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Article

Effect of the Postural Challenge on the Dependence of the Cardiovascular Control Complexity on Age

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Abstract: Short-term complexity of heart period (HP) and systolic arterial pressure (SAP) was computed to detect age and gender influences over cardiovascular control in resting supine condition (REST) and during standing (STAND). Healthy subjects (n = 110, men = 55) were equally divided into five groups (21–30; 31–40; 41–50; 51–60; and 61–70 years of age). HP and SAP series were recorded for 15 min at REST and during STAND. A normalized complexity index (NCI) based on conditional entropy was assessed. At REST we found that both NCI_{HP} and NCI_{SAP} decreased with age in the overall population, but only women were

responsible for this trend. During STAND we observed that both NCI_{HP} and NCI_{SAP} were unrelated to age in the overall population, even when divided by gender. When the variation of NCI in response to STAND (ΔNCI = NCI at REST-NCI during STAND) was computed individually, we found that ΔNCI_{HP} progressively decreased with age in the overall population, and women were again responsible for this trend. Conversely, ΔNCI_{SAP} was unrelated to age and gender. This study stresses that the complexity of cardiovascular control and its ability to respond to stressors are more importantly lost with age in women than in men.

Keywords: aging; gender; standing; heart rate variability; blood pressure variability; complexity; corrected conditional entropy; autonomic nervous system

1. Introduction

Aging is associated with a reduced ability of the physiological regulatory mechanisms to interact, leading to less flexible cardiovascular control [1,2]. These changes are mirrored by a reduction in heart period (HP) variability [2–9], by an increase of systolic blood pressure (SAP) variability [10–12], and by a reduction in complexity of physiological dynamics [2–4,13–23]. Although the abovementioned studies indicate that aging reduces the complexity of the cardiovascular control and prove the gender dependence, it is still unclear whether the complexity reduction and the gender relation are similarly observable from HP and SAP variabilities and if it persists during a cardiovascular control challenge. Indeed, protocols assessing the effect of aging on the complexity of the cardiovascular control are mainly limited to the evaluation of the complexity of the HP variability [13–15,19–23] and mostly do not deliberately challenge the cardiovascular control according to an experimental maneuver or pharmacological intervention [13–19,22,23]. In addition, those studies challenging autonomic nervous system regulation have a limited power because age ranges are inadequate [20] or they are mainly based on biased indexes of complexity such as approximate entropy [21].

The aim of this study was to evaluate the effect of a postural challenge on the dependence of the cardiovascular control complexity on age. The aging and gender effects on the complexity of HP and SAP variability are assessed by means of a univariate approach assessing irregularity as the amount of information carried by a series that cannot be derived from the knowledge of its own past values through the corrected conditional entropy [24] in several age groups in resting supine condition and during sympathetic activation induced by active standing in healthy subjects.

2. Methods

2.1. Study Population

The study was carried out at the Laboratory of Cardiovascular Physiotherapy, Department of Physiotherapy of the Federal University of São Carlos, São Carlos, Brazil. The study was performed according to the Declaration of Helsinki for medical research involving humans and approved by the Human Research Ethics Committee of the Federal University of São Carlos (protocol number 173/2011). Written informed consent was obtained from all subjects.

One hundred and ten subjects were studied and divided into five groups (n = 22 for each group, 11 men), according to the following age ranges: 21–30; 31–40; 41–50; 51–60; 61–70. All subjects were apparently healthy, were not taking medicine that influenced cardiovascular system, and had no history and no clinical evidence of any disease based on clinical and physical examinations, laboratory tests, a standard ECG, and a maximum cardiopulmonary exercise test conducted by a physician. Smokers, habitual drinkers, and obese subjects (body mass index larger than 30 kg/m^2) were excluded from this study. Only women without contraceptive medication or hormone replacement therapy were included. All women 51–60 and 61–70 were in the menopausal phase.

2.2. Experimental Protocol

All subjects were evaluated in the afternoon. The experiments were carried out in a climatically controlled room (22–23 °C), with relative air humidity of 40–60%. Subjects were instructed to not ingest caffeinated or alcoholic beverages as well as to not perform strenuous exercise on the day before the protocol application. They were also instructed to ingest a light meal at least 2 h prior to the test. On the experimental day, the subjects were interviewed and examined before the test to verify that they were in good health, had had a regular night's sleep, and had a heart rate and systemic blood pressure within the normal range. Prior to the experiment, the volunteers were familiarized with the equipment and the experimental procedure.

2.3. Data Acquisition

Prior to the experimental procedure, the subjects were maintained in the resting supine condition for 10 min. Then, signals were acquired for 15 min in the resting supine position (REST) and 15 min in the standing position (STAND). The subjects were instructed to breathe spontaneously but they were not allowed to talk. All subjects completed STAND without experiencing any sign of pre-syncope. The electrocardiographic signal (modified lead I) was captured by a bioamplificator (BioAmp FE132, ADInstruments, Sydney, Australia). Noninvasive continuous blood pressure waveform monitoring (Finometer-PRO, Finapres Medical System, Amsterdam, The Netherlands) was obtained from the middle finger of the right hand, which was maintained at the level of the heart by fixing the subject's arm to his thorax during the whole of the experiment. The auto-calibration procedure of the device was switched off after the first automatic calibration at the onset of the protocol. All signals were sampled at 400 Hz (Power Lab 8/35, ADInstruments, Sydney, Australia).

2.4. Time Series Extraction

After detecting the QRS complex of the electrocardiogram and locating the R-wave apex using parabolic interpolation, the HP was approximated as the time distance between two consecutive R-wave apexes. The maximum of arterial pressure inside HP was taken as SAP. The occurrences of QRS and SAP peaks were carefully checked to avoid erroneous detections or missed beats. The series HP = {HP(i), i = 1, …, N} and SAP = {SAP(i), i = 1, …, N}, where i is the progressive cardiac beat number, were linearly detrended. Since the analysis focuses on short-term cardiovascular control [25], the series length N was set to 256 (*i.e.*, recordings of a few minutes). The stationarity of the selected

sequence was tested according to [26] over the original series after linear detrending. If the test for the steadiness of mean and variance was not fulfilled, a new selection was carried out again until the fulfillment of the prerequisites for restricted weak stationarity [26]. Time domain parameters such as the HP mean, HP variance, SAP mean, and SAP variance, indicated as μ_{HP}, σ^2_{HP}, μ_{SAP}, and σ^2_{SAP}, respectively, were computed to assess their relation with age.

2.5. Complexity Analysis

Complexity was estimated via corrected conditional entropy, as described by Porta *et al.* [14,24]. The approach measures the amount of information carried by a time series that cannot be derived from the knowledge of its previous samples. If HP or SAP sequences are regular, the series are more predictable and less complex. In the opposite case, if the heart (or vessels) receives a large amount of information due to the action of many control subsystems, HP (or SAP) exhibits a higher degree of unpredictability. As a function of the number of previous samples, the corrected conditional entropy: (i) remains constant in the case of white noise; (ii) decreases to zero in the case of fully predictable signals; and (iii) shows a minimum if repetitive patterns are embedded in noise. In the corrected conditional entropy, the number of previous conditioning samples was not *a priori* fixed, as it occurs in sample entropy [27] and approximate entropy [28], but it is optimized on a case-by-case basis. Conversely, the level of coarse graining was fixed according to the number of levels utilized in the uniform quantization procedure (here, 6). The optimal embedding dimension for HP and SAP ranged from 2 to 6 at REST and from 2 to 7 during STAND. The minimum of the corrected conditional entropy with respect to the number of past conditioning values was taken as a complexity index (CI). CI ranges between 0, indicating null information, and the Shannon entropy of the series, indicating the maximum amount of information carried by the sequence of data. This index was normalized by the Shannon entropy to obtain a normalized CI (NCI), thus expressing complexity in terms of dimensionless units. This index ranges from 0 (null information, maximal predictability) to 1 (maximum information, minimal predictability) [29]. The greater the CI and NCI, the less predictable and regular the time series.

2.6. Statistical Analysis

Normality of the distributions was tested by the Kolmogorov–Smirnov test. One-way analysis of variance (Tukey test for multiple comparisons), or Kruskal–Wallis one-way analysis of variance on ranks (Dunn's method for multiple comparisons) when appropriate, was applied to check the significance of the differences among parameters in different age groups. Assigned the age group, unpaired t-test, or the Mann–Whitney rank sum test when appropriate, was applied to test the significance of the difference between genders. Bonferroni's correction was utilized to account for multiple comparisons. The Pearson correlation analysis was performed to check the linear association of any parameter on age. The same analysis was also used to assess the association between NCI and time domain parameters.

Linear regression analysis on age was performed only if a significant difference between the 21–30 and 61–70 groups was detected according to one-way analysis of variance. A $p < 0.05$ was considered significant. Statistical analyses were carried out using a commercial statistical program (SigmaPlot 11.0, Systat, Chicago, IL, USA). Data are shown as median (1st quartile–3rd quartile) in all tables and figures.

3. Results

3.1. Characteristics of the Population

Table 1 shows the anthropometric characteristics and aerobic capacity of our 110 studied subjects. Males and females are equally divided into five groups. The anthropometric characteristics (height and weight) did not show significant differences among groups. The body mass index (BMI) was significantly higher in the 41–50 and 61–70 groups compared to 21–30. The VO_2 peak was lower in the older groups (51–60 and 61–70) compared to 31–40.

When the data were analyzed by gender, only women exhibited a difference in BMI (41–50 and 61–70 were different compared to 21–30, and 61–70 was different in relation to 31–40). Men showed lower values of VO_2 peak in 41–50, 51–60, and 61–70 compared to 31–40, while women had lower values in 51–60 and 61–70 compared to 21–30 and 31–40, and also lower values in 61–70 compared to 41–50.

Table 1. Characteristics of the population.

Age bin	**21–30**	**31–40**	**41–50**	**51–60**	**61–70**
110 volunteers	11 M/11 F	11 M/11 F	11 M/11 F	11 M/11 F	11 M/11 F
Age (years)	26 (24–29)	33 (32–37)	44 (43–46)	55 (51–57)	64 (62–66)
Height (cm)	169 (164–178)	168 (163–174)	170 (160–174)	168 (159–172)	163 (155–167)
Weight (kg)	67.5 (59.5–73.0)	65.1 (60.0–75.0)	73.0 (64.1–83.0)	65.2 (58.0–73.0)	67.5 (62.0–73.0)
BMI (kg/m^2)	23 (21–24)	24 (22–25)	26 (23–28) *§	25 (23–25)	25 (24–27) *
VO_2 peak (mL/kg/min)	34 (28–40)	36 (28–43)	29 (24–38)	27 (23–34)§	24 (19–30) §
Men	11 M	11 M	11 M	11 M	11 M
Age (years)	26 (25–30)	33 (32–37)	44 (43–47)	55 (51–56)	64 (63–66)
Height (cm)	174 (170–182)	172 (168–178)	174 (170–181)	171 (168–177)	166 (164–176)
Weight (kg)	73.0 (68.6–80.0)	75.0 (69.1–78.2)	80.0 (76.1–86.7)	72.0 (69.0–80.9)	68.2 (65.5–76.6)
BMI (kg/m^2)	24 (23–26)	24 (23–26)	26 (25–28)	25 (22–25)	24 (24–27)
VO_2 peak (mL/kg/min)	37 (36–41)	42 (36–45)	38 (30–42) §	33 (30–38) §	29 (26–31) §
Women	11 F	11 F	11 F	11 F	11 F
Age (years)	25 (24–27)	33 (31–37)	44 (43–46)	56 (52–59)	65 (62–66)
Height (cm)	164 (159–167)	163 (159–165)	160 (158–165)	159 (151–165)	155 (148–162)
Weight (kg)	59.5 (51.9–63.5)	60.0 (53.1–62.7)	64.1 (62.0–71.5)	58.5 (57.1–61.1)	66.3 (56.5–70.1)
BMI (kg/m^2)	22 (20–23)	22 (20–25)	25 (24–26) *	24 (23–25)	27 (25–27) *§
VO_2 peak (mL/kg/min)	28 (25–31)	27 (25–36)	27 (22–29)	22 (20–25) *§	19 (18–21) $^{*\S\#}$

Values are expressed as median (1st quartile–3rd quartile). F: female; M: male; BMI: body mass index; VO_2 peak: peak oxygen uptake. * $p < 0.05$ compared to 21–30; § $p < 0.05$ compared to 31–40; $^{\#}$ $p < 0.05$ compared to 41–50.

3.2. Time Domain Parameters: Aging and Gender Effects

Table 2 shows the results of the linear regression analysis of time domain parameters (*i.e.*, μ_{HP}, σ^2_{HP}, μ_{SAP}, and σ^2_{SAP}) on age in the overall group of subjects and after the separation of the two genders. At REST in the entire group we found that σ^2_{HP}, μ_{SAP}, and σ^2_{SAP} were significantly correlated with age, while μ_{HP} was not. The correlation coefficient was positive in the case of μ_{SAP} and σ^2_{SAP} and negative in

the case of σ^2_{HP}. Similar results were obtained in women. In men the positive correlations of μ_{SAP} and σ^2_{SAP} were lost. During STAND in the whole group we found that μ_{HP}, σ^2_{HP}, and μ_{SAP} were linearly related to age and the sign of the correlation coefficient was positive in the case of μ_{HP} and μ_{SAP} and negative in the case of σ^2_{HP}. Similar results were obtained in women. In men the positive correlations of μ_{HP} and μ_{SAP} were lost.

Table 2. Linear regression analysis of time domain parameters on age.

Experimental Condition		REST		STAND	
Index		r	significance	r	significance
All (55 M/55 F)	μ_{HP}	0.027	No	0.258	Yes
	σ^2_{HP}	−0.378	Yes	−0.433	Yes
	μ_{SAP}	0.287	Yes	0.324	Yes
	σ^2_{SAP}	0.339	Yes	−0.003	No
Men (55 M)	μ_{HP}	−0.037	No	0.015	No
	σ^2_{HP}	−0.415	Yes	−0.546	Yes
	μ_{SAP}	0.064	No	0.153	No
	σ^2_{SAP}	0.220	No	−0.137	No
Women (55 F)	μ_{HP}	0.106	No	0.579	Yes
	σ^2_{HP}	−0.348	Yes	−0.326	Yes
	μ_{SAP}	0.477	Yes	0.480	Yes
	σ^2_{SAP}	0.424	Yes	0.148	No

μ_{HP}: HP mean; σ^2_{HP}: HP variance; μ_{SAP}: SAP mean; σ^2_{SAP}: SAP variance; r: Pearson correlation coefficient; Yes/No: detection of a significant correlation with $p < 0.05$.

3.3. NCI_{HP} and NCI_{SAP} at REST: Aging and Gender Effects

Figure 1 shows that NCI_{HP} decreased with age in the overall population at REST. However, when the groups were divided by gender, a significant negative correlation of NCI_{HP} on age was observed only in women. These data suggest that the decrease of HP complexity in women was the main cause of the decrease of NCI_{HP} in the overall population.

In men there was a tendency for NCI_{HP} to decrease, as suggested by the decreasing tendency in 41–50 compared to 31–40 and by the significantly lower values of 51–60 compared to 31–40, but this tendency was weak and finally did not produce a significant change from 21–30 to 61–70. Conversely, in women NCI_{HP} was significantly lower in 61–70 compared to 21–30, 31–40 and 41–50 and a clear crossover was observed at 51–60 (NCI_{HP} remained stable in 21–30, 31–40 and 41–50).

Similarly, Figure 2 shows that NCI_{SAP} decreased with age in the overall population. Again the analysis by gender showed that the decrease of NCI_{SAP} in women was responsible for the decrease of this variable in the overall population (NCI_{SAP} did not decrease in men). In women the decrease of NCI_{SAP} was particularly relevant in 61–70.

Figure 1. Box-and-whisker plots of NCI_{HP} at REST (upper panels) and linear regression of NCI_{HP} on age (lower panels). The linear regression over all values (*i.e.*, solid circles: age 21–30; open circles: age 31–40; solid triangles pointing down: age 41–50; open triangles pointing up: age 51–60; solid squares: age 61–70) and its 95% confidence interval are plotted when a significant change from 21–30 to 61–70 is detected according to one-way analysis of variance. The symbol * indicates $p < 0.05$.

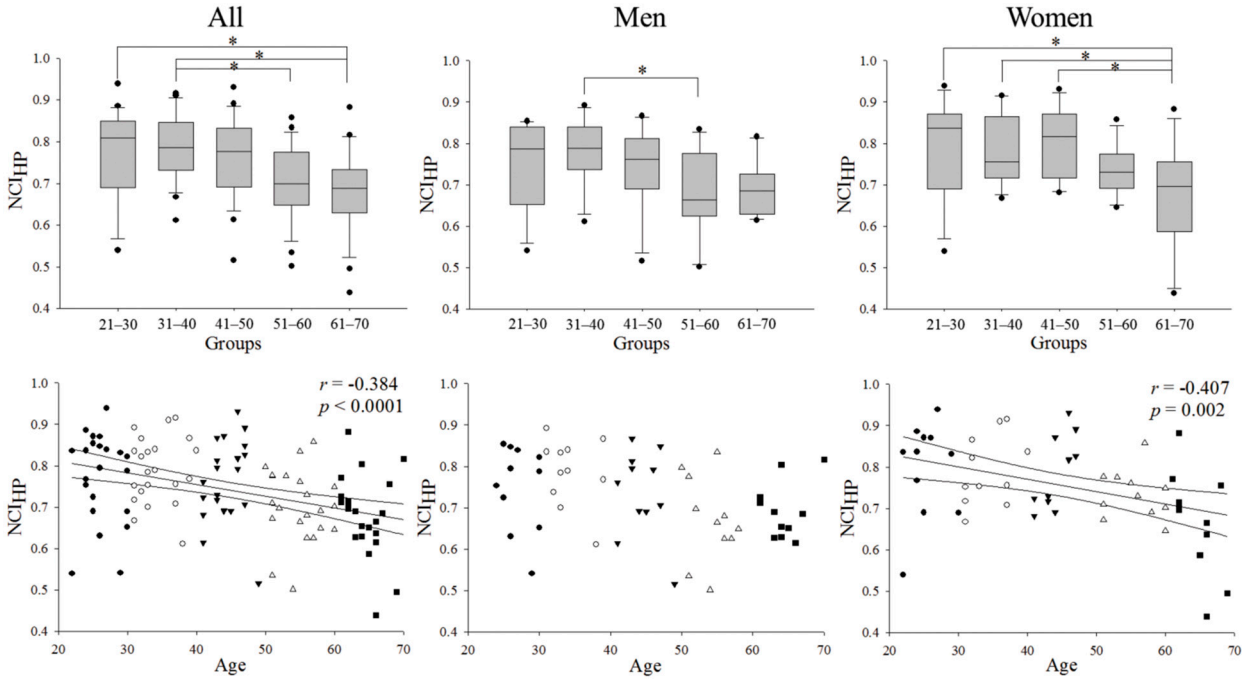
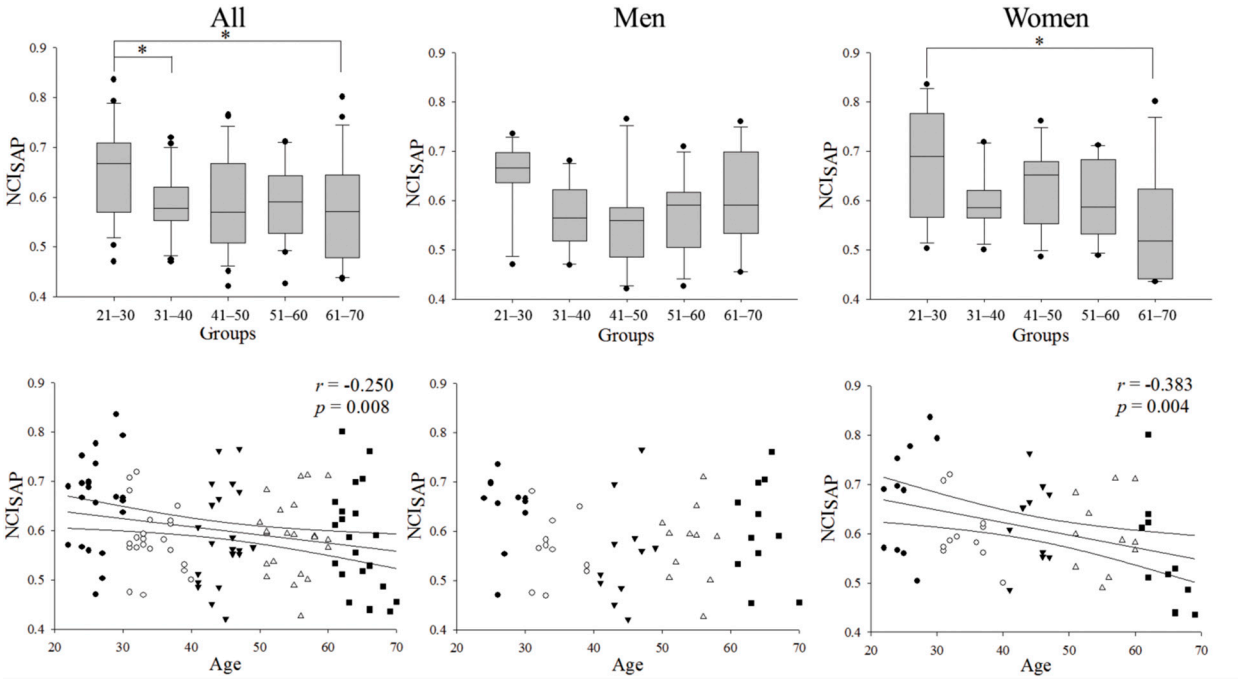


Figure 2. Box-and-whisker plots of NCI_{SAP} at REST (upper panels) and linear regression of NCI_{SAP} on age (lower panels). The linear regression over all values (*i.e.*, solid circles: age 21–30; open circles: age 31–40; solid triangles pointing down: age 41–50; open triangles pointing up: age 51–60; solid squares: age 61–70) and its 95% confidence interval are plotted when a significant change from 21–30 to 61–70 is detected according to one-way analysis of variance. The symbol * indicates $p < 0.05$.



3.4. NCI_{HP} and NCI_{SAP} during STAND: Aging and Gender Effects

Figure 3 shows that during STAND NCI_{HP} did not vary with age and this result was independent of gender (*i.e.*, NCI_{HP} did not show any relation to age in either men or women). The same finding held in the case of NCI_{SAP} (Figure 4).

3.5. ΔNCI_{HP} and ΔNCI_{SAP}: Aging and Gender Effects

Figure 5 shows the correlation of ΔNCI_{HP} (upper panel) and ΔNCI_{SAP} (lower panel) to age. ΔNCI was assessed as the NCI at REST minus NCI during STAND and represents the individual response of complexity to the orthostatic challenge.

A significant change between 21–30 and 61–70 was detected in the case of ΔNCI_{HP} in the overall population and in the subgroup of women. Linear correlation analysis of ΔNCI_{HP} on age confirmed that the change of ΔNCI_{HP} between 21–30 and 61–70 was related to aging. Therefore, the decrease of ΔNCI_{HP} with age in the overall population was explained by the decrease of ΔNCI_{HP} in women. Since no significant change between 21–30 and 61–70 was detected in the case of ΔNCI_{SAP}, linear association with age was not tested.

Figure 3. Box-and-whisker plots of NCI_{HP} during STAND (upper panels) and linear regression of NCI_{HP} on age (lower panels). The linear regression over all values (*i.e.*, solid circles: age 21–30; open circles: age 31–40; solid triangles pointing down: age 41–50; open triangles pointing up: age 51–60; solid squares: age 61–70) and its 95% confidence interval are plotted when a significant change from 21–30 to 61–70 is detected according to one-way analysis of variance. The symbol * indicates $p < 0.05$.

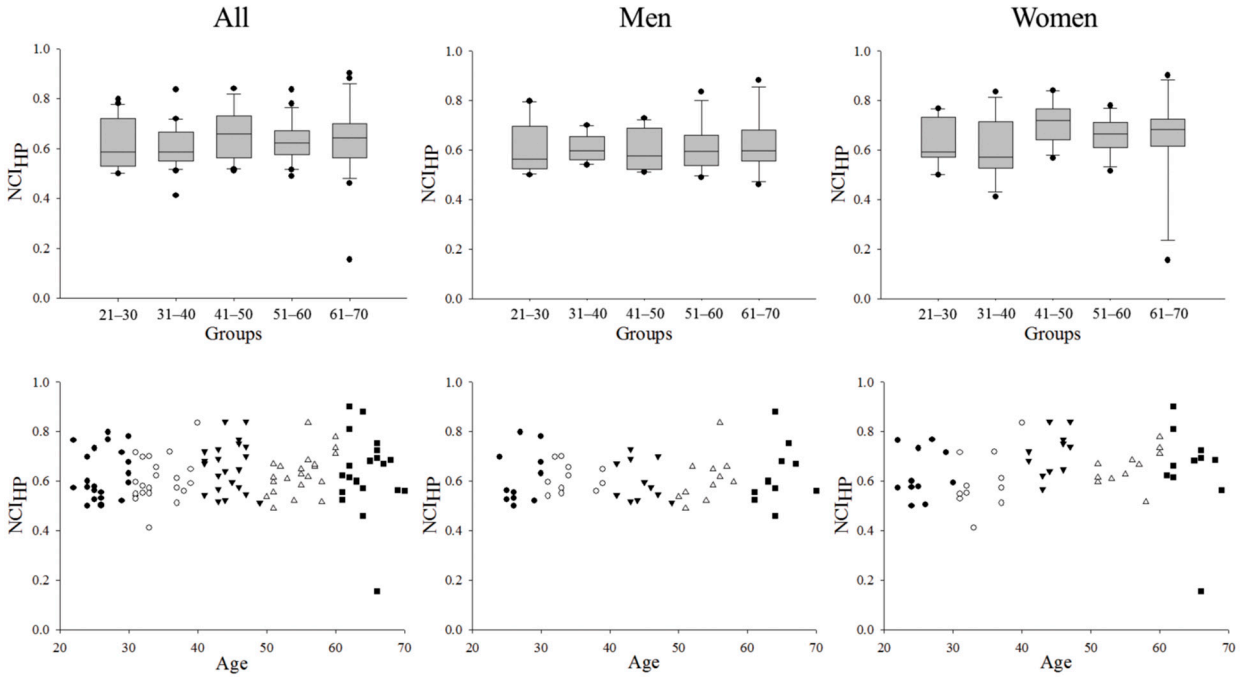


Figure 4. Box-and-whisker plots of NCI_{SAP} during STAND (upper panels) and linear regression of NCI_{SAP} on age (lower panels). The linear regression over all values (*i.e.*, solid circles: age 21–30; open circles: age 31–40; solid triangles pointing down: age 41–50; open triangles pointing up: age 51–60; solid squares: age 61–70) and its 95% confidence interval are plotted when a significant change from 21–30 to 61–70 is detected according to one-way analysis of variance. The symbol * indicates $p < 0.05$.

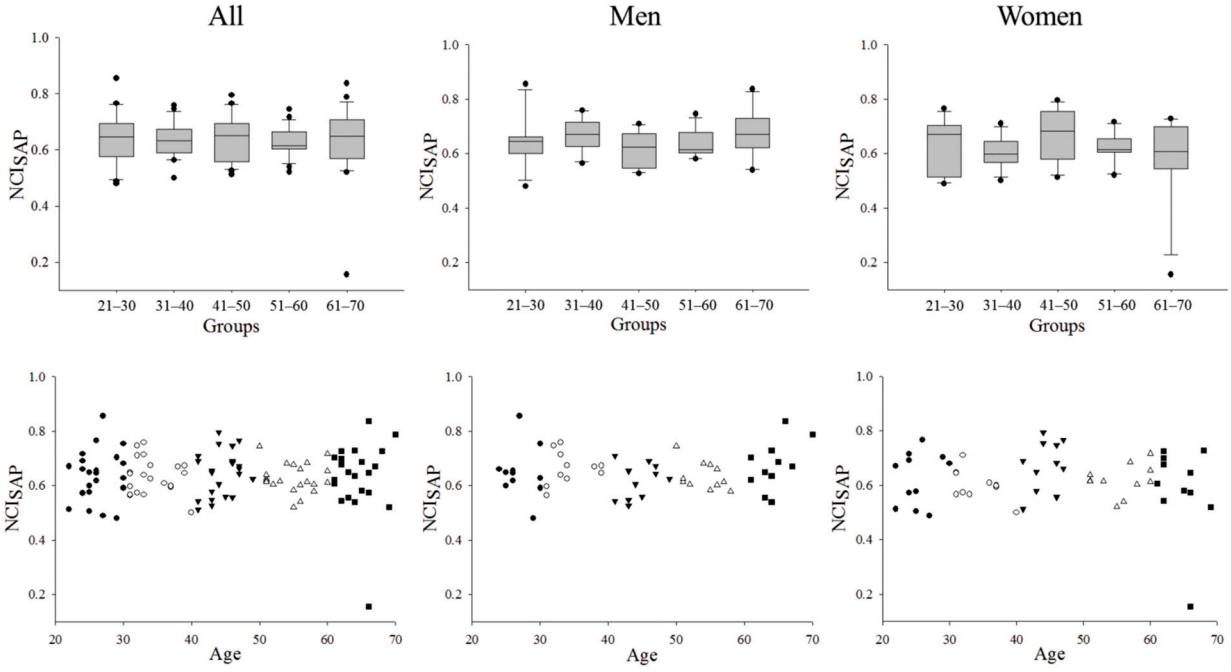
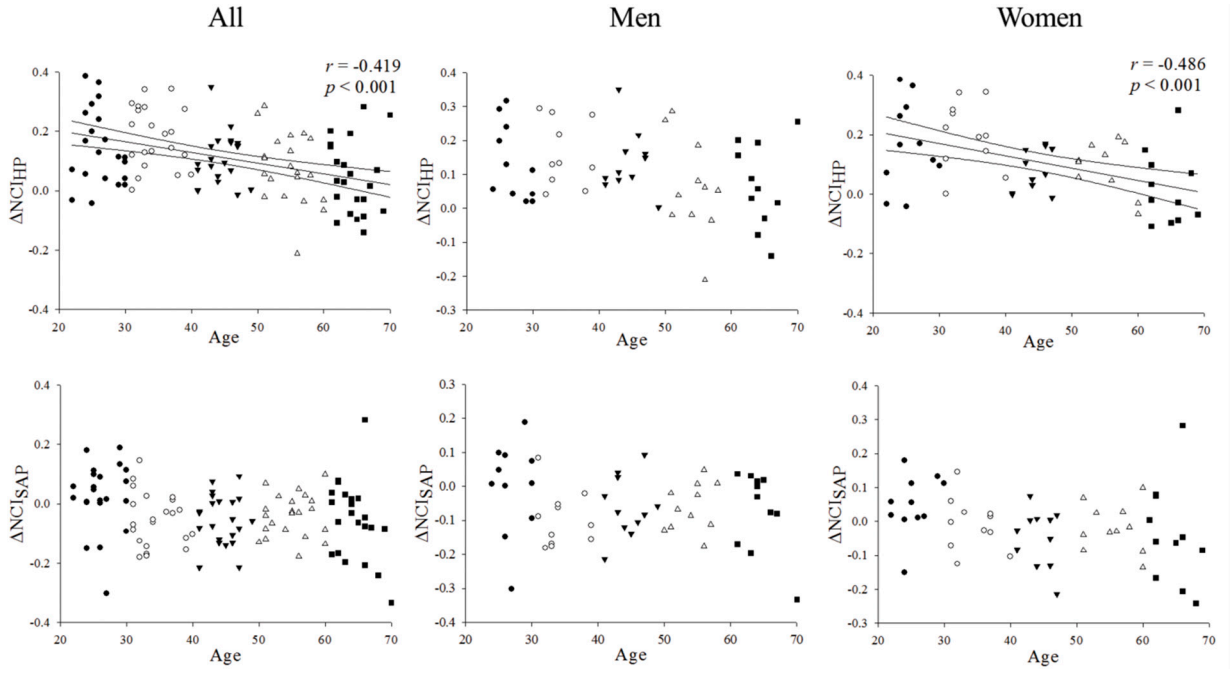


Figure 5. Linear regression of ΔNCI_{HP} (upper panels) and ΔNCI_{SAP} (lower panels) on age. The linear regression over all values (*i.e.*, solid circles: age 21–30; open circles: age 31–40; solid triangles pointing down: age 41–50; open triangles pointing up: age 51–60; solid squares: age 61–70) and its 95% confidence interval are plotted when a significant change from 21–30 to 61–70 is detected according to one-way analysis of variance. ΔNCI is assessed as NCI at REST minus NCI during STAND.



3.6. Linear Correlation Analysis between NCI and Time Domain Parameters

Table 3 shows the results of the linear regression analysis of time domain parameters (*i.e.*, μ_{HP}, σ^2_{HP}, μ_{SAP}, and σ^2_{SAP}) on NCI in the overall group of subjects and after separation of the two genders. The correlation analysis was performed in the planes (μ_{HP}, NCI_{HP}), (σ^2_{HP}, NCI_{HP}), (μ_{SAP}, NCI_{SAP}), and (σ^2_{SAP}, NCI_{SAP}). At REST in the entire group we found that μ_{HP} and σ^2_{HP} are significantly correlated with NCI_{HP}, while σ^2_{SAP} were significantly correlated with NCI_{SAP}. The correlation coefficient was positive in the case of μ_{HP} and σ^2_{HP} and negative in the case of σ^2_{SAP}. The same findings were obtained after separation of the two genders. During STAND in the overall group and in men the relations between μ_{HP} and NCI_{HP} and between σ^2_{SAP} and NCI_{SAP} were confirmed. In women the correlation between σ^2_{SAP} and NCI_{SAP} was lost.

Table 3. Linear regression analysis of time domain parameters on NCI.

Experimental Condition		**REST**		**STAND**	
Index		**r**	**significance**	**r**	**significance**
All (55 M/55 F)	μ_{HP}	0.422	Yes	0.394	Yes
	σ^2_{HP}	0.343	Yes	–0.046	No
	μ_{SAP}	–0.161	No	0.070	No
	σ^2_{SAP}	–0.493	Yes	–0.333	Yes
Men (55 M)	μ_{HP}	0.585	Yes	0.546	Yes
	σ^2_{HP}	0.432	Yes	0.090	No
	μ_{SAP}	–0.143	No	–0.034	No
	σ^2_{SAP}	–0.503	Yes	–0.550	Yes
Women (55 F)	μ_{HP}	0.388	Yes	0.401	Yes
	σ^2_{HP}	0.294	Yes	–0.127	No
	μ_{SAP}	–0.178	No	0.109	No
	σ^2_{SAP}	–0.5	Yes	–0.205	No

μ_{HP}: HP mean; σ^2_{HP}: HP variance; μ_{SAP}: SAP mean; σ^2_{SAP}: SAP variance; r: Pearson correlation coefficient; Yes/No: detection of a significant correlation with $p < 0.05$. μ_{HP} and σ^2_{HP} were tested against NCI_{HP}, while μ_{SAP} and σ^2_{SAP} against NCI_{SAP}.

4. Discussion

The main findings of this study are: (i) at REST NCI_{HP} and NCI_{SAP} decreased with age in the overall population and only women was responsible for this decrease; (ii) in women at REST the decrease of NCI_{HP} started in 51–60 and became significant in 61–70, while in men a tendency toward a decrease was observable in 41–50; (iii) during STAND NCI_{HP} and NCI_{SAP} was unrelated to age in the overall population and this trend held in both men and women; (iv) ΔNCI_{HP} decreased with age in the overall population and again only women were responsible for this decrease; and (v) ΔNCI_{SAP} was unrelated to age in the overall population and analysis by gender confirmed this trend.

4.1. Selection of the Population

No remarkable differences among groups were detectable. Only a tendency to decrease the functional capacity evaluated by VO_2 peak in both genders and a trend towards the increase of BMI in women were

observed. These tendencies are expected, because the normal aging process is associated with a reduction in functional capacity of the main organs (lung, heart, and skeletal muscles) involved in oxygen transportation, delivery, and utilization beyond the loss of ability to perform aerobic exercise [30–32] and with a propensity to overweight in women in the menopause period [33].

4.2. Complexity of the HP Variability at REST: Aging and Gender Effects

A significant decrease of HP variance and complexity was observed. These findings suggest that the magnitude of the HP changes became smaller and HP dynamics turned out to be more regular and predictable as a function of age. These results are in agreement with previous studies regardless of whether they are based on entropy rates [14,15,17,18,22,23,28,34] or on different metrics [2,13,15,16,19,22,23,29,34].

The most striking result of this study came out when the overall group was divided by gender: indeed, we found that the negative correlation of NCI_{HP} at REST was present only in women, thus suggesting that the decrease of HP complexity in women is responsible for the decrease of HP complexity in the overall population. Men had a tendency to decrease NCI_{HP} but this tendency did not lead to a significant change from 21–30 to 61–70. This observation is in agreement with the conclusion drawn by a recently proposed symbolic analysis approach [19]. Some studies using different nonlinear HP variability indexes detected the gender dependency of the relation of cardiac control complexity to age [13,16,17] but they rejected the statement that women have a significantly steeper negative relation with age than men. This discrepancy might be mostly the effect of the calculation of a biased entropy rate (*i.e.*, ApEn) that might limit the statistical power in distinguishing groups [35]. It is worth noting that, despite the decrease of functional capacity evaluated by VO_2 peak was observed in both men and women, the decline of the HP complexity was found only in women, thus suggesting that the changes of the HP complexity with age cannot be completely explained by modifications of the functional capacity. In addition, although in women there was a clear tendency to increase BMI (below the obesity threshold), the BMI variation was not significantly correlated with HP complexity modifications (REST: $r = -0.10$, $p = 0.278$; STAND: $r = -0.01$, $p = 0.898$).

It is not surprising to find out that gender is a factor modulating the relation of the complexity of the cardiac control with age at REST. Previous studies have reported that gender is one of the factors that influence cardiovascular autonomic regulation. Differences in adrenoreceptor responsiveness, arterial baroreflex sensitivity, cardiopulmonary baroreflex, and cardiac vagal and sympathetic activity and/or modulation [20,36–40] contribute to differentiating the cardiovascular control of men and women. These differences might play a role in the different relation of complexity of the cardiac control with age. We attribute to menopause the main responsibility of the observed difference between genders. Ryan *et al.* [21] observed difference in HP complexity between men and women at REST. Women showed a HP complexity greater than men. This result was interpreted as the effect of estrogen in women, making cardiac control in women more complex than that in men. Since the level of estrogen in women dramatically drops during menopause, it is not surprising to find that this decrease is mirrored by a decrease of HP complexity in women. Since the decrease of estrogen is associated to a reduction in vagal function as well [38,42], the vagal withdrawal might have driven the observed reduction of HP complexity [35,43]. The analysis of the differences between men and women in a specific age group

corroborated the observation that menopause is a key factor in decreasing HP complexity in women. Indeed, although no significant statistical differences between HP complexity in men and women were detected in any age group, likely as a result of the low statistical power of the study for this specific endpoint, we observed that NCI_{HP} was systematically larger in women than in men in any age group except 61–70, thus suggesting that after menopause complexity of the HP series in women dramatically dropped. This result confirmed the progressive decrease of gender differences with age reported in [13,19,23,38,41].

Additionally, our data suggest that, while women showed a relevant drop in 51–60, men started to exhibit a tendency toward a decrease of NCI_{HP} one decade before. Although this tendency was clear in men, it did not result in a significant change of NCI_{HP} from 21–30 to 61–70. The more stable behavior of HP complexity with age in women in the decades before 51–60 suggests that until menopause the preserved levels of estrogen might contribute to keeping the HP complexity high, thus playing a protective role against adverse cardiac events in women [13,21,44,45]. Therefore, our data support the view that menopause identifies a significant turning point in cardiovascular physiology in women [46]. Indeed, it is known that women have lower incidence of cardiovascular disease compared to men until menopause [46], and this protective effect is mirrored by a tendency to have a higher complexity of cardiac control. After menopause an increased incidence of cardiovascular disease has been observed [46,47], and the observed significant reduction of complexity of cardiac control in women might be a hallmark of the increased risk of occurrence of cardiovascular events [5].

4.3. Complexity of the SAP Variability at REST: Aging and Gender Effects

Earlier studies described an increase of SAP mean [7,48] and SAP variability with aging [10–12,49–51]. These results have been interpreted as a consequence of the increase of sympathetic activity and/or modulation directed to the vessels with age [48]. However, a modified cardiac reserve [7], changes of the structure of the arteries (*i.e.*, increased stiffness, decreased compliance, and endothelial dysfunction) [52], and changes of diastolic filling and increases in collagen in the left ventricle might play a role in the modification of SAP mean and variance with age [7,31]. Due to the abovementioned modifications all having a possible impact on SAP dynamics, a modification of the complexity of the SAP series at REST with age is expected.

In the present study we observed at REST a decrease of NCI_{SAP} with age in the overall population. This finding is in agreement with [18]. However, after separating women from men we found that the negative correlation of NCI_{SAP} with age was significant only in women. Therefore, it appears that the decrease of SAP complexity in women seems to be mainly responsible for the decrease of NCI_{SAP} in the overall population because the SAP complexity in men did not significantly decrease. The observed decrease in women is relevant as emphasized by the significantly lower NCI_{SAP} in 61–70 compared to 21–30. Similarly to the HP complexity, the relation of the SAP complexity with age observed in women cannot be fully explained as a result of the reduction of the functional capacity evaluated by VO_2 peak with age, given that a similar VO_2 peak decrease in men did not produce a significant change of the SAP complexity from 21–30 to 61–70. Again similarly to the HP complexity, the increase of BMI in women was not found to be correlated to SAP complexity variations (REST: $r = -0.08$, $p = 0.405$; STAND: $r = -0.02$, $p = 0.800$).

We speculate that, with the advent of menopause, the decrease in estrogen production leads to an increase in sympathetic tone [44,45,53,54] and sympathetic overactivity is mainly responsible for the reduction of SAP complexity with age in women [18]. Since the sympathetic drive increased with age even in men but was not associated to a reduction of the SAP complexity with age, we conclude that sympathetic control directed to the vessel is different in old men and women and this difference might be related to the sensitivity of cardiovascular control mechanisms to estrogen levels in women. Even though the abovementioned explanations are plausible, we cannot exclude the possibility that the progressive reduction of the SAP variability complexity with age might simply be a reflection of the decrease of HP variability complexity transferred to the SAP variability via the feedforward pathway, accounting for the Frank–Starling mechanism and the Windkessel effect [18].

4.4. Complexity of HP and SAP Variabilities during STAND: Aging and Gender Effects

During STAND, NCI_{HP} and NCI_{SAP} did not vary with age. This result was in agreement with [18] when a similar metric (*i.e.*, an entropy rate) was considered. Since this result held even when the overall group was divided by gender, we suggest that cardiovascular adjustments occurring during senescence in a healthy population in relation to one of the most important challenges for human beings (*i.e.*, STAND) is responsible for the lack of any gender-dependent effect.

4.5. Individual Changes in the Complexity of HP and SAP Variabilities in Response to STAND

The study assessed the individual response to the orthostatic challenge by computing the difference between the complexity markers assessed at REST and during STAND (*i.e.*, ΔNCI = NCI at REST–NCI during STAND). We confirm that in the youngest group (*i.e.*, 21–30) ΔNCI was positive in the case of ΔNCI_{HP} and was about 0 in the case of ΔNCI_{SAP} [43,55]. One of the original findings of this study is that ΔNCI_{HP} decreased with age in the overall population, while ΔNCI_{SAP} was not affected by age. Since at REST both NCI_{HP} and NCI_{SAP} decreased with age, the observed trends of ΔNCI cannot be considered exclusively a reflection of those of NCI at REST. This observation suggests that a mere decrease of vagal activity and/or sympathetic overactivation with age at REST might be insufficient to explain *per se* the progressive reduction of ΔNCI_{HP} during senescence. We suggest that the decrease of ΔNCI_{HP} with age is a sign of the difficulty that older individuals have in dealing with sympathetic stressors, such as the orthostatic challenge [37]: indeed, older subjects seem to lose their ability to decrease HP complexity by synchronizing the activity of several control mechanisms [35], likely via a sympathetic activation [56]. This loss of capability was not evident at the level of complexity of the vascular control, mainly because ΔNCI_{SAP} was already low in young subjects [43,55]. These findings are in agreement with those reported in [18,57,58] and can be explained by the reduced effect of the change of posture on the cardiovascular variables with age. The progressively limited influence of the orthostatic challenge has been attributed to the impairment of beta-adrenergic receptor stimulation [7,31], reduced efficiency of post-synaptic-adrenergic signaling [52], reduced vagal autonomic modulation to the sinus node [3,5–9,59], reduced sympathetic modulation to the vessels [48], and decreased baroreflex efficiency [48,58,60].

Remarkably, when the overall group was subdivided by gender we found that only women showed a significant negative correlation of ΔNCI_{HP} on age. In men only, a tendency toward a decrease of ΔNCI_{HP} was

observed. However, this tendency did not produce a significant drop of ΔNCI_{HP} from 21–30 to 61–70. Therefore, men did not contribute to the reduction of the individual response of HP complexity to postural challenge with age observed in the overall population, thus stressing further that the relation of the complexity of cardiac control with age is gender-dependent. It appears that the ability to synchronize several control mechanisms via a sympathetic activation was more importantly lost in women than in men. We speculate that the reduction of estrogen during menopause leads to a higher sympathetic tone at REST [20,21] and, thus, to a cardiovascular control less fit to react to the change of posture.

4.6. Linear Regression Analysis of NCI on Time Domain Parameters

From a methodological perspective, time domain parameters, such as mean and variance describing, respectively, the position and the dispersion of the HP and SAP distributions, provide completely different information compared to NCI quantifying the degree of irregularity of the series based on their unpredictability. Despite the different focus, we found an important association between time domain and complexity indexes. For example, the positive association between HP variance and HP complexity is due to their relation with vagal control increasing both HP variance and HP complexity [25,35] and the negative association between SAP variance and SAP complexity might be due to their relation with sympathetic control increasing SAP variance but decreasing SAP complexity, possibly via synchronization of peripheral vasomotion in different districts [56]. However, having a significant association between a time domain parameter and a complexity index does not imply that the two variables exhibit the same relation (significant or nonsignificant) with age. For example, despite HP mean and HP complexity being correlated at REST, the HP mean was not significantly related to age, while HP complexity was. This observation leads us to conclude that, although correlated, time domain parameters and complexity indexes carry non-redundant information.

5. Conclusions

Our study stresses that the relation of the complexity of cardiovascular control to age, as measured via the computation of an entropy rate over HP and SAP variabilities, is gender-dependent. Therefore, gender should be accounted for in any study assessing the complexity of the cardiovascular regulation. We observed that women exhibited a bigger drop in the complexity of cardiovascular control than men and this relevant decrease occurred after menopause, thus suggesting its association with the reduction of estrogen production during menopause. In addition, only women showed a progressively limited response to the orthostatic challenge with age, thus stressing again the dependence of complexity indexes on gender and suggesting that women tend to lose the physiological ability to reduce the complexity of cardiac control during orthostatic challenge at a faster rate with age than men, especially during menopause. This study suggests that complexity markers derived from cardiovascular variability are more helpful in following the aging process in women than in men. Similarly, orthostatic challenge appears to be more suitable in women than in men for emphasizing the difficulty that elderly subjects have in reacting to a postural challenge.

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Author Contributions

Aparecida M. Catai: conception and design of the work, analysis of the data, interpretation of the data, drafting the article, critical revision of the manuscript.

Anielle C.M. Takahashi: conception of the work, analysis of the data, critical revision of the manuscript.

Natália M. Perseguini, Juliana C. Milan, Vinicius Minatel, Patrícia Rehder-Santos: acquisition of the data, analysis of the data.

Vlasta Bari, Andrea Marchi: analysis of the data.

Alberto Porta: conception and design of the work, interpretation of the data, drafting the article, critical revision of the manuscript.

All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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