Heart rate variability parameters and the PNNx family as discriminators between athletes and sedentary people

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Abstract:

To characterize the heart rate variability parameters which best discriminate sedentary from athletes, in order to comprehend the regulatory mechanisms involved. Chronotropic activity is modulated by the autonomic nervous system through its sympathetic and parasympathetic branches, as a result of this dynamic interaction cardiac cycles are affected, changing heart rate variability in both time and frequency domains. A 16-minute ECG recording was collected from each one, and classic parameters of heart rate variability and the pNNx family were calculated. 16 athletes and 20 sedentary were analysed. LH, HF, average RR, maximum RR interval, minimum RR interval, SDNN, RMSSD, SDRR and pNN from 40 to 80% were higher in athletes than in sedentary, and pNN from 0 to 20% were higher in sedentary than in athletes; the parameters with the highest discriminatory power between the two groups were average RR, maximum RR Interval, minimum RR interval, SDNN, LF, and pNNx between 0 and 80% (where x is less than 80 ms for acceleration and x from 40 to 80 ms for decelerations). Athletes had higher consecutive RRs settings by magnitudes which range from 40 to 80 ms (regardless of whether the adjustment was in acceleration or deceleration), while sedentary ones had higher consecutive RRs settings by magnitudes between 10 and 20 ms, suggesting mechanisms related to vagal activation and "withdrawal".

Key Words: Athletes, sedentary, exercise, Heart Rate, Autonomic Nervous System, adaptability

Introduction

Chronotropic activity is determined by intrinsic characteristics of cardiac pacemaker cells and the Autonomic Nervous System (ANS) activity through reflex circuits involving their sympathetic and parasympathetic subsystems. These subsystems stimulate or inhibit the sinus node depending on the regulated frequencies and the kinetics of the neurotransmitters involved (Aubert, Seps and Beckers, 2003), which results in variations of the heart rate (HR). The Parasympathetic System (PS) slows down the heart rate by releasing acetylcholine, a neurotransmitter with a very short latency activation period and fast metabolism. Meanwhile, the Sympathetic System (SS) increases the HR, the conduction speed and the inotropismo by using as neurotransmitters reabsorbed and slowly metabolised noradrenaline and adrenaline. Thus, the quick response of the parasympathetic system regulates beat to beat the cardiac function (Pumpela, Howorka and Groves, 2002).

The dynamic interaction of the above listed components produces variations in the periodicity of the cardiac cycles. Some effects might be identified soon in the cycle to cycle variations, and some others may be associated to modulations with longer lasting effects (tonics) and probably more stable. In this context, the temporal evolution of the R-R intervals, whose representation is known as tachogram, might be analysed numerically from different mathematical approaches, two of the most frequently used are a) time domain and b) frequency domain analysis. The first methods are based on statistical indicators of the time variation of consecutive cycles (differences between two consecutive R-R intervals, RMSSD, pNNx etc.) or measuring features of the cycles, analysed as a set, (such as mean, standard deviation, frequency distributions, etc.); meanwhile, the frequency approach analyse the energy or power content of the components associated with very low (<0.04 Hz), low (0.04 to 0.15 Hz) or high (.15 to 0.4 Hz) frequency, constituting tachogram. In general, the study of temporal variations of cardiac cycles recorded on an electrocardiogram, known as Heart Rate Variability (HRV), has become a useful strategy not only to infer regulatory processes but also to forecast health (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Previous research on HRV have provided prognostic parameters and indicators of sudden death risk in different clinical settings (4,5), mainly after myocardial infarction (Bigger JT, Fleiss JL, Steinman RC, 1992), (Kleiger RE, Miller JP, Bigger JT, 1987). In practice, the processing of these measures is

relatively complex and sometimes does not clearly explain the basic regulatory mechanisms involved.

Moreover, time domain measurements exhibit quick adjustments of the ANS, which are primarily related to vagal activity (Zaza and Lombardi, 2001), as well as the High-Frequency component (HF), while Low Frequency parameters (LF) and LF / HF ratio has been interpreted as a mixture of sympathetic and parasympathetic activity. Additionally, the Very Low Frequency component (VLF) has been associated to adjustment during respiratory cycles and it usually requires extended electrocardiographic records in order to cover several phases of such oscillations (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). From which it follows that time domain parameters can be as effective as frequency domain parameters (especially HF) when analysing control mechanisms of the RR variability in periods of short duration records.

Literature has converged that when bradycardia is monitored in athletes at rest, it shows a systematic decrease in heart rate after sustained training (Aubert, Seps and Beckers, 2003), providing evidence of the influence of both subsystems of the ANS over heart chronotropism. However, a small number of these studies have focused on the analysis of the chronotropic regulatory mechanisms and very few have assessed which of the classic parameters, derived from HRV analysis, discriminate better between athletes and sedentary people at rest (Olivassé, Paulo and Jurandir N, 2014), (Shin, Minamitani and Onishi S, 1995), (Aubert, Beckers and Ramaekers, 2000); only one of these works provides new measures related to slowdowns in two consecutive RR intervals through the phase-rectified signal averaging (PSRA) in athletes and sedentary during rest, comparing these new measures against classical parameters of vagal modulation as HF and RMSSD, in order to better illustrate the behaviour of the SNA (Olivassé, Paulo and Jurandir, 2014). Nonetheless, there is a growing trend towards exploring HRV parameters in the frequency domain able to explain the behaviour of the SNA (leaving behind time domain parameters), which could give an approach to the vagal system behaviour and also be useful discrimination indicators to characterize athletes, reflecting risk of mortality and morbidity. The aim of this paper is to describe and analyse the most commonly used HRV parameters in athletes and sedentary people, seeking numerically practical elements such as the pNNx family (e.g. pNN50, pNN20) (Mietus, Peng and Henry I, 2002), (De la cruz and Naranjo, 2010), which may characterize athletes and obliquely supplement control models that contribute to a clear understanding of the regulatory mechanisms involved.

Material & methods

Sport teams members, who reside in Bucaramanga and college students who volunteered to be part of this study attended the call. The general inclusion criterion was: ages ranging from 18 to 35; and the specific criteria for the group of athletes: 2 hours of daily exercise for at least 6 days a week and with minimum training of 2 years. Whereas for non-athletes or sedentary people: having no sport in a time ranging from 3 weeks to 6 months before the physiological records. Exclusion criteria taken into account were some cardiovascular or neurological disease diagnosed by healthcare professionals, consumption of drugs even for therapeutic use, abuse and/or auto formulation of any medication, smoking and non-acceptance or quitting to be part of the study. Finally, 5 out of the 41 people who came to the event were excluded, for not meeting criteria of age and consumption of psychotropic drugs.

Procedure

Participants were appointed in the morning, after a previous night's rest, and abstinence from consumption of stimulating drinks within 2 hours prior to the recording. One electrocardiographic recording was obtained for each participant. The electrocardiographic signal was taken in recumbent supine position with ambient temperature of 22 ± 1 degrees Centigrade position. Each record was 16 minutes-length. The results of each explored variable were compared.

The signal is obtained with the "PowerLab 880 ML 16/30" device. A sampling frequency of 1 KHz was used, sensitivity equal to 5 mV and the bandpass filter was tuned between 0.3 and 200 hertz; further notch filter (60 Hz) was used. Demographic variables, as age, gender and sport practiced, were defined as well as electrocardiographic variables, both in the time and the frequency domain (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), including the most relevant parameters of the pNNx Family (Mietus, Peng and Henry I, 2002), (De la cruz and Naranjo, 2010), where x is the cutting time value to assess the fraction of consecutive RR intervals differing by more than "x" ms, taking "x" as multiples of 10 ranging from -200 ms to +200 ms. Negative values of pNNx indicate acceleration while positive ones indicate slowdowns. Frequency distributions of both discrete (frequency ranges between 10 ms) and accumulated intervals: positive and negative (smaller than "x" for negative and greater than "x" for positive) were performed. Finally, the most frequently pNNx variables reported in literature were calculated, the pNN₂₀ and pNN₅₀ corresponding to the absolute values of the consecutive RR differences greater than 20 and 50ms respectively.

After verifying the software was successfully detecting the QRS complexes, one tachogram was built from the R-R periods of the electrocardiogram and subsequently the HRV parameters were measured; those cases where ectopias were detected, we interpolated the data to the cubic spline method, at a sampling frequency of 5Hz since physiologically there are no heartbeats faster than 0.2 seconds. It was not necessary to exclude any

electrocardiographic record due to the fact that none of the 36 records had more than 20% of ectopias. Based on the tachogram computed, all the frequency components in the bands defined by the European Association of Cardiology (Fukuta, Hayano and Ishihara, 2003) were estimated. The autoregressive Yulewaker model (order 150) was used to determine the signal power distribution, shown in **Fig 1**.

In **Fig 1**, it can also be observed in detail the differences between the results given by the methods used. Since the signal's power is the area under the curve, an important difference between the thin and the gross line Yulewaker methods would result in significantly different measurements.

Fig 1. Heart Rate Variability.

Data Analysis

For those variables presenting normal distribution and homogeneous variance, the "t" test for independent samples was performed and the Mann-Whitney U test was used for asymmetric and inhomogeneous samples. The level of statistical significance was equal to 5%.

The Receiver Operator Characteristics (ROC) indexes were calculated in order to assess the discrimination ability between athletes and sedentary people for each electrocardiographic variable, and choosing as cut points of the continuous variable the one that gave a greater number of patients correctly classified.

In all the cases studied, the data analysed added up a length of 16 minutes per record.

Through informed consent each patient approved admission to this study, the collection of personal, clinical and electrocardiographic signals data and the confidentiality of the data collected was guaranteed, based on the ethical guidelines of the Helsinki Declaration. In this study neither pharmacological nor invasive intervention, which could lead to any pathological or physiological reaction in the participants, was made.

Results

Finally 36 persons were included in the study: 16 were sedentary and 20 athletes. In the sedentary group 25% were women and 75% men; meanwhile, in the athletes group 20% were women and 80% men. The average age of the men was the same in both athletes and sedentary (25 years) and the average age of women was 2 years older in the group of athletes than in the sedentary group (the latter 20 years on average), but no significant differences between the groups were found ($t = 0.268$, $p = 0.79$). **Table 1** shows the frequency distribution of sports the athletes practiced.

Table 1. Distribution of the athletes depending on the type of sport and gender.

Sport	Women	Men	Total
Basketball	1	2	3
Long-distance runner	1	2	3
Race-walker	1	-	1
Sprinter	-	3	3
Indoor football	1	9	10
Total	4	16	20

Initially, the HRV parameters most commonly reported in literature and some of the "pNNx" family were described.

The analysis of components of the HRV in the frequency domain showed that athletes had significantly higher values than sedentary only for LF and HF variables. (see **Fig 2**).

Fig 2. This figure depicts averages of the powers (ms^2) (top) and relative powers (bottom) resulting from the components analysis in the frequency domain, with standard error equal to ± 1 . *Significant differences ($p < 0.05$) when comparing Sedentary (S) to athletes (A).

The time domain analysis showed that athletes had significantly higher values than sedentary in the RR variables: Maximum RR interval, Minimum RR interval, SDNN, RMSSD, SDRR, pNN20, and pNN50. (**Fig 3, Fig 4**).

Fig 3. This figure depicts averages of the minimum, mean and maximum RR intervals with standard error equal to ± 1 . *Significant differences ($p < 0.05$) when comparing Sedentary (S) to athletes (A).

Fig 4: This figure depicts averages of the time domain variables with standard error equal to ± 1 . *Significant differences ($p < 0.05$) when comparing Sedentary (S) to athletes (A).

Table 2 shows a detailed description of the kind of test and its main statistical parameters by comparing each variable studied and obtained for athletes and sedentary people.

Table 2. Summary of the type of test and its main statistical parameters by comparing each of the variables studied and obtained from athletes and sedentary. The shaded rows highlight the variables in which no significant differences were found between the two groups of participants.

<i>VARIABLE</i>	<i>Test</i>	<i>t</i>	<i>T</i>	<i>P</i>
RR	t	4,971		<0.001
Max. RR	Mann-Whitney		267000	<0.001
Min.RR	t	3,62		<0.001
Rango.RR	Mann-Whitney		207000	0,139
SDNN	Mann-Whitney		255000	0,003
pNN20	Mann-Whitney		230000	0,027
pNN50	Mann-Whitney		230000	0,027
RMSSD	Mann-Whitney		227000	0,034
SD-DIF.RR	Mann-Whitney		227000	0,034
LF	t	3,89		<0.001
HF	Mann-Whitney		224000	0,023
LF/HF	t	0,101		0,92
LF.nu	t	0,003		0,998
HF.nu	t	0,003		0,998
TP	t	4,934		<0.001

Table 3 shows the average values (along with their standard deviations) of the HRV parameters in athletes and sedentary.

Table 3. Average values, with their standard deviations, of the HRV parameters in athletes and sedentary people.

<i>VARIABLE</i>	<i>S</i>	<i>A</i>
N	16	20
RR (s)	0,76 ± 0,11	0,94 ± 0,10 *
FC/min (s)	79,8 ± 11,6	64,1 ± 6,51 *
Max. RR (s)	1,01 ± 0,12	1,23 ± 0,22 *
Min.RR (s)	0,58 ± 0,08	0,68 ± 0,08 *
Rango RR (s)	0,42 ± 0,12	0,54 ± 0,25
SDNN (s)	0,06 ± 0,01	0,08 ± 0,02 *
pNN20 (%)	50,3 ± 22,5	66,2 ± 10,3 *
pNN50 (%)	20,6 ± 20,4	31,7 ± 15,0
RMSSD (s)	0,04 ± 0,02	0,06 ± 0,03
SD.DRR (s)	0,04 ± 0,02	0,06 ± 0,03
LF (ms2)	2,17 ± 0,70	3,57 ± 1,29 *
HF (ms2)	1,02 ± 0,73	1,70 ± 1,39
LF/HF	2,75 ± 1,10	2,71 ± 1,11
LF.nu (%)	70,5 ± 10,6	70,5 ± 9,48
HF.nu (%)	29,4 ± 10,6	29,4 ± 9,48

ROC analysis were performed in order to identify the discriminatory power of the above explored variables (**Table 4** only includes variables whose ROC is higher than 0.7), showing that the best ROC index and thus the variable that best discriminated athletes from non-athletes was the "average RR" (ROC = 0.87, sensitivity = 1 and specificity = 0.63) where the cutting value indicated that people with average RR ≥ 0.81 sg -less than 74

beats per minute (lpm)- was classified in the group of athletes and values less than this period were classified as non-athletes. The second best discriminatory variable was "LF" (ROC = 0.85, see details in the No. 4 box), followed by this, the Max-RR, Min-RR and SDNN variables (ROC = 0.83, 0.81 and 0.8, respectively) can be found.

Table 4: Summary ROC analysis for each of the variables explored. ROC: ROC index, S and E: Sensitivity and specificity calculated for the best cutoff value. VPP and VPN: positive and negative predictive values obtained with the best cutoff value. The shaded boxes belong to the variables whose ROC values are greater than 0.8.

<i>VARIABLE</i>	<i>ROC</i>	<i>Cutoff values</i>	<i>S</i>	<i>E</i>	<i>VPP</i>	<i>VPN</i>	<i>% right classified</i>
<i>HF (ms²)</i>	0,73	0,72	0,95	0,56	0,73	0,9	77,78
<i>LF (ms²)</i>	0,85	2,24	0,95	0,63	0,76	0,91	80,56
<i>SD-DRR</i>	0,71	0,03	1	0,44	0,69	1	75
<i>RMSSD</i>	0,71	0,03	1	0,44	0,69	1	75
<i>pNN50</i>	0,72	12,24	1	0,5	0,71	1	77,78
<i>pNN20</i>	0,72	48,5	1	0,5	0,71	1	77,78
<i>SDNN</i>	0,8	0,07	0,85	0,69	0,77	0,79	77,78
<i>Min RR</i>	0,81	0,62	0,8	0,75	0,8	0,75	77,78
<i>MaxRR</i>	0,83	1,17	0,6	1	1	0,67	77,78
<i>Prom RR</i>	0,87	0,81	1	0,63	0,77	1	83,33

Exploring the "pNNx family"

As shown in **Fig. 5**, the frequency distribution of pNNx was performed between each interval of 10 milliseconds, showing that sedentary people have a more "acute" and symmetrical distribution than athletes, which highlights that the most notable differences are located at the peak of the distribution and lateral intermediate values thereof (see **Table 5**: ROC for the discriminatory ability of each interval), so that the ratio of consecutive RR differences whose magnitude was between 0 and 20 ms for both accelerations (I.pNN-x) and slowdowns (I.pNN + x) was higher for non-athletes compared to athletes; in contrast, the ratios of consecutive RR differences whose magnitudes were between 40 and 80 ms (for both accelerations and decelerations) was higher in athletes than non-athletes. Moreover, the "symmetry" (skewness) and "acuity" (kurtosis) values of the distribution were higher for sedentary than for athletes. Fig 6 depicts the behaviour of the "conventional" pNNx, i.e., the percentage of RR consecutive absolute differences higher than "x", this involves the total percentage of the RR differences, regardless of whether such differences were in accelerations or decelerations. The results showed that the ROC index was higher than 0.7 for pNN between 10 and 80%.

Additionally the frequency distribution of the pNN+x and pNN-x was explored, finding again that these variables can discriminate the two groups of participants when for pNNx, x was less than 80 ms for acceleration and between 40 and 80 ms for slowdowns. The "acuity" (kurtosis) and the "symmetry" (skewness) gave ROC values greater than 0.83 and as the distribution by discrete intervals, these two parameters were higher in non-athletes than in athletes (see **Table 5**).

Table 5: Results of the ROC evaluation for pNNx variables. There were included variables calculated in discrete intervals (I.pNNx), negative and positive accumulated (pNN-x and pNN + x), consecutive absolutes greater than "x" (pNNx) and, kurtosis and skewness analysis of the first two distributions.

Discriminatory ability of the pNNx variables.

<i>VARIABLE</i>	<i>ROC</i>	<i>Cutoff value</i>	<i>S</i>	<i>E</i>	<i>VPP</i>	<i>VPN</i>	<i>% Right classified</i>
<i>pNN10</i>	0,72	73,16	1	0,56	0,74	1	80,56
<i>pNN20</i>	0,72	49,07	1	0,5	0,71	1	77,78
<i>pNN30</i>	0,72	35,64	0,95	0,63	0,76	0,91	80,56
<i>pNN40</i>	0,73	21,67	1	0,63	0,77	1	83,33
<i>pNN50</i>	0,72	12,4	1	0,5	0,71	1	77,78
<i>pNN60</i>	0,73	6,36	1	0,5	0,71	1	77,78
<i>pNN70</i>	0,72	4,35	0,95	0,56	0,73	0,9	77,78
<i>pNN80</i>	0,7	2,83	0,9	0,56	0,72	0,82	75
<i>kurtosispNNx</i>	0,83	19,97	0,69	0,95	0,92	0,79	83,33
<i>skewnesspNNx</i>	0,83	4,64	0,63	1	1	0,77	83,33
<i>kurtosisI.pNNx</i>	0,7	1,55	0,63	0,9	0,83	0,75	77,78
<i>skewnessI.pNNx</i>	0,7	1,68	0,63	0,9	0,83	0,75	77,78

<i>pNN+80</i>	0,71	1,51	1	0,38	0,67	1	72,22
<i>pNN+70</i>	0,71	2,94	1	0,44	0,69	1	75
<i>pNN+60</i>	0,72	5,34	1	0,5	0,71	1	77,78
<i>pNN+50</i>	0,72	9,62	1	0,56	0,74	1	80,56
<i>pNN+40</i>	0,72	16,17	1	0,63	0,77	1	83,33
<i>pNN-10</i>	0,74	38,71	0,95	0,56	0,73	0,9	77,78
<i>pNN-20</i>	0,73	27,19	0,95	0,63	0,76	0,91	80,56
<i>pNN-30</i>	0,72	18,44	0,95	0,63	0,76	0,91	80,56
<i>pNN-40</i>	0,72	11,61	1	0,56	0,74	1	80,56
<i>pNN-50</i>	0,72	6,52	1	0,5	0,71	1	77,78
<i>pNN-60</i>	0,72	3,35	1	0,5	0,71	1	77,78
<i>pNN-70</i>	0,72	1,51	1	0,5	0,71	1	77,78
<i>pNN-80</i>	0,72	0,79	1	0,44	0,69	1	75
<i>I.pNN0</i>	0,72	27,4	0,56	1	1	0,74	80,56
<i>I.pNN10</i>	0,7	22,14	0,63	0,85	0,77	0,74	75
<i>I.pNN40</i>	0,76	7,63	0,95	0,56	0,73	0,9	77,78
<i>I.pNN50</i>	0,73	5,38	1	0,63	0,77	1	83,33
<i>I.pNN60</i>	0,73	2,99	1	0,5	0,71	1	77,78
<i>I.pNN80</i>	0,73	1,11	1	0,69	0,8	1	86,11
<i>I.pNN+90</i>	0,73	0,56	1	0,69	0,8	1	86,11
<i>I.pNN+70</i>	0,7	1,21	1	0,44	0,69	1	75
<i>I.pNN+60</i>	0,75	2,18	1	0,63	0,77	1	83,33
<i>I.pNN+50</i>	0,76	3,03	1	0,44	0,69	1	75
<i>I.pNN+10</i>	0,73	11,54	0,69	0,9	0,85	0,78	80,56
<i>I.pNN-10</i>	0,7	11,84	0,56	1	1	0,74	80,56
<i>I.pNN-40</i>	0,76	4,32	0,85	0,75	0,81	0,8	80,56
<i>I.pNN-50</i>	0,73	2,85	1	0,56	0,74	1	80,56
<i>I.pNN-60</i>	0,75	1,45	1	0,56	0,74	1	80,56
<i>I.pNN-80</i>	0,74	0,54	1	0,56	0,74	1	80,56

Dicussion

In a comprehensive way, the present study evaluated different HRV parameters both in the time and the frequency domain, with results pointing consistently to a significant increase of the variability in athletes compared to sedentary; Indeed, this was true in virtually all parameters that are commonly used in the time domain (RR, maximum and minimum RR, SDNN, RMSSD, SD.DRR, pNN20 and pNN50). However, in the frequency domain only an increase in the power of the LF and HF variables was detected, but no in their normalized values (nu) nor the LF / HF ratio. These results are consistent with those obtained by other authors in similar research for variables in the time domain (Olivassé N, Paulo RB and Jurandir, 2014), (Shin, Minamitani and Onishi, 1995), (Aubert AE, Beckers F and Ramaekers, 2000), (Puig Freitas and Carvalho, 1993), and frequency (Puig Freitas and Carvalho, 1993), although in those studies verifying the discriminatory ability of such variables was not a purpose. Discrepancy was found in the results obtained in this study compared to those in Shin et al (Shin, Minamitani and Onishi, 1995), who found that there were significant differences in the LF / HF ratio, being lower for athletes than sedentary (at rest) due to an increase in HF unchanged at LF (regarding to non-athletes), reflecting an increase of this proportion. Possibly because of this, Gupta (Gupta Gupta and Gupta, 2014) and Dixon (Dixon, Kamath, McCartney and Fallen, 1992) also showed and increase in LF / HF, due to the increase of LF and decrease of HF in athletes regarding to sedentary people. Meanwhile, Furlan (Furlan, Piazza and Dell'Orto, 1993) and Pichot (Pichot, Roche and Gaspoz 2000) found that in athletes with increased physical load during their trainings: LFnu and LF / HF was increased and HFnu decreased; whereas in athletes with reduced physical load: LF nu and LF / HF was decreased and HFnu significantly increased in these parameters. This could be explained so that the values (nu) are weights relating to the total power of the frequency components involved in the calculation, that is, in electrocardiographic records longer than 30 minutes, the total power is usually obtained as the sum of the powers provided by VLF, LF and HF, whereas in short records, the contribution of VLF is usually excluded due to its calculation can be mathematically questionable and therefore the total power is the sum of VL and HF. Briefly, it can be said that much of the differences obtained by

different authors regarding the parameters of the frequency domain can be relate to: the patient position (e.g. standing or 90 ° Vs. decubitus) in physical activity conditions during the electrocardiography (during exercise, recovery or rest), with the level/type of physical training of the participants (high or low performance, with different aerobic sports participation) (Aubert, Beckers and Ramaekers, 2000),(Gupta, Gupta and Gupta, 2014), (Pichot , Roche and Gaspoz , 2000) or due to different durations/continuities of the records (2, 10, 15 minutes or 24 hours. continuous vs. discontinuous records). These variations in recording conditions might influence methodological issues, associated to the computing of the parameters, as well as physiologically regulated issues. From the methodological point of view of processing electrocardiographic signals, it would be useful to consider aspects as the contributions given by the different frequency components (e.g., magnitude and reliability of VLF related to the length of records), the stationary nature of the signal, interference ("noise") obtained during the electrocardiographic recordings which might lead to mistakes in the identification of the RR peaks, defects in the computation of the tachograms and the probability of occurrence and detection of ectopias, among others. From the point of view of the regulating mechanisms involved in the cardiovascular system: respiratory activity associated with posture and movements during recording, which in turn significantly changes VLF and LF components with consequent weighting of LF/HF (Aubert, Beckers and Ramaekers, 2000),(Mietus , Peng and Henry I, 2002),(Furlan, Piazza and Dell'Orto, 1993); the adaptation level reached by seniority and type of physical training performed by the athletes, which influences the various metabolic mechanisms and therefore electrophysiological typical of excitable systems as suggested by Martinelli et al (Martinelli, Chacon-Mikahil and Martins, 2005); patient's position during registration modulates the effects on baroreceptors and respiratory movements and in turn influences the autonomic inhibition/arousal. The duration and/or novelty of the record can also determine characteristic changes of the stress condition associated with the fact of being evaluated/analysed.

Overall, the above observations suggest that if the aim is to study HRV in order to contribute to the understanding of the regulatory mechanisms involved in athletes or in any other clinical condition, strictly controlled conditions are mandatory in order to assess the changes in HRV parameters against measurable stimulations or at rest on internally homogeneous populations; otherwise, the validity of the results will be strongly restricted to the specific conditions of each study and therefore the implementation of diagnostic criteria based on quantitative results would not be reliable.

With regard to the "pNNx family" authors like Mietus and De la cruz have used these parameters to assess whether it is possible to discriminate different groups of people, finding that thresholds with pNNx smaller than 20% discriminate better no cardiac from cardiac patients, and even patients during exercise with those at rest; however, so far no studies have attempted to assess which "pNNx" parameters discriminate better between athletes and sedentary.

About "The pNNx family" is not only important to state the possibility of increase or decrease of HRV but how these changes occurred. In that regard, the description of the percentage of consecutive RR differences by discrete and cumulative time intervals, allows to identify that athletes had lower proportions than non-athletes in the accelerations and slowdowns in consecutive cycles whose magnitude were between 0 and 20 ms while athletes had a higher proportion than non-athletes in magnitudes ranging from 40 to 80 ms. This indicates that the adjustability of non-athletes is at the expense of small changes in the RR intervals (0-20 ms), while athletes perform it better with intermediate adjustments (40-80 ms) and this is valid for both accelerations and slowdowns. Both groups had similar proportions for acceleration and (greater than 80 ms) extreme slowdowns. Since athletes adjusted consecutive RRs in intermediate ranges (40 to 80 ms) for both accelerations and slowdowns, it follows that there is only one mechanism involved that might be reversible; for example, a parasympathetic activation for the slowdown and the consequent "withdrawal" parasympathetic for acceleration, such capacity could have been modified by exercise.

As shown in **Fig 5**, "conventional" pNNx, the proportion of absolute and consecutive RRs differences consecutive absolute (regardless they were accelerations or slowdowns) higher than the "x" values are useful for discriminating the two groups of participants between pNN10 and pNN80.

However, pNNx close to zero, involve almost all ranges of the frequency distribution and this does not allow to observe what is possible with frequency distributions at intervals of 10 ms (**Fig 6**), i.e., how athletes accelerate or decelerate their chronotropism at the expense of RRs differences between 40 and 80 ms, while non-athletes do it better with acceleration or slowdowns from zero to 20 ms. Although the analysis to evaluate changes in consecutive RRs differences was not performed, it was estimated the implication of increasing or decreasing 40 and 80 ms in the RRs when the starting frequency were between 50 and 120 lpm. These calculations indicated that slowdowns could range from 3.2 lpm (for initial frequencies equal to 50 lpm) to 13.8 lpm (for initial frequencies equal to 120lpm). Meanwhile, accelerations could mean increases of 3.5 lpm for initial frequency of 50 bpm and 19 bpm for initial frequencies of 120 lpm. In estimating the pNN with x between 40 and 80 ms, based on the results, it was found that for non-athletes there would have about 24% of consecutive RRs differences between these ranges, while for non-athletes that percentage increase to 34%. This 10% difference between the groups could mean the magnitude of the adjustment given by exercise. Since athletes presented RRs means significantly higher than non-athletes, sudden changes between 40 and 80 ms could mean

that in this group, settings would be more efficient as to the maintenance of steady chronotropism.

Finally, it was found that HRV is higher in athletes than in sedentary people during rest when evaluated by most of the "classic" time domain parameters (RRs, RMSSD, SDNN, pNN20 and pNN50) and is also higher in the main parameters of the frequency domain (LF and HF).

Fig 5: Average ± 1 of the cumulative mean of the pNNx standard error. *ROC>0.7

Fig 6: Above: arithmetic mean ± 1 standard error of the mean of each point in the Probability Density Function for the consecutive RR differences. The upper limits of each interval are shown on the x-axis (e.g.: LpNN-20 represents the percentage of two consecutive RR differences between -10 and -20 milliseconds). * ROC > 0.7. Below: arithmetic mean ± 1 standard error of the skewness and kurtosis values, calculated for each participant in each group. *p < 0.05

Conclusions

It is concluded that the parameters that best discriminated athletes from non-athletes were: RR average in the time domain, LF in the frequency domain. The "kurtosis" and "skewness" of the PNN distribution in

accelerations and slowdowns showed discriminatory abilities similar to the obtained with the above mentioned classical parameters. Additionally, athletes had higher settings of consecutive RRS in magnitudes between 40 and 80 ms regardless of whether the adjustment was in accelerations or slowdowns, while non-athletes did at intervals between 10 and 20 ms, also for accelerations and slowdowns. This suggests mechanisms related to vagal activation and "withdrawal".

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