set at p<0.05.

RESULTS

The HRV indices that were studied are show in Figure 1 and 2. To begin with the results from the Kruskal-Wallis test were calculated and showed that there were statistically significant differences in the HRV variable of rMSSD ($x^2 = 19.085$, df = 3, p<0.001 or p<0.005?), and of LF/HF ($x^2 = 17.271$, df = 3, p<0.005) between the time intervals throughout the whole sample.

The results of the Wilcoxon test that was performed for the whole sample as well, showed that there were statistically significant differences in both rMSSD and LF/HF ratio within most of the time intervals (p<0.05) except for the differences found between the 5-min post1 and the 5-min post2 intervals which they were not statistically significant (p>0.05) for both of the pre-mentioned variables.

Further, statistically significant differences were found separately in the group of runners when interpreting the Kruskal-Wallis analysis between the time intervals and the rMSSD value ($x^2 = 16.938$, df = 3, p<0.005), as well as for the LF/HF value ($x^2 = 15.169$, df = 3, p<0.005).

The Wilcoxon test that was performed individually for the group of runners showed some statistically significant differences once again between the time intervals for both variables of HRV for runners.

In more detail, the value LF/HF showed statistically significant differences in most of the time intervals (p<0.05), except for the time interval 5-min post1 and the 5-min post2 that when being compared they didn't show any statistically significant differences with each other (p>0.05) unlike the rest of the time intervals (fig.2).

Concerning the rMSSD variable statistically significant differences were found between the time intervals 6mwt and 5-min pre, 5-min post2 and 5-min pre, 5min post1 and 6mwt and last but not least between the 5-min post2 and the 6mwt (p<0.05). The differences found within 5-min post two time interval and 5-min post1 were not statistically significant (p>0.05), as well for the 5-min post1 and the 5-min pre but in this case a trend appears towards statistically significant differences (p=0.063) (fig.1).

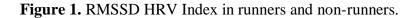
On the contrary, the Kruskal-Wallis test that was conducted *for the group of non-runners* there were not found statistically significant differences between both values rMSSD ($x^2 = 4.503$, df = 3, p>0.005) and LF/HF ($x^2 = 5.070$, df = 3, p>0.050) within the time intervals respectively.

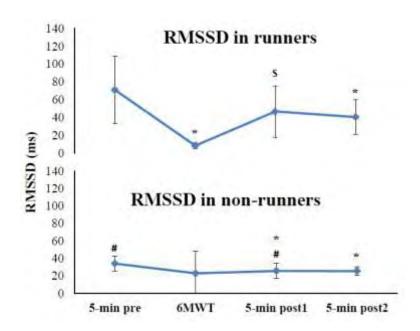
The analysis that were done with the Wilcoxon test separately for the group of non-runners comparing the rMSSD within the time intervals showed non-significant differences in most of them (p>0.05) except for the comparison between the time intervals 5-min post1 and the 5-min interval pre, the 5-min post2 and also the 5-min interval pre that statistically significant differences were found (p<0.05) (fig.1).

The analysis made for the LF/HF variable showed that between most of the time intervals there were not found statistically significant differences (p>0.05) except for the time intervals between the first 5-min recording after the 6MWT (5-min post1) and the recording prior to the 6MWT (5-min pre), as well as when comparing the 6MWT and the second 5-min interval during recovery (5-min post2) which statistically significant differences were found (p<0.05) (fig.2).

The results of the Mann-Whitney (U) analysis demonstrated statistically significant differences in the rMSSD value when comparing the group of runners and the group

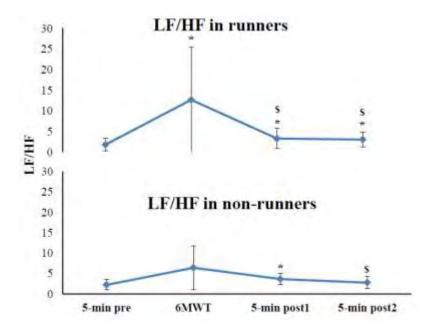
of non- runners at the 5-min pre time and at the 5-min post1 intervals (p<0.05). A trend to statistically significant differences also appeared (p= 0.94) when comparing once again the two groups with the same variable (rMSSD) at the 5-min post2 time interval (fig.1). Additionally, for the LF/HF variable there were not any statistically differences between the two groups for any time interval throughout the recordings (p>0.05) (fig.2).





^{*}Significant different from runners at the same time point; *Significant different from 5-min pre at the same group; *Significant different from 6MWT at the same group

Figure 2. LF/HF HRV Index in runners and non-runners.



*Significant different from 5-min pre at the same group; \$Significant different from 6MWT at the same group

DISCUSSION

The purpose of this study was to examine the differences that might appear on HRV measurements prior (at rest), during a submaximal test for aerobic capacity (6MWT) and after the exercise test (during recovery). Other studies have compared these two subgroups of population at rest using 24hour electrocardiogram recorded with Holter (Kiss et al., 2016; Sotiriou et al., 2013), during exercise protocols (Kaltsatou et al., 2011; Martinelli et al., 2005). However, there is a lack in the literature considering short-term HRV recordings prior, during and after exercise protocols; hence there are not yet standard measurements for the length of the time recordings and the exercise intensity. Although the non-standard exercise protocols for HRV measurements, this is the first time there is such an experimental protocol between endurance athletes and non-athletes that are undergoing a 6MWT and comparing the indices of HRV prior during and after the test throughout the recordings of HRV with polar.

Our results indicate that endurance training increases the parasympathetic modulation in the runners by increasing the time domain rMSSD variable of the HRV at rest (5-min recording prior the 6mwt) and recovery (post 5-min recording) since it has been linked with the parasympathetic activation (A. Zygmunt & J. Stanczyk, 2010) while supporting the hypothesis that there would be differences on HRV between runners and non-runners at rest and throughout the recovery time of the heart rate, presenting that runners had a faster heart rate recovery rate than non-runners. Other studies had similar findings with higher rMSSD values in athletes compared with non-athletes (Martinelli et al., 2005) while most of them suggest that this outcome is supporting the PNS enhancement at rest as a result of the increased endurance physical activity throughout different time recordings (Kiss et al., 2016; Sotiriou et al., 2013).

On the contrary, there were not found any statistical differences between the two groups in the LF/HF ratio which indicates the sympatho-vagal balance (Electrophysiology, 1996), throughout the recordings of HRV in the time intervals. Similar results found in another study with endurance athletes and sedentary people (Martinelli et al., 2005) which commonly they found an increase in the time domain parameters of the HRV (rMSSD) in endurance athletes but they did not correspond with the differences in the frequency domain parameters such as LF/HF ratio. One suggestion is that this outcome is related to intrinsic adaptations of endurance athletes in the sinus node instead of autonomic cardiovascular alterations.

Further research need to be done to study the association of the LF/HF ratio between endurance trained athletes and non-athletes.

Limitations of the study

One strong limitation of the present study would be the size of the sample. The specific size of the participants does not ensure 100% of statistical power. Moreover, the fact that there were not found statistically significant differences between the groups throughout some time intervals and between the LF/HF indice of HRV may be possibly due to the small sample size. Another limitation would be the male/female ratio in this study. Evidence in the literature show that it is needed to take into consideration the gender influences when performing analysis of the HRV since a relationship in gender and HRV indices in the short-term monitoring could be observed (Voss, Schroeder, Heitmann, Peters, & Perz, 2015).

Additional limitation would be the protocol used for the recording of the heart rate at rest. The method used, (polar with chest strap while seated) is not the most valid, since the best conditions would be for the participants to lie down on a supine position

in an isolated room to eliminate the possibility of strong movements or abnormal breathing caused by the environmental disturbances (noise etc.) that could interfere with the results. Furthermore, another drawback is the lack of confidence on whether our volunteers followed strictly the instructions considering the pre-measurement period. If someone for example would consume caffeine in the morning before their arrival at the lab that would interfere with the results without being aware of that. Finally, a restriction would be the fact that two female participants had thyroid problem and they were under treatment. Although we were informed they have been having their hormones under control and stable the last years once again there is no certainty that it did not alter our results in any level, since according to previous studies thyroid hyper/hypoactivity might affect the autonomic nervous system and it has been showed that hypothyroidism is associated with a decreased sympatho-vagal modulation of the heart rate (Foley, McAllister, & Hasser, 2001; Galetta et al., 2008).

Conclusions

Our results demonstrate that the time domain indicator of HRV, rMSSD is higher in athletes compared to the group of non-athletes during rest and at recovery after the submaximal test of 6MWT, showing a faster cardiovascular recovery time than non-runners. This may indicate the cardiovascular autonomic adaptation of the runners with higher PNS activation based on their endurance training compared with the group of non-runners at a similar age.

We failed to show an accordance of the time domain variable (rMSSD) and the frequency domain (LF/HF). Further research needs to be done to reveal the underlying association between time domain variable (rMSSD) and the frequency domain (LF/HF) in the endurance athletes (runners) and non-athletes.

Our study suggests that aerobic physical activity is associated with a better health profile and a higher HRV in runners and with a lower risk of cardiovascular diseases in the future.

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Conflict of Interest

The authors have no conflict of interest.

References:

- Abad, C. C., do Nascimento, A. M., Gil, S., Kobal, R., Loturco, I., Nakamura, F. Y., . . . Irigoyen, M. C. (2014). Cardiac autonomic control in high level Brazilian power and endurance track-and-field athletes. *Int J Sports Med*, *35*(9), 772-778. doi: 10.1055/s-0033-1363268
- Antelmi, I., de Paula, R. S., Shinzato, A. R., Peres, C. A., Mansur, A. J., & Grupi, C. J. (2004). Influence of age, gender, body mass index, and functional capacity on heart rate variability in a cohort of subjects without heart disease. *Am J Cardiol*, *93*(3), 381-385. doi: 10.1016/j.amjcard.2003.09.065
- Bigger, J. T., Fleiss, J. L., Steinman, R. C., Rolnitzky, L. M., Kleiger, R. E., & Rottman, J. N. (1992). Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation*, 85(1), 164-171. doi: 10.1161/01.cir.85.1.164
- Bilchick, K. C., Fetics, B., Djoukeng, R., Fisher, S. G., Fletcher, R. D., Singh, S. N., . . . Berger, R. D. (2002). Prognostic value of heart rate variability in chronic congestive heart failure (Veterans Affairs' Survival Trial of Antiarrhythmic Therapy in Congestive Heart Failure). *Am J Cardiol*, 90(1), 24-28. doi: S0002914902023809 [pii]
- Booth, F. W. (2012). Lack of exercise is a major cause of chronic diseases. 2(2), 1143-1211. doi: 10.1002/cphy.c110025
- Camm, A. J., Pratt, C. M., Schwartz, P. J., Al-Khalidi, H. R., Spyt, M. J., Holroyde, M. J., . . . Brum, J. M. (2004). Mortality in patients after a recent myocardial infarction: a randomized, placebo-controlled trial of azimilide using heart rate variability for risk stratification. *Circulation*, 109(8), 990-996. doi: 10.1161/01.CIR.0000117090.01718.2A
- 01.CIR.0000117090.01718.2A [pii]
- Carter, J. B., Banister, E. W., & Blaber, A. P. (2003). Effect of endurance exercise on autonomic control of heart rate. *Sports Med*, *33*(1), 33-46.
- Dekker, J. M., Crow, R. S., Folsom, A. R., Hannan, P. J., Liao, D., Swenne, C. A., & Schouten, E. G. (2000). Low Heart Rate Variability in a 2-Minute Rhythm Strip Predicts Risk of Coronary Heart Disease and Mortality From Several Causes. *The ARIC Study*, 102(11), 1239-1244. doi: 10.1161/01.cir.102.11.1239
- Dekker, J. M., Schouten, E. G., Klootwijk, P., Pool, J., Swenne, C. A., & Kromhout, D. (1997). Heart Rate Variability from Short Electrocardiographic Recordings Predicts Mortality from All Causes in Middle-aged and Elderly MenThe Zutphen Study. *Am J*

- *Epidemiol,* 145(10), 899-908. doi: 10.1093/oxfordjournals.aje.a009049
- Electrophysiology, T. F. o. t. E. S. o. C. t. N. A. S. o. P. (1996). Heart Rate Variability. *Standards of Measurement, Physiological Interpretation, and Clinical Use, 93*(5), 1043-1065. doi: 10.1161/01.cir.93.5.1043
- Flouris, A. D., & Cheung, S. S. (2009). Influence of thermal balance on cold-induced vasodilation. *J Appl Physiol*, 106(4), 1264-1271.
- Foley, C. M., McAllister, R. M., & Hasser, E. M. (2001). Thyroid status influences baroreflex function and autonomic contributions to arterial pressure and heart rate. *American Journal of Physiology Heart and Circulatory Physiology*, 280(5), H2061-H2068.
- Franciosi, S., Perry, F. K. G., Roston, T. M., Armstrong, K. R., Claydon, V. E., & Sanatani, S. (2017). The role of the autonomic nervous system in arrhythmias and sudden cardiac death. *Auton Neurosci*, 205, 1-11. doi: 10.1016/j.autneu.2017.03.005
- Freeman, J. V., Dewey, F. E., Hadley, D. M., Myers, J., & Froelicher, V. F. (2006). Autonomic Nervous System Interaction With the Cardiovascular System During Exercise. *Progress in Cardiovascular Diseases*, 48(5), 342-362. doi: http://dx.doi.org/10.1016/j.pcad.2005.11.003
- Galetta, F., Franzoni, F., Fallahi, P., Tocchini, L., Braccini, L., Santoro, G., & Antonelli, A. (2008). Changes in heart rate variability and QT dispersion in patients with overt hypothyroidism. *Eur J Endocrinol*, 158(1), 85-90. doi: 10.1530/eje-07-0357
- Galetta, F., Franzoni, F., Femia, F. R., Roccella, N., Pentimone, F., & Santoro, G. (2005). Lifelong physical training prevents the agerelated impairment of heart rate variability and exercise capacity in elderly people. *J Sports Med Phys Fitness*, 45(2), 217-221.
- Gordan, R., Gwathmey, J. K., & Xie, L. H. (2015). Autonomic and endocrine control of cardiovascular function. *World J Cardiol*, 7(4), 204-214. doi: 10.4330/wjc.v7.i4.204
- Hayano, J., Yamada, M., Sakakibara, Y., Fujinami, T., Yokoyama, K., Watanabe, Y., & Takata, K. (1990). Short- and long-term effects of cigarette smoking on heart rate variability. *Am J Cardiol*, 65(1), 84-88.
- Kaikkonen, K. M., Korpelainen, R. I., Tulppo, M. P., Kaikkonen, H. S., Vanhala, M. L., Kallio, M. A., . . . Korpelainen, J. T. (2014). Physical activity and aerobic fitness are positively associated with heart rate variability in obese adults. *J Phys Act Health*, 11(8), 1614-1621. doi: 10.1123/jpah.2012-0405
- Kaltsatou, A., Kouidi, E., Fotiou, D., & Deligiannis, P. (2011). The use of pupillometry in the assessment of cardiac autonomic function in

- elite different type trained athletes. *Eur J Appl Physiol*, 111(9), 2079-2087. doi: 10.1007/s00421-011-1836-0
- Kiss, O., Sydo, N., Vargha, P., Vago, H., Czimbalmos, C., Edes, E., . . . Merkely, B. (2016). Detailed heart rate variability analysis in athletes. *Clin Auton Res*, 26(4), 245-252. doi: 10.1007/s10286-016-0360-z
- La Rovere, M. T., Pinna, G. D., Maestri, R., Mortara, A., Capomolla, S., Febo, O., . . . Cobelli, F. (2003). Short-term heart rate variability strongly predicts sudden cardiac death in chronic heart failure patients. *Circulation*, 107(4), 565-570.
- Laboratories, A. T. S. C. o. P. S. f. C. P. F. (2002). ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med*, 166(1), 111-117. doi: 10.1164/ajrccm.166.1.at1102
- Lipkin, D. P., Scriven, A. J., Crake, T., & Poole-Wilson, P. A. (1986). Six minute walking test for assessing exercise capacity in chronic heart failure. *Br Med J (Clin Res Ed)*, 292(6521), 653-655.
- Martinelli, F. S., Chacon-Mikahil, M. P., Martins, L. E., Lima-Filho, E. C., Golfetti, R., Paschoal, M. A., & Gallo-Junior, L. (2005). Heart rate variability in athletes and nonathletes at rest and during head-up tilt. *Braz J Med Biol Res*, *38*(4), 639-647. doi: /S0100-879x2005000400019
- Papathanasiou, G., Georgoudis, G., Papandreou, M., Spyropoulos, P., Georgakopoulos, D., Kalfakakou, V., & Evangelou, A. (2009). Reliability measures of the short International Physical Activity Questionnaire (IPAQ) in Greek young adults. *Hellenic J Cardiol*, 50(4), 283-294.
- Penttila, J., Helminen, A., Jartti, T., Kuusela, T., Huikuri, H. V., Tulppo, M. P., . . . Scheinin, H. (2001). Time domain, geometrical and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns. *Clin Physiol*, 21(3), 365-376.
- Prinsloo, G. E., Rauch, H. G., & Derman, W. E. (2014). A brief review and clinical application of heart rate variability biofeedback in sports, exercise, and rehabilitation medicine. *Phys Sportsmed*, 42(2), 88-99. doi: 10.3810/psm.2014.05.2061
- Rennie, K. L., Hemingway, H., Kumari, M., Brunner, E., Malik, M., & Marmot, M. (2003). Effects of moderate and vigorous physical activity on heart rate variability in a British study of civil servants. *Am J Epidemiol*, *158*(2), 135-143.
- Shivkumar, K., Ajijola, O. A., Anand, I., Armour, J. A., Chen, P. S., Esler, M., . . . Zipes, D. P. (2016). Clinical neurocardiology defining the value of neuroscience-based cardiovascular therapeutics. *J Physiol*, *594*(14), 3911-3954. doi: 10.1113/JP271870

- Sotiriou, P., Kouidi, E., Samaras, T., & Deligiannis, A. (2013). Linear and non-linear analysis of heart rate variability in master athletes and healthy middle-aged non-athletes. *Med Eng Phys*, *35*(11), 1676-1681. doi: 10.1016/j.medengphy.2013.06.003
- Tapanainen, J. M., Thomsen, P. E., Kober, L., Torp-Pedersen, C., Makikallio, T. H., Still, A. M., . . . Huikuri, H. V. (2002). Fractal analysis of heart rate variability and mortality after an acute myocardial infarction. *Am J Cardiol*, 90(4), 347-352. doi: S0002914902024888 [pii]
- Thomas, S., Reading, J., & Shephard, R. J. (1992). Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci*, 17(4), 338-345.
- Troosters, T., Gosselink, R., & Decramer, M. (1999). Six minute walking distance in healthy elderly subjects. *Eur Respir J*, 14(2), 270-274.
- Tsuji, H., Larson, M. G., Venditti, F. J., Manders, E. S., Evans, J. C., Feldman, C. L., & Levy, D. (1996). Impact of Reduced Heart Rate Variability on Risk for Cardiac Events. *The Framingham Heart Study*, *94*(11), 2850-2855. doi: 10.1161/01.cir.94.11.2850
- Verlinde, D., Beckers, F., Ramaekers, D., & Aubert, A. E. (2001). Wavelet decomposition analysis of heart rate variability in aerobic athletes. *Auton Neurosci*, 90(1-2), 138-141. doi: 10.1016/S1566-0702(01)00284-3
- Voss, A., Schroeder, R., Heitmann, A., Peters, A., & Perz, S. (2015). Short-Term Heart Rate Variability—Influence of Gender and Age in Healthy Subjects. *PLoS One*, 10(3). doi: 10.1371/journal.pone.0118308
- Yadav, R. L., Yadav, P. K., Yadav, L. K., Agrawal, K., Sah, S. K., & Islam, M. N. (2017). Association between obesity and heart rate variability indices: an intuition toward cardiac autonomic alteration a risk of CVD. *Diabetes Metab Syndr Obes*, 10, 57-64. doi: 10.2147/dmso.s123935
- Yamamoto, K., Miyachi, M., Saitoh, T., Yoshioka, A., & Onodera, S. (2001). Effects of endurance training on resting and post-exercise cardiac autonomic control. *Med Sci Sports Exerc*, 33(9), 1496-1502.
- Zhang, J. (2007). Effect of age and sex on heart rate variability in healthy subjects. *J Manipulative Physiol Ther*, 30(5), 374-379. doi: 10.1016/j.jmpt.2007.04.001
- Zulfiqar, U., Jurivich, D. A., Gao, W., & Singer, D. H. (2010). Relation of high heart rate variability to healthy longevity. *Am J Cardiol*, 105(8), 1181-1185. doi: 10.1016/j.amjcard.2009.12.022

- Zygmunt, A., & Stanczyk, J. (2010). Methods of evaluation of autonomic nervous system function. *Archives of Medical Science*, *6*(1), 11-18. doi: 10.5114/aoms.2010.13500
- Zygmunt, A., & Stanczyk, J. (2010). Methods of evaluation of autonomic nervous system function. *Arch Med Sci*, 6(1), 11-18. doi: 10.5114/aoms.2010.13500