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RESEARCH ARTICLE

Influence of age and gender on the phase and strength of the relation between heart period and systolic blood pressure spontaneous fluctuations

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Milan-Mattos JC, Porta A, Perseguini NM, Minatel V, Rehder-Santos P, Takahashi AC, Mattiello SM, Catai AM. Influence of age and gender on the phase and strength of the relation between heart period and systolic blood pressure spontaneous fluctuations. *J Appl Physiol* 124: 791–804, 2018. First published December 6, 2017; doi:10.1152/japplphysiol.00903.2017.—Aging affects baroreflex regulation. The effect of senescence on baroreflex control was assessed from spontaneous fluctuations of heart period (HP) and systolic arterial pressure (SAP) through the HP-SAP gain, while the HP-SAP phase and strength are usually disregarded. This study checks whether the HP-SAP phase and strength, as estimated, respectively, via the phase of the HP-SAP cross spectrum ($Ph_{HP\text{-}SAP}$) and squared coherence function ($K^2_{HP\text{-}SAP}$), vary with age in healthy individuals and trends are gender-dependent. We evaluated 110 healthy volunteers (55 males) divided into five age subgroups (21–30, 31–40, 41–50, 51–60, and 61–70 yr). Each subgroup was formed by 22 subjects (11 males). HP series was extracted from electrocardiogram and SAP from finger arterial pressure at supine resting (REST) and during active standing (STAND). $Ph_{HP\text{-}SAP}$ and $K^2_{HP\text{-}SAP}$ functions were sampled in low-frequency (LF, from 0.04 to 0.15 Hz) and in high-frequency (HF, above 0.15 Hz) bands. Both at REST and during STAND $Ph_{HP\text{-}SAP}$(LF) showed a negative correlation with age regardless of gender even though values were more negative in women. This trend was shown to be compatible with a progressive increase of the baroreflex latency with age. At REST $K^2_{HP\text{-}SAP}$(LF) decreased with age regardless of gender, but during STAND the high values of $K^2_{HP\text{-}SAP}$(LF) were more preserved in men than women. At REST and during STAND the association of $Ph_{HP\text{-}SAP}$(HF) and $K^2_{HP\text{-}SAP}$(HF) with age was absent. The findings points to a greater instability of baroreflex control with age that seems to affect to a greater extent women than men.

NEW & NOTEWORTHY Aging increases cardiac baroreflex latency and decreases the degree of cardiac baroreflex involvement in regulating cardiovascular variables. These trends are gender independent but lead to longer delays and asmaller degree of cardiac baroreflex involvement in women than in men, especially during active standing, with important implications on the tolerance to an orthostatic stressor.

active standing; aging; autonomic nervous system; cardiac baroreflex sensitivity; cardiovascular control; coherence; heart rate variability; latency

INTRODUCTION

The characterization of the cardiac baroreflex from spontaneous fluctuations of heart period (HP) and systolic arterial pressure (SAP) was frequently carried out in the frequency domain based on the cross-spectral method estimating the transfer function from SAP to HP (7, 10, 13, 16, 28, 38, 40, 46, 54). This method allows the computation of the gain of the HP-SAP relation, usually referred to as cardiac baroreflex sensitivity (BRS), the strength of the HP-SAP relation via the squared coherence function ($K^2_{HP\text{-}SAP}$), and the phase of the HP-SAP relation ($Ph_{HP\text{-}SAP}$) as a function of the frequency (37). The BRS, usually expressed in milliseconds per millimeters of mercury, provides the HP lengthening (or shortening), expressed in milliseconds, in response to a SAP rise (or fall) of 1 mmHg, $K^2_{HP\text{-}SAP}$ measures the degree of association between HP and SAP spontaneous fluctuations, and $Ph_{HP\text{-}SAP}$ can be exploited to infer the baroreflex latency (BL). Despite the powerful characterization of the HP-SAP relation given by the cross-spectral analysis, solely BRS is usually monitored in experimental protocols aiming at the characterization of cardiac baroreflex. As a matter of fact, $K^2_{HP\text{-}SAP}$ and $Ph_{HP\text{-}SAP}$ are computed mainly to check the reliability of the BRS computation requiring a significant $K^2_{HP\text{-}SAP}$ and a $Ph_{HP\text{-}SAP}$ compatible with HP changes lagging behind SAP variations (i.e., negative phase values with the most frequently utilized convention for HP-SAP cross-spectrum estimation) (13). This attitude neglects that $K^2_{HP\text{-}SAP}$ and $Ph_{HP\text{-}SAP}$ can have a pathophysiological value per se, limits the characterization of cardiac baroreflex and reduces the information about cardiac baroreflex control. This position is particularly evident in protocols assessing the effect of aging on cardiac baroreflex control. Indeed, while it is well-known that BRS progressively decreases with age (1, 14, 16, 24, 26, 32, 52), as a likely result of the progressive sympathetic activation and vagal withdrawal observed during senescence (1, 6, 12, 14, 18, 24, 34, 47, 51), the effects of age on $K^2_{HP\text{-}SAP}$ and $Ph_{HP\text{-}SAP}$ are not explored from spontaneous HP and SAP variabilities. The sole data on BL modification with age are based on carotid baroreceptor stimulation via neck suction or pressure (17). Similarly, while the influence of gender on the decline of BRS with age has been evaluated (1, 52), it is unclear whether $K^2_{HP\text{-}SAP}$ and $Ph_{HP\text{-}SAP}$ might show differences in relation to gender. Since the sympathetic drive increases with age and sympathetic

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activation is known to affect BL (7, 25, 41) and the degree of the SAP-HP coupling (36, 44), it can be hypothesized that $Ph_{HP\text{-}SAP}$ and $K^2_{HP\text{-}SAP}$ could vary with age. Since the sympathetic overactivation during senescence is gender dependent (34), it can be hypothesized that modifications of $Ph_{HP\text{-}SAP}$ and $K^2_{HP\text{-}SAP}$ with age could be gender specific. This information is relevant because an augmented BL might play a role in postural-related syncope in old individuals (21) and increase the likelihood of cardiovascular events (45) by inducing instabilities in arterial pressure regulatory loop and increasing arterial pressure variability (9, 22, 29). On the other hand, a reduced strength of HP-SAP relation indicates a reduced involvement of the cardiac baroreflex in controlling arterial pressure variations (2, 35, 38) with again implications on the ability of the individual to cope with the orthostatic challenge and buffer arterial pressure variations. Increased BL and reduced baroreflex involvement might play a role in the observed gender-related differences in orthostatic tolerance (20, 53).

Therefore, the aim of this study is to provide the full characterization of the dependence of $Ph_{HP\text{-}SAP}$ and $K^2_{HP\text{-}SAP}$ on age and gender as computed from HP and SAP spontaneous beat-to-beat variabilities. We considered a group of 110 healthy individuals aged from 21 to 70 yr and composed by 22 subjects in each decade. The entire group and the subgroups were gender balanced. Subjects underwent recordings at rest in supine position (REST) and during sympathetic activation induced by active standing (STAND) (8, 43). BRS was monitored as well.

METHODS

Study population. Data belonged to a database specifically designed to track the modifications of the cardiovascular control and its response to an orthostatic stressor with age (8, 43). We make reference to Catai et al. (8) and Porta et al. (43) for a full description of the population characteristics. Briefly, we evaluated 110 apparently healthy humans (55 males and 55 females) aged from 21 and 70 yr. The overall range of age was divided into five decades: from 21 to 30 yr (21–30), from 31 to 40 yr (31–40), from 41 to 50 yr (41–50), from 51 to 60 yr (51–60), and from 61 to 70 yr (61–70). Twenty-two subjects (11 men and 11 women) fell in each decade. Subjects exhibiting pathological alterations of cardiac electrical activity, as assessed via surface electrocardiogram (ECG) at REST and during maximal dynamic exercise, as well as suffering from cardiovascular diseases, orthopedic, respiratory, neurological, inflammatory, and vascular dysfunctions were not enrolled in the study. Smokers, drinkers, and users of illicit drugs or any medication that could interfere with the autonomic control, individuals with arterial hypertension or diabetes, and people with body mass index >30 kg/m^2 were excluded. Women under contraceptive medication or hormone replacement therapy were not included as well.

The study was approved by the Ethics Committee of Federal University of São Carlos (São Carlos, SP, Brazil; Protocol No. 1.293.582) and was performed according to the principles of the Declaration of Helsinki for medical research involving humans. All participants gave their informed written consent.

Experimental protocol. Experiments were carried out in the afternoon in a climatically controlled room (22–23°C) with relative air humidity of 40–60%. Subjects were instructed to avoid caffeinated or alcoholic beverages as well as strenuous exercise on the day before the experiment. They were also instructed to have a light meal 2 h before the experiment. All volunteers were familiarized with the equipment and the experimental procedures before starting the acquisition session. Surface ECG (lead II) was acquired via a differential amplifier (BioAmp FE132; ADInstruments), finger arterial pressure via a photoplethysmographic device (Finometer PRO; Finapres Medical System) and respiratory movements through a piezoelectric belt (Marazza, Monza, Italy). Signals were recorded for 15 min at REST and during STAND. Ten minutes at REST were allowed to the subject for stabilizing cardiovascular variables before starting the REST session. STAND followed always REST. During the data collection, subjects were instructed to breathe spontaneously and they were not allowed to talk. All subjects completed STAND without experiencing any sign of presyncope. The signals were sampled at 400 Hz using a commercial acquisition device (Power Laboratory 8/35; ADInstruments). The arterial pressure was obtained from the middle finger of the right hand, which was maintained at the level of heart by fixing the subject's arm to his/her thorax during the experimental protocol.

Beat-to-beat series extraction. After the QRS complex was detected from the ECG and the R-wave apex was located using parabolic interpolation, the HP was approximated as the time distance between two consecutive R-wave apexes and taken as the i-th HP [i.e., HP(i)], where i the progressive cardiac beat counter. The maximum of arterial pressure inside HP(i) was taken as i-th SAP [i.e., SAP(i)]. Respiratory movement signal was sampled in correspondence of the first R-wave delimiting HP(i) and denoted as RESP(i). The occurrences of R-wave and SAP peaks were carefully checked to avoid erroneous detections or missed beats. The series HP $= \{HP(i), i = 1,...,N\}$, SAP $= \{SAP(i), i = 1,...,N\}$, and RESP $= \{RESP(i), i = 1,...,N\}$, where N is the series length, was linearly detrended. Cross-spectral analysis was carried out over HP and SAP series, while RESP series was utilized to estimate the dominant respiratory rate f_{RESP} expressed in hertz. Since the analysis focuses on short-term cardiovascular control, N was fixed to 256 (i.e., recordings of a few minutes) (49). The stationarity of the selected sequence was tested according to Magagnin et al. (30) 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 (30). Time domain parameters such as the HP mean, HP variance, SAP mean, and SAP variance were computed, labeled as μ_{HP}, σ^2_{HP}, μ_{SAP}, and σ^2_{SAP}, and expressed in milliseconds, milliseconds squared, millimeters of mercury, and millimeters of mercury squared, respectively.

HP-SAP cross-spectral analysis. Transfer function estimation was carried according to Porta et al. (39). The approach was grounded on the computation of the HP-SAP cross spectrum and HP and SAP power spectra via a model-based bivariate autoregressive approach. The model order was fixed to 10. The coefficients of the linear regressions describing the dynamics of HP and SAP according to previous past samples of both the series and the variance of the white noises impinging the model were identified via the least-squares approach. The transfer function from SAP to HP was estimated as the ratio of the HP-SAP cross spectrum to the SAP power spectrum. The transfer function modulus (i.e., the gain of the HP-SAP relation) was taken as an estimate of BRS as a function of the frequency. The transfer function phase (i.e., the phase of the HP-SAP cross spectrum), labeled as $Ph_{HP\text{-}SAP}$, was taken as an estimate of the phase shift between HP and SAP in the frequency domain. The squared coherence function $K^2_{HP\text{-}SAP}$ computed as the ratio of the square cross-spectrum modulus divided by product of the power spectra of HP and SAP series was taken as an estimate of the degree of the HP-SAP association as a function of the frequency. A low value of $K^2_{HP\text{-}SAP}$ suggests a weak involvement of baroreflex in regulating HP-SAP dynamical interactions. By definition, BRS was larger than 0; $K^2_{HP\text{-}SAP}$ ranged from 0 to 1, indicating the perfect HP-SAP uncorrelation and the full HP-SAP correlation, respectively; and $Ph_{HP\text{-}SAP}$ ranged from $-\pi$ to $+\pi$, indicating equivalent out-of-phase behaviors between HP and SAP. $Ph_{HP\text{-}SAP}$ equal to 0 indicated no delay between HP and SAP series and, according to the convention for the computation of the HP-SAP cross spectrum, a negative phase suggested that HP changes lagged behind SAP variations. BRS is expressed in

milliseconds per millimeters of mercury, $Ph_{HP\text{-}SAP}$ in radians, and $K^2_{HP\text{-}SAP}$ is dimensionless. BRS, $Ph_{HP\text{-}SAP}$, and $K^2_{HP\text{-}SAP}$ depended on frequency. Therefore, they were sampled at the maximum of the coherence function in the low-frequency (LF, from 0.04 to 0.15 Hz) and high-frequency (HF, above 0.15 Hz) bands. The indexes were labeled as BRS(LF), $Ph_{HP\text{-}SAP}$(LF), $K^2_{HP\text{-}SAP}$(LF), BRS(HF), $Ph_{HP\text{-}SAP}$(HF), and $K^2_{HP\text{-}SAP}$(HF) in the analyses that followed. According to Porta et al. (40), BRS(LF) was computed regardless the values of $Ph_{HP\text{-}SAP}$(LF) and $K^2_{HP\text{-}SAP}$(LF). The same strategy was followed to compute BRS(HF) in relation to $Ph_{HP\text{-}SAP}$(HF) and $K^2_{HP\text{-}SAP}$(HF). $Ph_{HP\text{-}SAP}$(LF) and $Ph_{HP\text{-}SAP}$(HF) were converted into delays or advancements, $\tau_{HP\text{-}SAP}$(LF) and $\tau_{HP\text{-}SAP}$(HF), respectively, according to the transformations $\tau_{HP\text{-}SAP}(LF) = Ph_{HP\text{-}SAP}(LF)/(2\pi LF)$ and $\tau_{HP\text{-}SAP}(HF) = Ph_{HP\text{-}SAP}(HF)/(2\pi HF)$, respectively (41). Since $Ph_{HP\text{-}SAP}$(LF) and $Ph_{HP\text{-}SAP}$(HF) were known at multiples of 2π, phase multiples should be considered as well [i.e., $Ph_{HP\text{-}SAP}(LF) + 2\pi k$ and $Ph_{HP\text{-}SAP}(HF) + 2\pi k$ with $k = 0, \pm1, \pm2, \ldots$]. Only multiples with $k = 0$ and $k = \pm1$ were considered, since only those values of k produced time shifts that might be physiologically plausible. The calculated values of $\tau_{HP\text{-}SAP}$(LF) and $\tau_{HP\text{-}SAP}$(HF) were compared with the shortest BL between a single arterial carotid baroreceptor stimulus and the vagally mediated HP prolongation (i.e., 0.24 s) (15) and the time after a single arterial carotid baroreceptor stimulus occurring to the HP to decline to control level (i.e., effects of stimulation are exhausted by 4 s) (5). If the calculated delay was longer than 0.24 s and shorter than 4 s, it was assumed to be compatible with a working baroreflex (41) and indicated as τ_{BL}(LF) and τ_{BL}(HF) in the analyses that followed.

Statistical analysis. The null hypothesis of normal distribution was tested according to Shapiro-Wilk test. After all the extracted parameters were pooled together [i.e., BRS(LF), $Ph_{HP\text{-}SAP}$(LF), $K^2_{HP\text{-}SAP}$(LF), BRS(HF), $Ph_{HP\text{-}SAP}$(HF), and $K^2_{HP\text{-}SAP}$(HF)] regardless of age, unpaired *t*-test, or Mann-Whitney rank sum test when appropriate, was utilized to check the significance of the gender-related differences. One-way ANOVA (Dunnett's method for multiple comparisons), or Kruskal-Wallis one-way ANOVA on ranks (Dunnett's method for multiple comparisons) when appropriate, was applied to check the significance of the differences of all parameters vs. the 21–30 subgroup. The linear correlation analysis was performed to check the association of any parameter on age. The Pearson product moment correlation coefficient *r* and the probability of type I error *P* was calculated. The presence of a more general form of correlation (i.e., nonlinear) was tested using Spearman rank order correlation coefficient. Statistical analyses were carried out using a commercial statistical program (SigmaPlot 11.0; Systat, Chicago, IL). $P < 0.05$ was always considered as significant.

RESULTS

Time domain parameters and breathing rate at REST and during STAND. Table 1 shows time domain parameters (i.e., μ_{HP}, σ^2_{HP}, μ_{SAP}, and σ^2_{SAP}) and f_{RESP} in the overall population and in the two subgroups composed only by men and women at REST. Values are expressed as median (1st quartile/3rd quartile) as a function of the decade. μ_{HP} and f_{RESP} remained constant with age. Conversely, σ^2_{HP} decreased with age in the overall population and the decline was significant in both genders. Both μ_{SAP} and σ^2_{SAP} increased in the overall group. While the increase of μ_{SAP} was mainly due to women, the increase of σ^2_{SAP} was manifest in both genders.

Table 2 has the same structure of Table 1 but reports the values of the parameters during STAND. σ^2_{SAP} and f_{RESP} were not affected by age. Conversely, μ_{SAP} lengthened with age in the overall population and this trend was more evident in women. σ^2_{HP} decreased with age in the overall population, and the decline was significant in both genders. μ_{SAP} increased in the overall group and its rise was mainly due to women.

Table 3 compares μ_{HP}, σ^2_{HP}, μ_{SAP}, σ^2_{SAP}, and f_{RESP} in men and women. Values are reported as median (1st quartile/3rd quartile) after pooling together all values regardless of decade both at REST and during STAND. No gender-related differences were detected except for μ_{HP} and f_{RESP}: more specifically, women were more tachycardic than men both at REST and during STAND and breathed at a faster rate at REST.

Cross-spectral indexes at REST and during STAND. Table 4 shows the results of HP-SAP cross-spectral analysis in the overall population and in the two subgroups composed only by men and women at REST. Indexes were reported as median (first quartile/third quartile) as a function of decade. In the

Table 1. *Time domain indexes and respiratory rate as a function of age at REST*

Decade/Sex	μ_{HP}, ms	σ^2_{HP}, ms^2	μ_{SAP}, mmHg	σ^2_{SAP}, mmHg^2	f_{RESP}, Hz
21–30					
All	901 (782/1,016)	2,316 (1,325/3,743)	110 (106/119)	13.69 (7.70/25.06)	0.31 (0.28/0.33)
Men	961 (865/1,053)	2,469 (1,398/3,302)	117 (110/122)	13.98 (11.59/35.43)	0.3 (0.26/0.31)
Women	859 (767/915)	2,163 (1,372/4,230)	108 (104/111)	13.40 (7.03/20.00)	0.32 (0.31/0.34)
31–40					
All	930 (848/1,018)	1,292 (950/1,907)	117 (110/124)	18.65 (15.07/22.88)	0.29 (0.27/0.32)
Men	962 (858/1,020)	1,667 (849/2,511)	119 (112/126)	18.84 (15.66/21.85)	0.29 (0.27/0.32)
Women	873 (848/955)	1,256 (1,067/1,678)	116 (109/117)	18.45 (10.10/26.85)	0.30 (0.27/0.32)
41–50					
All	934 (851/1,053)	1,177 (947/2,291)	114 (108/120)	19.69 (13.35/36.17)	0.28 (0.25/0.32)
Men	1,004 (876/1,127)	1,184 (931/3,129)	113 (107/120)	21.39 (14.48/31.04)	0.26 (0.23/0.30)
Women	919 (859/982)	1,171 (958/1,948)	114 (111/119)	14.86 (11.11/36.08)	0.30 (0.27/0.32)
51–60					
All	895 (823/953)	866 (596/1,740)*	124 (123/139)*	19.60 (14.27/26.86)	0.28 (0.25/0.29)
Men	949 (897/1,077)	1,232 (799/1,845)	123 (120/125)	19.85 (14.68/32.47)	0.25 (0.23/0.28)
Women	830 (811/895)	819 (466/1,078)*	135 (124/146)*	19.34 (14.10/22.91)	0.29 (0.28/0.30)
61–70					
All	880 (842/989)	897 (520/1,144)*	116 (113/124)	29.02 (17.44/52.64)*	0.28 (0.26/0.30)
Men	881 (867/987)	919 (682/1,067)*	115 (111/120)	28.27 (15.60/44.72)	0.26 (0.26/0.29)
Women	850 (805/1,001)	876 (474/1,156)*	117 (113/127)*	29.78 (21.18/77.61)	0.29 (0.27/0.31)

Values are expressed as median (1st quartile/3rd quartile). HP, heart period; SAP, systolic arterial pressure; μ_{HP}, HP mean; σ^2_{HP}, HP variance; μ_{SAP}, SAP mean; σ^2_{SAP}, SAP variance; f_{RESP}, respiratory rate. *$P < 0.05$ vs. 21–30.

Table 2. *Time domain indexes and respiratory rate as a function of age at STAND*

Decade/Sex	μ_{HP}, ms	σ^2_{HP}, ms^2	μ_{SAP}, mmHg	σ^2_{SAP}, mmHg^2	f_{RESP}, Hz
21–30					
All	698 (690/777)	1,782 (893/2,599)	114 (108/120)	32.67 (18.60/40.69)	0.30 (0.26/0.33)
Men	739 (708/822)	2,469 (1512/3,195)	117 (113/123)	38.21 (26.33/51.46)	0.28 (0.27/0.32)
Women	685 (659/698)	1,447 (911/2,281)	104 (99/118)	25.61 (20.72/35.89)	0.32 (0.26/0.33)
31–40					
All	767 (726/822)	1,582 (749/2,091)	117 (113/129)	23.27 (17.93/36.72)	0.28 (0.26/0.31)
Men	825 (799/962)	1,667 (628/2,253)	125 (113/135)	16.23 (13.28/31.07)	0.27 (0.26/0.28)
Women	723 (710/737)	1,498 (964/2,080)	114 (112/122)	26.56 (22.51/36.45)	0.30 (0.26/0.31)
41–50					
All	791 (730/872)	1,370 (549/2,569)	120 (118/126)	21.92 (19.86/36.03)	0.28 (0.26/0.30)
Men	807 (706/905)	1,245 (968/2,355)	121 (117/130)	22.92 (19.11/41.27)	0.29 (0.27/0.31)
Women	787 (739/855)*	1,609 (501/2,691)	118 (113/124)	20.03 (15.71/26.41)	0.28 (0.25/0.30)
51–60					
All	786 (732/824)	909 (407/1,713)*	137 (119/146)*	40.88 (20.85/52.77)	0.28 (0.26/0.30)
Men	772 (730/941)	1,615 (716/1,783)	133 (120/141)	45.16 (24.86/51.47)	0.27 (0.26/0.29)
Women	789 (772/803)*	765 (458/1,606)	143 (134/150)*	24.80 (22.49/55.14)	0.29 (0.28/0.31)
61–70					
All	814 (732/871)*	669 (375/900)*	121 (112/127)	26.21 (17.29/40.41)	0.31 (0.26/0.33)
Men	793 (726/868)	746 (303/911)*	122 (112/128)	22.51 (20.73/38.04)	0.31 (0.29/0.34)
Women	828 (756/892)*	431 (387/868)*	119 (114/125)*	29.92 (15.43/41.96)	0.30 (0.26/0.32)

Values are expressed as median (1st quartile/3rd quartile). μ_{HP}, HP mean; σ^2_{HP}, HP variance; μ_{SAP}, SAP mean; σ^2_{SAP}: SAP variance; f_{RESP}, respiratory rate. *$P < 0.05$ vs. 21–30.

entire population $Ph_{HP\text{-}SAP}$(LF) became more negative in the 41–50, 51–60, and 61–70 groups compared with 21–30. In men, the decline in $Ph_{HP\text{-}SAP}$(LF) was significant when the 61–70 decade was compared with 21–30, while in women differences were significant when the 51–60 and 61–70 decades were compared with 21–30. $Ph_{HP\text{-}SAP}$(HF) decreased significantly in the 51–60 decade compared with 21–30 in both overall population and men. No between-group difference in $Ph_{HP\text{-}SAP}$(HF) was observed in women. In both the entire population and men, $K^2_{HP\text{-}SAP}$(LF) was lower in the 61–70 group compared with 21–30, while in women between-decade changes were not significant. In the entire population, $K^2_{HP\text{-}SAP}$(HF) did not show any significant variation and this result held regardless of gender. The BRS(LF) of the 41–50, 51–60, and 61–70 groups was lower than that of 21–30 in the overall population and in men, while in women significant differences were detected solely when the 61–70 decade was compared with 21–30. The BRS(HF) decreased in the 51–60 and 61–70 decades compared with 21–30 in the entire population and in women. In men, the decline of BRS(HF) was observed in the 61–70 decade compared with 21–30.

Table 3. *Time domain indexes and respiratory rate in men and women*

Index/Experimental Condition	Men	Women
μ_{HP}, ms		
REST	947 (867/1,062)	873 (806/940)*
STAND	793 (729/910)	758 (709/809)*
σ^2_{HP}, ms^2		
REST	1,210 (859/2,456)	1,132 (729/1,875)
STAND	1,245 (752/2,170)	1,107 (513/1,867)
μ_{SAP}, mmHg		
REST	119 (110/123)	116 (109/125)
STAND	122 (114/133)	119 (111/129)
σ^2_{SAP}, mmHg^2		
REST	19.85 (14.07/36.65)	19.34 (11.32/31.47)
STAND	31.07 (17.83/46.02)	24.85 (19.34/39.72)
f_{RESP}, Hz		
REST	0.27 (0.25/0.31)	0.30 (0.28/0.32)*
STAND	0.28 (0.26/0.31)	0.30 (0.26/0.32)

Values are expressed as median (1st quartile/3rd quartile). μ_{HP}, HP mean; σ^2_{HP}, HP variance; μ_{SAP}, SAP mean; σ^2_{SAP}, SAP variance; f_{RESP}, respiratory rate. *$P < 0.05$ vs. men.

Table 5 has the same structure of Table 4 but reports the parameters during STAND. The $Ph_{HP\text{-}SAP}$(LF) of the 51–60 and 61–70 groups was lower than that of 21–30 in the overall population and in women. This decrease was visible in men as well when the 61–70 decade was compared with 21–30. In the overall population, $Ph_{HP\text{-}SAP}$(HF) did not show significant variations and this result held in men and women. In the entire population and in women, the decrease of $K^2_{HP\text{-}SAP}$(LF) was visible when 51–60 decades was compared with 21–30. In men, $K^2_{HP\text{-}SAP}$(LF) remained high and stable. In the overall population $K^2_{HP\text{-}SAP}$(HF) was similar to that of 21–30 decades and this result held regardless of gender. In the overall population, the BRS(LF) of 51–60 and 61–70 groups was lower than that of 21–30. In men, BRS(LF) declined in the 61–70 decade, while in women the fall was visible in the 51–60 decade. In the overall population, the BRS(HF) fell in the 61–70 decade but this decline was not observable when analysis was carried out separately in men and women.

Table 6 compares the results of HP-SAP cross-spectral analysis in men and women. Values are reported as median (1st quartile/3rd quartile) after all values were pooled together regardless of decades both at REST and during STAND. Regardless of the experimental condition, Ph_{HPSAP}(LF) was significantly more negative in women than in men both at REST and during STAND, while no gender-related differences were found in the HF band. This result was accompanied by a significant decrease of $K^2_{HP\text{-}SAP}$(LF) in women observable solely during STAND. Regardless of the experimental condition $K^2_{HP\text{-}SAP}$(HF), BRS(LF), and BRS(HF) were similar in men and women.

Association of cross-spectral indexes with age at REST and during STAND. Figure 1 shows the individual values of $Ph_{HP\text{-}SAP}$(LF) as open circles (Fig. 1, *A*, *B*, and *C*) and

Table 4. *HP-SAP cross-spectral indexes in the LF and HF bands as a function of age at REST*

	$Ph_{HP\text{-}SAP}$(LF), rad	$K^2_{HP\text{-}SAP}$(LF)	BRS(LF), ms/mmHg	$Ph_{HP\text{-}SAP}$(HF), rad	$K^2_{HP\text{-}SAP}$(HF)	BRS(HF), ms/mmHg
21–30						
All	−0.82 (−1.08/−0.66)	0.86 (0.81/0.90)	17.21 (9.89/19.98)	0.08 (−0.38/0.25)	0.94 (0.91/0.97)	19.39 (12.47/31.26)
Men	−0.89 (−1.03/−0.70)	0.85 (0.81/0.88)	17.45 (16.30/19.52)	0.23 (−0.12/0.26)	0.93 (0.90/0.96)	18.48 (11.66/28.16)
Women	−0.78 (−1.15/−0.62)	0.88 (0.81/0.92)	11.48 (9.68/20.99)	−0.17 (−0.54/0.17)	0.96 (0.93/0.97)	19.75 (16.81/31.81)
31–40						
All	−0.97 (−1.08/−0.67)	0.83 (0.72/0.87)	11.92 (7.67/15.07)	−0.19 (−0.40/0.15)	0.93 (0.84/0.97)	20.25 (16.49/29.66)
Men	−0.82 (−1.05/−0.59)	0.85 (0.81/0.87)	12.12 (11.44/15.00)	−0.20 (−0.46/0.01)	0.82 (0.79/0.93)	26.78 (15.13/32.97)
Women	−1.04 (−1.30/−0.83)	0.77 (0.68/0.86)	11.41 (4.73/15.96)	−0.14 (−0.30/0.14)	0.97 (0.94/0.98)	20.03 (16.99/23.45)
41–50						
All	−1.20 (−1.50/−1.04)*	0.80 (0.75/0.88)	8.75 (7.83/11.06)*	−0.26 (−0.54/0.11)	0.91 (0.88/0.97)	16.87 (11.03/29.67)
Men	−1.20 (−1.61/−0.86)	0.80 (0.76/0.88)	10.85 (8.19/12.88)*	−0.26 (−0.56/−0.02)	0.87 (0.81/0.91)	17.78 (9.33/29.25)
Women	−1.20 (−1.39/−1.16)	0.80 (0.76/0.89)	8.26 (7.02/9.73)	−0.24 (−0.50/0.12)	0.94 (0.90/0.97)	15.97 (11.85/28.42)
51–60						
All	−1.20 (−1.48/−1.03)*	0.77 (0.71/0.85)	8.24 (5.21/9.12)*	−0.51 (−0.82/−0.17)*	0.94 (0.90/0.95)	13.03 (8.18/17.46)*
Men	−1.06 (−1.22/−0.98)	0.77 (0.75/0.84)	8.93 (7.64/10.15)*	−0.50 (−1.24/−0.23)*	0.93 (0.90/0.95)	16.38 (9.12/19.86)
Women	−1.33 (−1.74/−1.12)*	0.77 (0.61/0.86)	6.46 (4.06/8.48)	−0.66 (−0.75/−0.08)	0.95 (0.92/0.96)	11.90 (8.10/15.61)*
61–70						
All	−1.32 (−1.73/−1.08)*	0.72 (0.64/0.79)*	6.59 (4.55/9.91)*	−0.05 (−0.51/0.04)	0.91 (0.82/0.95)	7.96 (5.62/10.63)*
Men	−1.27 (−1.66/−0.99)*	0.72 (0.69/0.77)*	6.48 (5.01/7.06)*	−0.07 (−0.55/0.07)	0.89 (0.75/0.94)	7.96 (5.66/9.17)*
Women	−1.47 (−1.73/−1.19)*	0.72 (0.62/0.81)	7.53 (4.27/10.75)*	−0.04 (−0.19/0.00)	0.93 (0.85/0.96)	7.68 (5.53/11.47)*

Values are expressed as median (1st quartile/3rd quartile). LF, low frequency; HF, high frequency; $Ph_{HP\text{-}SAP}$(LF) and $Ph_{HP\text{-}SAP}$(HF), HP-SAP phase in the LF and HF bands; $K^2_{HP\text{-}SAP}$(LF) and $K^2_{HP\text{-}SAP}$(HF), HP-SAP squared coherence in the LF and HF bands; BRS(LF) and BRS(HF), baroreflex sensitivity in the LF and HF bands. *$P < 0.05$ vs. to 21–30.

$Ph_{HP\text{-}SAP}$(HF) (Fig. 1, *D*, *E*, and *F*) as a function of age at REST. The regression line (solid line) and its 95% confidence interval (dotted lines) are plotted as well if a significant association with age is found. The zero offset is marked as a medium dashed line. Indexes are reported in all volunteers (Figs. 1, *A* and *D*), only in men (Fig. 1, *B* and *E*), and only in women (Fig. 1, *C* and *F*). $Ph_{HP\text{-}SAP}$(LF) was significantly and negatively correlated with age in the overall population (Fig. 1*A*: $r = -0.457$, $P = 5.16 \times 10^{-7}$) and the result held even when only men (Fig. 1*B*: $r = -0.497$, $P = 1.14 \times 10^{-4}$) and only women (Fig. 1*C*: $r = -0.438$, $P = 8.19 \times 10^{-4}$) were considered. No significant relation of $Ph_{HP\text{-}SAP}$(HF) on age was found in the overall population and women (Fig. 1, *D* and *F*), while men exhibited a weak but and significant negative relation with age (Fig. 1*E*: $r = -0.303$, $P = 2.44 \times 10^{-2}$).

Figure 2 shows the scatter plots of $\tau_{HP\text{-}SAP}$(LF), obtained after the transformation of phase multiples $Ph_{HP\text{-}SAP}$(LF) + $2\pi k$ into delays or advancements, on age at REST. $\tau_{HP\text{-}SAP}$(LF) is reported in all volunteers, only in men and only in women, respectively, in Fig. 2, *A*, *B*, and *C*. Only phase multiples with k equal to 0 (open circles), +1 (open down triangles), and −1 (open up triangles) were converted. The range of $\tau_{HP\text{-}SAP}$(LF) compatible with the BL is delimited by two medium dashed lines. The $\tau_{HP\text{-}SAP}$(LF) values compatible with the BL, τ_{BL}(LF), as detected in all volunteers, only in men, and only in women are reported as a function of age in Fig. 2, *D*, *E*, and *F*.

Table 5. *HP-SAP cross-spectral indexes in the LF and HF bands as a function of age during STAND*

	$Ph_{HP\text{-}SAP}$(LF), radian	$K^2_{HP\text{-}SAP}$(LF)	BRS(LF), ms/mmHg	$Ph_{HP\text{-}SAP}$(HF), radian	$K^2_{HP\text{-}SAP}$(HF)	BRS(HF), ms/mmHg
21–30						
All	−0.87 (−1.01/−0.76)	0.91 (0.87/0.95)	8.09 (6.88/9.72)	−0.50 (−0.73/−0.01)	0.87 (0.64/0.90)	6.32 (5.27/10.97)
Men	−0.89 (−1.00/−0.80)	0.93 (0.92/0.96)	8.37 (6.85/9.34)	−0.32 (−0.77/0.09)	0.88 (0.74/0.89)	5.88 (5.21/8.03)
Women	−0.83 (−0.98/−0.73)	0.87 (0.83/0.91)	7.55 (7.00/9.82)	−0.54 (−0.71/−0.03)	0.81 (0.65/0.92)	6.47 (5.75/11.97)
31–40						
All	−1.03 (−1.19/−0.81)	0.88 (0.77/0.95)	7.84 (6.83/11.34)	−0.33 (−0.73/−0.13)	0.84 (0.68/0.91)	6.56 (4.15/12.80)
Men	−0.83 (−1.06/−0.79)	0.90 (0.77/0.95)	8.74 (7.79/12.63)	−0.24 (−0.60/−0.13)	0.82 (0.73/0.91)	13.82 (5.18/16.34)
Women	−1.07 (−1.29/−0.92)	0.85 (0.77/0.93)	7.23 (6.21/7.97)	−0.33 (−0.72/−0.21)	0.87 (0.63/0.91)	5.16 (3.54/7.53)
41–50						
All	−1.03 (−1.22/−0.77)	0.83 (0.75/0.91)	7.17 (5.54/11.03)	−0.59 (−0.92/−0.11)	0.82 (0.71/0.92)	7.29 (5.55/12.30)
Men	−1.03 (−1.14/−0.72)	0.89 (0.75/0.93)	7.43 (6.69/12.00)	−0.58 (−1.00/−0.18)	0.75 (0.65/0.88)	5.88 (3.44/10.35)
Women	−1.04 (−1.25/−0.87)	0.82 (0.74/0.86)	6.32 (5.45/9.73)	−0.59 (−0.88/−0.16)	0.82 (0.78/0.93)	10.39 (6.41/12.47)
51–60						
All	−1.19 (−1.41/−1.06)*	0.78 (0.68/0.88)*	4.80 (2.87/7.68)*	−0.49 (−0.75/0.25)	0.88 (0.78/0.93)	5.08 (2.72/6.40)
Men	−1.12 (−1.24/−0.86)	0.86 (0.75/0.95)	5.56 (3.65/8.05)	−0.55 (−0.75/0.13)	0.92 (0.79/0.96)	4.66 (2.72/6.14)
Women	−1.38 (−1.66/−1.19)*	0.75 (0.66/0.79)*	3.85 (2.47/6.76)*	−0.35 (−0.75/0.30)	0.87 (0.80/0.89)	5.33 (3.71/7.73)
61–70						
All	−1.30 (−1.47/−1.12)*	0.85 (0.71/0.90)	4.55 (3.62/5.64)*	0.16 (−0.66/0.45)	0.83 (0.69/0.95)	4.08 (2.79/5.99)*
Men	−1.30 (−1.46/−1.21)*	0.89 (0.84/0.92)	4.12 (2.19/4.90)*	0.16 (−0.31/0.57)	0.79 (0.70/0.92)	3.55 (1.92/4.87)
Women	−1.22 (−1.44/−1.07)*	0.79 (0.69/0.87)	5.72 (4.26/7.09)	−0.61 (−0.78/0.35)	0.92 (0.73/0.95)	4.24 (3.66/7.97)

Values are expressed as median (1st quartile/3rd quartile). LF, low frequency; HF, high frequency; $Ph_{HP\text{-}SAP}$(LF) and $Ph_{HP\text{-}SAP}$(HF), HP-SAP phase in the LF and HF bands; $K^2_{HP\text{-}SAP}$(LF) and $K^2_{HP\text{-}SAP}$(HF), HP-SAP squared coherence in the LF and HF bands; BRS(LF) and BRS(HF), baroreflex sensitivity in the LF and HF bands. *$P < 0.05$ vs. to 21–30.

Table 6. *HP-SAP cross-spectral indexes in the LF and HF bands in men and women*

Index/Experimental Condition	Men	Women
$Ph_{HP\text{-}SAP}$(LF), radian		
REST	−1.05 (−1.23/−0.80)	−1.20 (−1.51/−0.93)*
STAND	−1.05 (−1.20/−0.81)	−1.10 (−1.37/−0.90)*
$K^2_{HP\text{-}SAP}$(LF)		
REST	0.81 (0.73/0.86)	0.80 (0.66/0.88)
STAND	0.90 (0.81/0.95)	0.82 (0.72/0.89)*
BRS(LF), ms/mmHg		
REST	10.48 (7.24/15.00)	8.62 (5.33/11.51)
STAND	7.24 (4.96/9.34)	6.69 (5.17/8.33)
$Ph_{HP\text{-}SAP}$(HF), radian		
REST	−0.24 (−0.52/0.15)	−0.17 (−0.54/0.13)
STAND	−0.33 (−0.74/0.18)	−0.45 (−0.80/0.07)
$K^2_{HP\text{-}SAP}$(HF)		
REST	0.90 (0.82/0.94)	0.95 (0.91/0.97)
STAND	0.85 (0.67/0.92)	0.87 (0.72/0.93)
BRS(HF), ms/mmHg		
REST	15.23 (9.02/26.47)	15.33 (10.01/20.25)
STAND	5.34 (3.46/8.96)	6.25 (4.46/9.93)

Values are expressed as median (1st quartile/3rd quartile). LF, low frequency; HF, high frequency; REST, supine resting; STAND, active standing; $Ph_{HP\text{-}SAP}$(LF) and $Ph_{HP\text{-}SAP}$(HF), HP-SAP phase in the LF and HF bands; $K^2_{HP\text{-}SAP}$(LF) and $K^2_{HP\text{-}SAP}$(HF), HP-SAP squared coherence in the LF and HF bands; BRS(LF) and BRS(HF), baroreflex sensitivity in the LF and HF bands. *$P < 0.05$ vs. men.

The regression line (solid line) and its 95 percent confidence interval (dotted lines) are plotted as well if a significant association with age is found. The percentage of subjects exhibiting $\tau_{HP\text{-}SAP}$(LF) values in agreement with the BL were 87% in the overall population, 81% in men, and 84% in women. τ_{BL}(LF) was significantly and negatively correlated with age in the overall population (Fig. 2*D*: $r = -0.352$, $P = 4.44 \times 10^{-4}$), and the result held in men (Fig. 2*E*: $r = -0.394$, $P = 4.60 \times 10^{-3}$) and women (Fig. 2*F*: $r = -0.310$, $P = 3.62 \times 10^{-2}$). The same procedure was adopted to convert $Ph_{HP\text{-}SAP}$(HF) into $\tau_{HP\text{-}SAP}$(HF) and to find τ_{BL}(HF). Although the percentages of subjects with $\tau_{HP\text{-}SAP}$(HF) matching the BL were similar to those reported in the case of τ_{BL}(LF), linear regression analysis was not significant (data were not drawn).

The results of the linear correlation analysis of $Ph_{HP\text{-}SAP}$(LF) and $Ph_{HP\text{-}SAP}$(HF) on age during STAND are shown, respectively, in Fig. 3 *A*, *B*, and *C* and Fig. 3, *D*, *E*, and *F*. Figure 3 has the same structure of Fig. 1 reporting the indexes in all volunteers in Fig. 3, *A* and *D*, only in men in Fig. 3, *B* and *E*, and only in women in Fig. 3, *C* and *F*. $Ph_{HP\text{-}SAP}$(LF) was significantly and negatively correlated with age in the overall population (Fig. 3*A*: $r = -0.475$, $P = 1.62 \times 10^{-7}$) and gender-specific subgroups (Fig. 3, *B* and *C*: $r = -0.568$, $P = 6.08 \times 10^{-6}$ and $r = -0.412$, $P = 1.80 \times 10^{-3}$). $Ph_{HP\text{-}SAP}$(HF) was unrelated to age regardless of group (Fig. 3, *D*, *E*, and *F*).

Figure 4 shows the scatter plots of $\tau_{HP\text{-}SAP}$(LF), obtained after the transformation of phase multiples $Ph_{HP\text{-}SAP}$(LF) + $2\pi k$ into delays or advancements, on age during STAND. Figure 4 has the same structure as Fig. 2 reporting $\tau_{HP\text{-}SAP}$(LF) in all volunteers, only in men, and only in women in Fig. 4, *A*, *B*, and *C* and the $\tau_{HP\text{-}SAP}$(LF) values compatible with BL, τ_{BL}(LF), in the different groups in Fig. 4, *D*, *E*, and *F*. The percentage of subjects exhibiting $\tau_{HP\text{-}SAP}$(LF) values in agreement with the BL were 97% in the overall population, 98% in men, and 96% in women. Values in agreement with the latency of the baroreflex were significantly and negatively correlated with age in the overall population (Fig. 4*D*: $r = -0.483$, $P = 1.37 \times 10^{-7}$), and the result held in men (Fig. 4*E*: $r = -0.545$, $P = 1.99 \times 10^{-5}$) and women (Fig. 4*F*: $r = -0.429$, $P = 1.34 \times 10^{-3}$). Computation of $\tau_{HP\text{-}SAP}$(HF) and selection of τ_{BL}(HF) led to conclusions similar to those reported at REST (data were not drawn).

Figure 5 shows the individual values of $K^2_{HP\text{-}SAP}$(LF) and $K^2_{HP\text{-}SAP}$(HF) as function of age at REST, respectively, in Fig. 5, *A*, *B*, and *C* and Fig. 5, *D*, *E*, and *F*. Figure 5 has the same structure of Fig. 1 reporting the indexes in all volunteers in Fig.

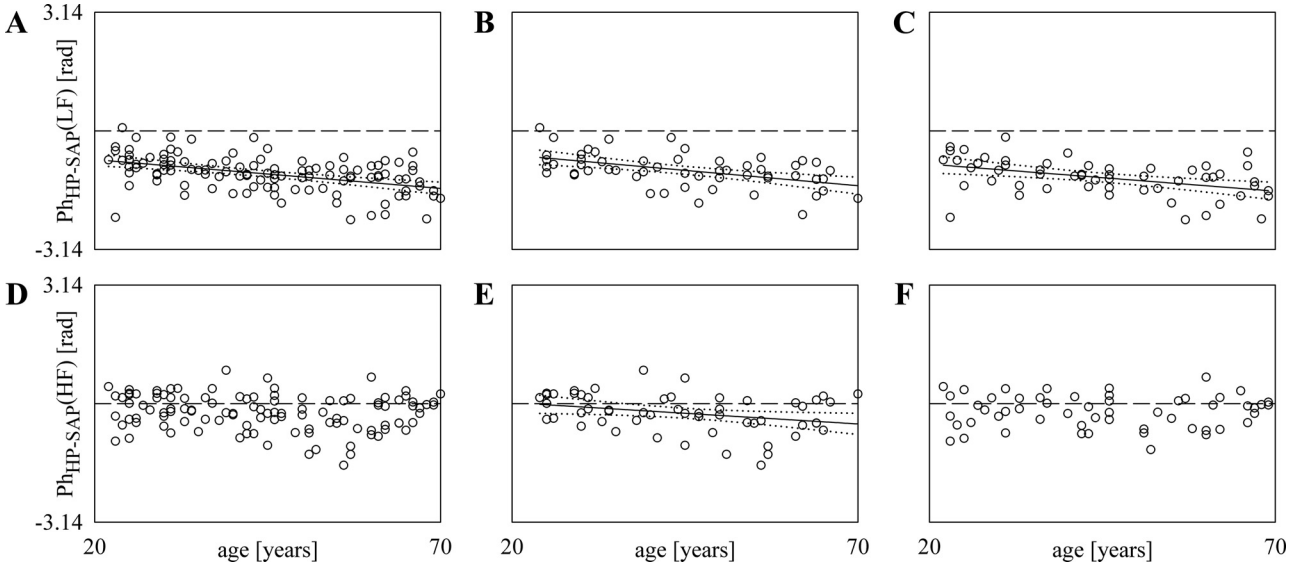

Fig. 1. Gaphs show the individual values of phase between heart period (HP) and systolic arterial pressure (SAP) at low-frequency [$Ph_{HP\text{-}SAP}$(LF)] and high-frequency [$Ph_{HP\text{-}SAP}$(HF)] (open circles) as function of age at REST. $Ph_{HP\text{-}SAP}$(LF) in all volunteers, only in men, and only in women are reported *A*, *B*, and *C*, respectively, while $Ph_{HP\text{-}SAP}$(HF) in all volunteers, only in men and only in women are reported in *D*, *E*, and *F*, respectively. The regression line (solid line), its 95% confidence interval (dotted lines), and the 0 (medium dashed line) are plotted as well if a significant association with age was found with $P < 0.05$.

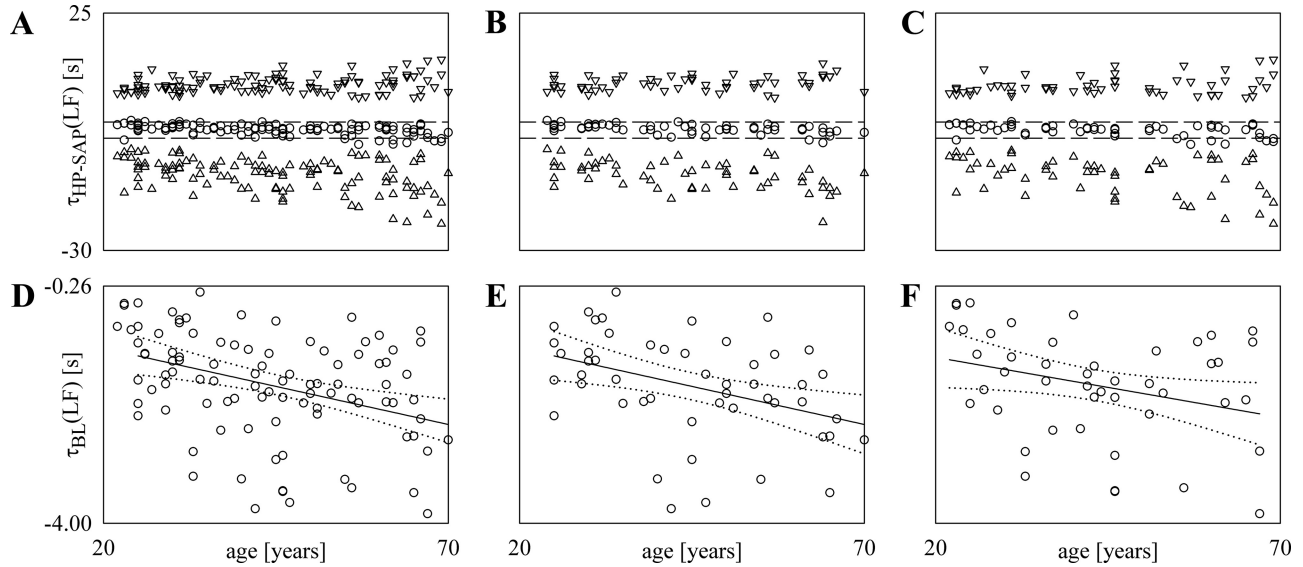

Fig. 2. Scatter plots show $\tau_{HP\text{-}SAP}$(LF), obtained after the transformation of phase multiples $Ph_{HP\text{-}SAP}$(LF) + 2πk into delays or advancements, as function of age at REST. $\tau_{HP\text{-}SAP}$(LF) are reported in all volunteers, only in men, and only in women in *A*, *B*, and *C*. Only phase multiples with k equal to 0 (open circles), +1 (open down triangles), and −1 (open up triangles) were converted. The range of $\tau_{HP\text{-}SAP}$(LF) compatible with the baroreflex latency (BL) is delimited by 2 medium dashed lines. The $\tau_{HP\text{-}SAP}$(LF) values compatible with BL, τ_{BL}(LF), as detected in all volunteers, only in men, and only in women were reported as a function of age in *D*, *E*, and *F*. The regression line (solid line) and its 95 percent confidence interval (dotted lines) are plotted as well if a significant association with age was found with $P < 0.05$.

5, *A* and *D*, only in men in Fig. 5, *B* and *E*, and only in women in Fig. 5, *C* and *F*. $K^2_{HP\text{-}SAP}$(LF) was significantly and negatively correlated with age regardless of group (Fig. 5, *A*, *B*, and *C*: $r = -0.331$ with $P = 4.08 \times 10^{-4}$, $r = -0.386$ with $P = 3.61 \times 10^{-3}$, and $r = -0.292$ with $P = 3.06 \times 10^{-2}$). Conversely $K^2_{HP\text{-}SAP}$(HF) was unrelated to age and this finding held regardless of group (Fig. 5, *D*, *E*, and *F*).

The results of the linear correlation analysis of $K^2_{HP\text{-}SAP}$(LF) and $K^2_{HP\text{-}SAP}$(HF) on age during STAND are shown, respectively, in Fig. 6, *A*, *B*, and *C* and Fig. 6, *D*, *E*, and *F*. Figure 6 has the same structure of Fig. 1 reporting the indexes in all volunteers in Fig. 6, *A* and *D*, only in men in Fig. 6, *B* and *E*, and only in women in Fig. 6, *C* and *F*. $K^2_{HP\text{-}SAP}$(LF) was significantly and negatively correlated with age in the overall population (Fig. 6*A*: $r = -0.307$, $P = 1.33 \times 10^{-3}$) and in women (Fig. 6*C*: $r = -0.40$, $P = 2.48 \times 10^{-3}$) but unrelated to age in men (Fig. 6*B*). No association of $K^2_{HP\text{-}SAP}$(HF) with age was found regardless of group (Fig. 6, *D*, *E*, and *F*).

Figure 7 shows the individual values of BRS(LF) and BRS(HF) as function of age at REST in Fig. 7, *A*, *B*, and *C*, and Fig. 7, *D*, *E*, and *F*, respectively. Figure 7 has the same structure of Fig. 1 reporting the indexes in all volunteers in Fig.

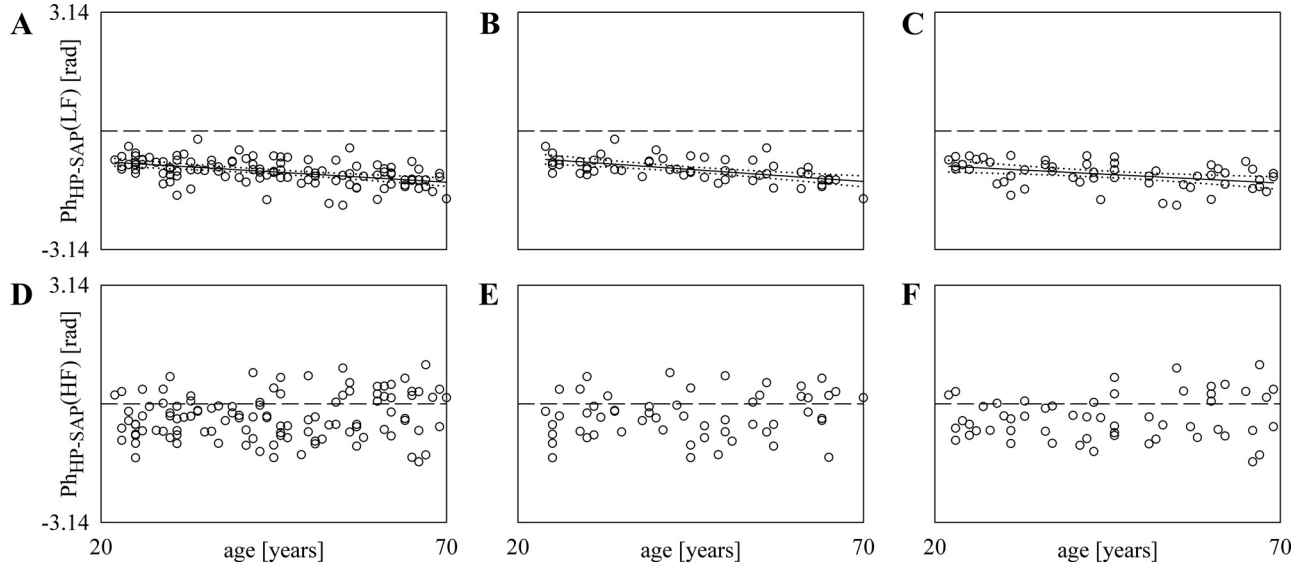

Fig. 3. Graphs show the individual values of $Ph_{HP\text{-}SAP}$(LF) and $Ph_{HP\text{-}SAP}$(HF) (open circles) as function of age during STAND. $Ph_{HP\text{-}SAP}$(LF) in all volunteers, only in men, and only in women are reported in *A*, *B*, and *C*, respectively, while $Ph_{HP\text{-}SAP}$(HF) in all volunteers, only in men, and only in women are reported in *D*, *E*, and *F*, respectively. The regression line (solid line), its 95 percent confidence interval (dotted lines), and the 0 (medium dashed line) are plotted as well if a significant association with age was found with $P < 0.05$.

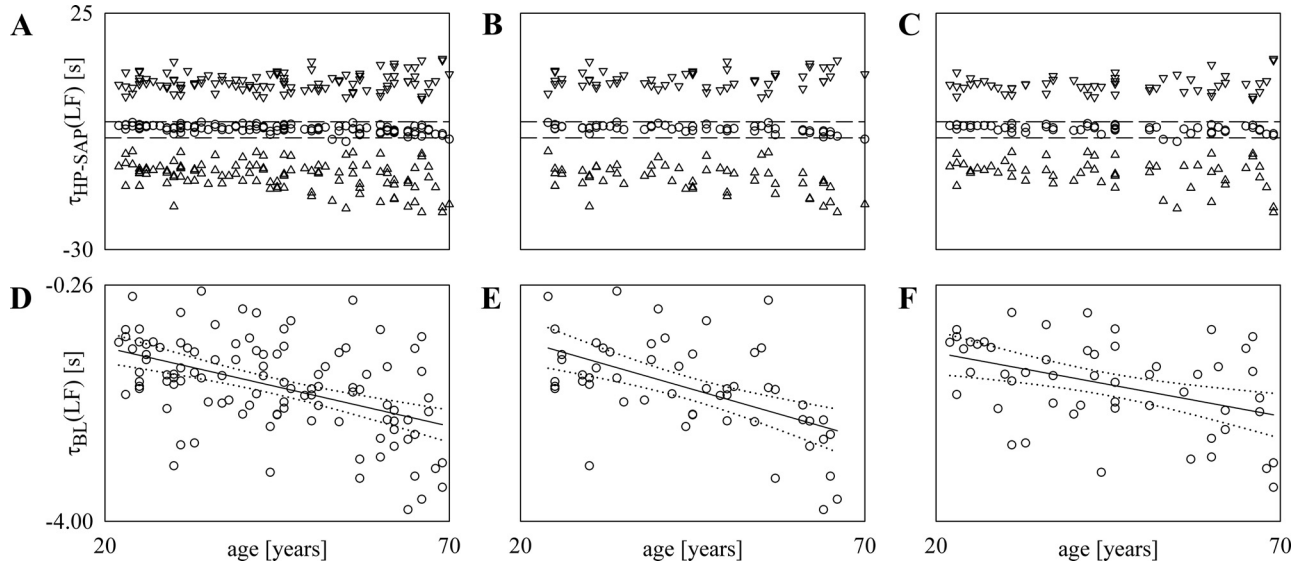

Fig. 4. Scatter plots show $\tau_{HP\text{-}SAP}$(LF), obtained after the transformation of phase multiples $Ph_{HP\text{-}SAP}$(LF) + 2πk into delays or advancements, as function of age during STAND. $\tau_{HP\text{-}SAP}$(LF) are reported in all volunteers, only in men, and only in women in *A*, *B*, and *C*. Only phase multiples with k equal to 0 (open circles), +1 (open down triangles), and −1 (open up triangles) were converted. The range of $\tau_{HP\text{-}SAP}$(LF) compatible with the BL is delimited by 2 medium dashed lines. The $\tau_{HP\text{-}SAP}$(LF) values compatible with BL, τ_{BL}(LF), as detected in all volunteers, only in men, and only in women were reported as a function of age in *D*, *E*, and *F*. The regression line (solid line) and its 95 percent confidence interval (dotted lines) are plotted as well if a significant association with age was found with $P < 0.05$.

7, *A* and *D*, only in men in Fig. 7, *B* and *E*, and only in women in Fig. 7, *C* and *F*. Both BRS(LF) and BRS(HF) were significantly and negatively correlated with age in the overall population (Fig. 7, *A* and *D*: $r = -0.533$, $P = 2.08 \times 10^{-9}$ and $r = -0.524$, $P = 4.31 \times 10^{-9}$). These relations were confirmed even when only men (Fig. 7, *B* and *E*: r = −0.643, $P = 1.21 \times 10^{-7}$ and $r = -0.50$, $P = 1.02 \times 10^{-4}$) and only women (Fig. 7, *C* and *F*: $r = -0.444$, $P = 6.88 \times 10^{-4}$ and $r = -0.549$, $P = 1.41 \times 10^{-5}$) were considered.

The results of the linear correlation analysis of BRS(LF) and BRS(HF) on age during STAND are shown in Fig. 8 *A*, *B*, and *C*, and Fig. 8 *D*, *E*, and *F*, respectively. Figure 8 has the same structure of Fig. 1 reporting the indexes in all volunteers in Fig. 8, *A* and *D*, only in men in Fig. 8, *B* and *E*, and only in women in Fig. 8, *C* and *F*. Both BRS(LF) and BRS(HF) were significantly and negatively correlated with age in the overall population (Fig. 8, *A* and *D*: $r = -0.475$, $P = 1.63 \times 10^{-7}$ and $r = -0.281$, $P = 2.90 \times 10^{-3}$) and in men (Fig. 8, *B* and *E*: $r = -0.563$, $P = 7.56 \times 10^{-6}$ and $r = -0.393$, $P = 3.0 \times 10^{-3}$). In women, only the negative association of BRS(LF) on age was detected (Fig. 8*C*: $r = -0.387$, $P = 3.55 \times 10^{-3}$).

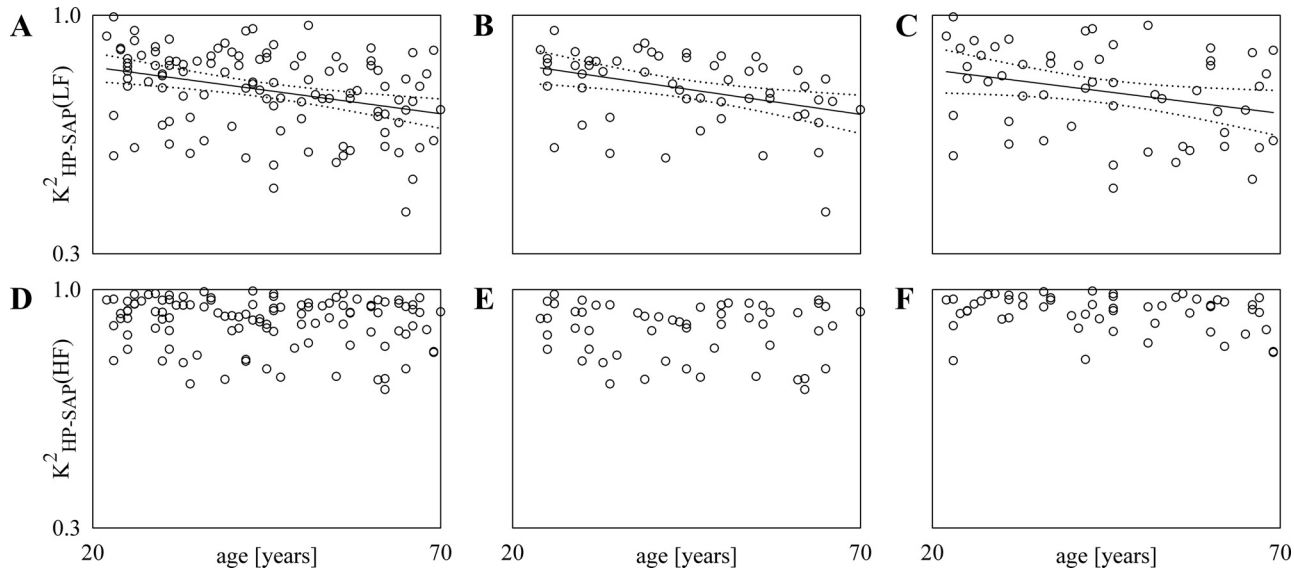

Fig. 5. Graphs show the individual values of squared coherence function [$K^2_{HP\text{-}SAP}$(LF) and $K^2_{HP\text{-}SAP}$(HF)] (open circles) as function of age at REST. $K^2_{HP\text{-}SAP}$(LF) in all volunteers, only in men and only in women are reported in *A*, *B*, and *C*, respectively, while $K^2_{HP\text{-}SAP}$(HF) in all volunteers, only in men, and only in women are reported in *D*, *E*, and *F*, respectively. The regression line (solid line) and its 95 percent confidence interval (dotted lines) are plotted as well if a significant association with age was found with $P < 0.05$.

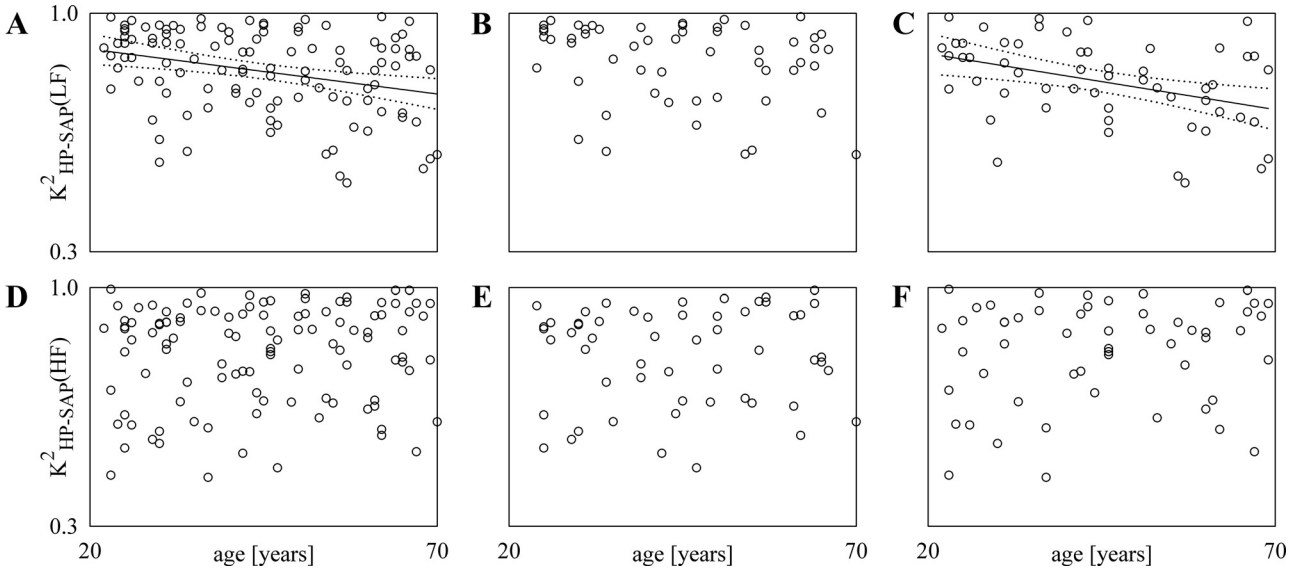

Fig. 6. Graphs show the individual values of $K^2_{HP\text{-}SAP}$(LF) and $K^2_{HP\text{-}SAP}$(HF) (open circles) as function of age during STAND. $K^2_{HP\text{-}SAP}$(LF) in all volunteers, only in men and only in women are reported in *A*, *B*, and *C* respectively, while $K^2_{HP\text{-}SAP}$(HF) in all volunteers, only in men and only in women are reported in *D*, *E*, and *F*, respectively. The regression line (solid line) and its 95 percent confidence interval (dotted lines) are plotted as well if a significant association with age was found with $P < 0.05$.

The presence of a more general form of correlation was tested using Spearman correlation analysis. When a significant linear correlation was found, as detected via Pearson correlation analysis, the Spearman correlation test confirmed the presence of a significant association. Conversely, when no significant linear correlation was found, even the application of Spearman correlation analysis accounting for the eventual presence of a nonlinear relation did not allow us to reject the null hypothesis of uncorrelation with age.

DISCUSSION

The main findings of this study can be summarized as follows: *1*) in addition to the BRS also $Ph_{HP\text{-}SAP}$ and $K^2_{HP\text{-}SAP}$ varied with age; *2*) both at REST and during STAND in the LF band $Ph_{HP\text{-}SAP}$ became progressively more negative with age, and this result was compatible with a progressive increase of BL; *3*) at REST $K^2_{HP\text{-}SAP}$ in the LF band decreased with age regardless of gender but during STAND was more preserved in men than women; *4*) in the LF band $Ph_{HP\text{-}SAP}$ was more negative in women than in men both at REST and during STAND, while in the same band $K^2_{HP\text{-}SAP}$ was smaller during STAND; *5*) both at REST and during STAND in HF band $Ph_{HP\text{-}SAP}$ and $K^2_{HP\text{-}SAP}$ were unrelated to age and no gender-related differences were found; and *6*) BRS decreased with age regardless of frequency band, experimental condition, and gender.

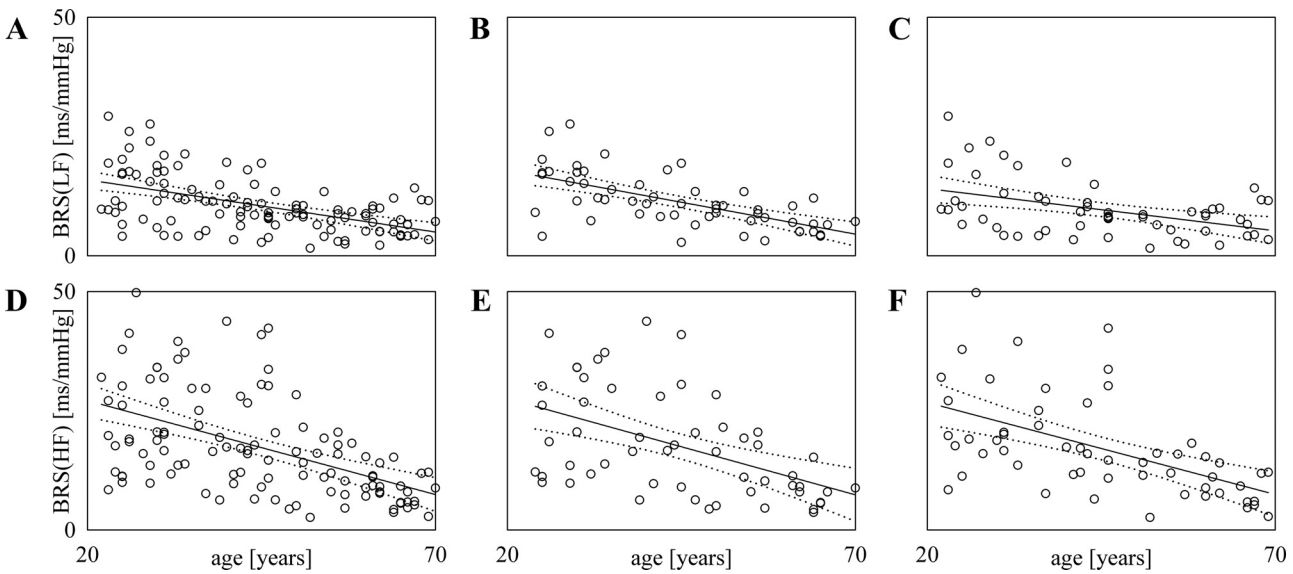

Fig. 7. Graphs show the individual values of baroreflex sensitivity [BRS (LF) and BRS(HF)] (open circles) as function of age at REST. BRS(LF) in all volunteers, only in men and only in women are reported in *A*, *B*, and *C*, respectively, while BRS(HF) in all volunteers, only in men, and only in women are reported in *D*, *E*, and *F*, respectively. The regression line (solid line) and its 95 percent confidence interval (dotted lines) are plotted as well if a significant association with age is found with $P < 0.05$.

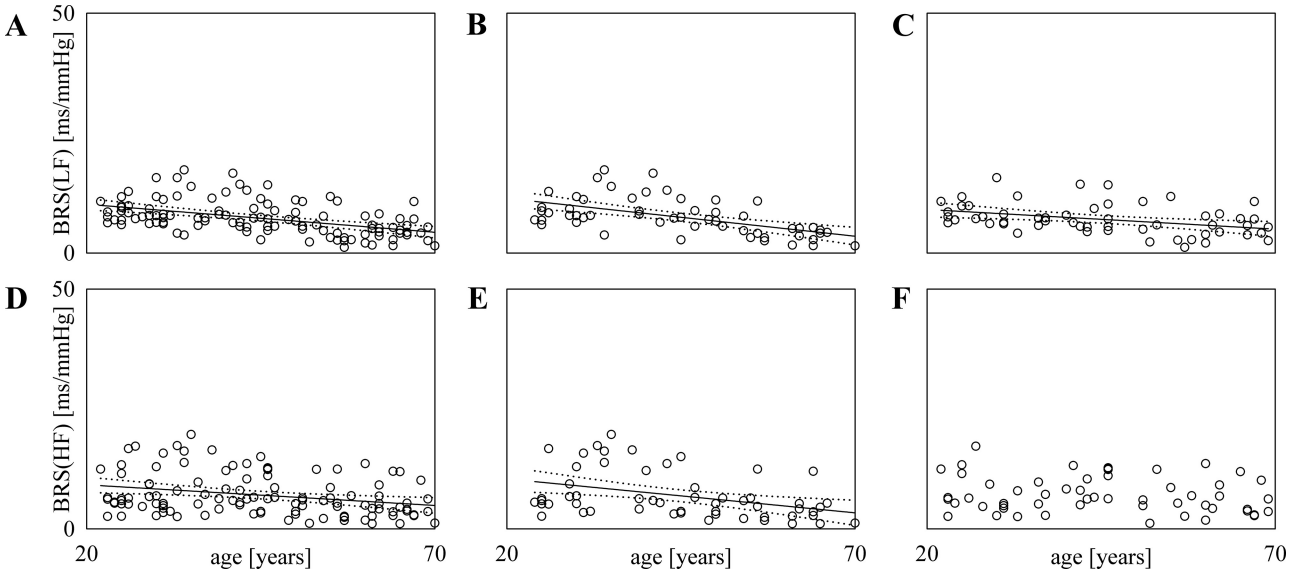

Fig. 8. Graphs show the individual values of BRS(LF) and BRS(HF) (open circles) as function of age during STAND. BRS(LF) in all volunteers, only in men, and only in women are reported in *A*, *B*, and *C*, respectively, while BRS(HF) in all volunteers, only in men and only in women are reported in *D*, *E*, and *F*, respectively. The regression line (solid line) and its 95 percent confidence interval (dotted lines) are plotted as well if a significant association with age was found with $P < 0.05$.

Influences of aging and gender on time domain parameters. Time domain parameters confirmed well-known trends with age (6, 8, 26, 43, 50) such as the increase of HP mean during STAND, the decrease of HP variance at REST and during STAND, the increase of SAP mean at REST and during STAND, and the rise of SAP variance at REST. These findings are compatible with the progressive vagal withdrawal, the gradual augmentation of tonic sympathetic activity, the reduced responsiveness of the sinus node to sympathetic outflow, and a greater instability of baroreflex control becoming less and less able to buffer arterial pressure fluctuations. Also, the finding that women were more tachycardic than men at REST and during STAND was expected (3).

Influences of aging and gender on HP-SAP phase and latency. The phase shift between HP and SAP in the LF band, $Ph_{HP\text{-}SAP}(LF)$, became more and more negative as a function of the age, thus suggesting that the latency of the cardiac baroreflex (i.e., the time elapsing from the arterial pressure sensing via stretch-sensitive mechanoreceptors and the consequent HP variation) increased with aging. This conjecture held when $Ph_{HP\text{-}SAP}(LF)$ was converted into $\tau_{HP\text{-}SAP}(LF)$, and only values compatible with the BL [i.e., $\tau_{BL}(LF)$] were considered in the regression analysis. It is well-known that a sympathetic activation, such as the one associated to a central hypovolemia induced by head-up tilt or lower body negative pressure, leads to more and more negative phase values and longer BL with the relevance of the stimulus (7, 25). Therefore, it is not surprising to find out that the progressive sympathetic activation with age (1, 12, 14, 18, 34, 47) is accompanied by more and more negative $Ph_{HP\text{-}SAP}(LF)$ and longer $\tau_{BL}(LF)$. Remarkably, this finding is gender independent. This observation supports that sympathetic overactivity plays a major role in determining the observed phase and latency modification with age: indeed, a progressive increase of the sympathetic drive was observed in both genders, even though the characteristics of the sympathetic surge are gender specific (34). This conclusion is in agreement with, and complements the frequency domain, data obtained in the time domain by unloading and loading carotid baroreceptors, respectively, with pulses of neck pressure and suction (17). The augmentation of $\tau_{BL}(LF)$ might have relevant consequences in old subjects. Indeed, the control systems theory proves that increasing the delay in a closed loop negative feedback system might drive it toward instability (9, 29). The migration of the system toward the instability region is characterized by large SAP oscillations increasing the likelihood of cardiovascular adverse events in elderly population (45) and of complex regulatory behaviors occurring along time scales different from the typical frequency of the baroreflex control loop (~0.1 Hz) (4). The increase of SAP variance with age observed at REST provides further support to this conclusion. The gradual modification of $Ph_{HP\text{-}SAP}(LF)$ with age was observable even during STAND and confirmed by the trend of $\tau_{BL}(LF)$. This finding supports again the conclusion that the progressive shift of $Ph_{HP\text{-}SAP}(LF)$ toward more negative values and longer $\tau_{BL}(LF)$ could be linked to structural modifications of the sympathetic control that is more intensively activated during STAND (11, 19, 31). Remarkably, both at REST and during STAND $Ph_{HP\text{-}SAP}(LF)$ were more negative in women than in men. This finding suggests that women might be more at risk to exhibit the arterial pressure regulatory system closer to the instability region and supports the lower orthostatic tolerance of women compared with their male counterparts (20, 53). The observed trends are unlikely to be the consequence of modifications of the breathing rate because they pertained to parameters computed in the LF band and breathing rate did not change with age in both experimental conditions.

The phase shift between HP and SAP in the HF band, $Ph_{HP\text{-}SAP}(HF)$, appeared to be unrelated to age both at REST and during STAND. Only $Ph_{HP\text{-}SAP}(HF)$ at REST in men suggests that some relation with age could be present. The weakness of $Ph_{HP\text{-}SAP}(HF)$ in showing a dependency on age is not surprising. Indeed, the main periodical driver in the HF band is respiration that it is usually considered a confounding

factor for the assessment of cardiac baroreflex both via pharmacological and nonpharmacological methods. As a result, BRS was estimated via pharmacological procedures based on the administration of a vasoactive agent in expiratory phase (48), and model-based approaches adopt modeling classes able to describe the direct effects of respiration on HP unmediated by cardiac baroreflex (39, 40). The possible confounding effect of respiration on the estimate of the HP-SAP relation is particularly evident during STAND: indeed, the variability of $Ph_{HP\text{-}SAP}(HF)$ is much higher than that of $Ph_{HP\text{-}SAP}(LF)$. The absence of a relation of $Ph_{HP\text{-}SAP}(HF)$ to age is in agreement with Halámek et al. (22), who sampled the HP-SAP phase always in correspondence with the respiratory frequency during a protocol of spontaneous respiration and controlled breathing at different rates. The weakness of the respiratory band for the assessment of $Ph_{HP\text{-}SAP}$ might explain also the absence of a gender-related difference in $Ph_{HP\text{-}SAP}(HF)$ both at REST and during STAND.

Influences of aging and gender on the degree of HP-SAP association. At REST, the degree of the association between HP and SAP in the LF band, $K^2_{HP\text{-}SAP}(LF)$, estimating the degree of the involvement of cardiac baroreflex in regulating arterial pressure, progressively decreased with age. This result indicates a loss of ability of the cardiovascular control in responding to SAP variations with suitable HP changes and must be considered as a marker of the increased difficulty of cardiac baroreflex to regulate arterial pressure with age. This trend is likely to be the consequence of factors contributing to HP-SAP uncoupling such as the progressive sympathetic activation with age reducing more and more the HP response to SAP changes, vascular structural remodeling and functional changes impairing the ability of stretch-sensitive mechanoreceptors to respond to arterial pressure changes, and reduced cardiac cholinergic responsiveness leading to a reduction of the HP variation to neural vagal stimulation (27, 33). For the same reasons listed in *Influences of aging and gender on HP-SAP phase and latency*, the observed trend is unlikely to be exclusively attributable to modifications of the breathing rate. Remarkably, the progressive decline of $K^2_{HP\text{-}SAP}(LF)$ is gender independent at REST likely because modifications responsible for the HP-SAP uncoupling are observable in both genders. Conversely, during STAND, gender-related differences were detected, with $K^2_{HP\text{-}SAP}(LF)$ decreasing only in women. This finding suggests that the ability to cope with postural stressor could be impaired more remarkably in females than in their male counterparts given that HP changes appeared to be less linked to SAP variations with age in females. This result was confirmed even after $K^2_{HP\text{-}SAP}(LF)$ was pooled together regardless of age: indeed, during STAND $K^2_{HP\text{-}SAP}(LF)$ was smaller in females than in males.

The degree of association between HP and SAP in the HF band, $K^2_{HP\text{-}SAP}(HF)$, appeared to be unrelated to age both at REST and during STAND. Respiratory drive, acting on both HP and SAP variabilities (4, 40), tends to keep high the HP-SAP correlation at the respiratory rate regardless of age. Since $K^2_{HP\text{-}SAP}(HF)$ might account for factors of nonbaroreflex origin (39, 40), mechanisms unrelated to baroreflex might play a role in maintaining high $K^2_{HP\text{-}SAP}(HF)$ values with age. The result held regardless of gender. The absence of gender-related difference was confirmed both at REST and during STAND after pooling together $K^2_{HP\text{-}SAP}(HF)$ values.

Influences of aging and gender on BRS. It is well-known that BRS decreases with age (1, 14, 16, 24, 26, 32, 52). Our data confirmed this finding using the transfer function approach in LF band regardless of the experimental condition. This trend is likely to be the consequence of the progressive sympathetic activation with age and vagal withdrawal increasing SAP variability and reducing the HP response to SAP variations (1, 6, 12, 14, 18, 24, 34, 43, 47, 51). However, the sympathetic overactivation could not be considered the unique determinant of the decrease of BRS during senescence. Indeed, vascular structural remodeling and functional changes, increased levels of oxidative stress, reduced cardiac cholinergic responsiveness, and decreased baroreflex buffering (24, 27, 34) can contribute to the diminution of BRS during senescence. BRS(LF) was similar in men and women both at REST and during STAND. This result suggests that the lower tolerance of females to orthostatic challenge (20, 53) is more evident over phase and coherence parameters than gain markers.

Similarly to BRS(LF), BRS(HF) decreased during senescence and this decrease was observed regardless of gender at REST. Conversely, during STAND, the decrease of BRS(HF) was detectable only in men. This result is mainly the effect of higher values of BRS(HF) in the oldest group of females compared with their male counterparts. The reduced ability of BRS(HF) to respond to STAND in the oldest group of females can be taken as a further indication that vagal tone tends to remain higher during STAND in old women than in men (1, 23) and this situation might have some impact in the ability of old females to cope with the orthostatic challenge compared with their male counterparts. However, this observation held only for the last decade given that, after BRS(HF) values were pooled together regardless of age, no gender-related difference was found both at REST and during STAND, again stressing the weak statistical power of parameters based on the HP-SAP gain to reveal gender-related differences.

While the trends of BRS(LF) and BRS(HF) with age might be linked to the drift of the set point of the HP-SAP relation with age, this explanation is unlikely to hold in the case of the observed trends of phase and coherence indexes given that these markers are unrelated to absolute HP and SAP values and amplitude of their variability.

Limitations of the study and future developments. The present interpretation is based on the assumption that modifications of the phase (and consequently latency) with age could be totally attributed to the baroreflex feedback. This supposition does not hold manifestly given that, in addition to the baroreflex feedback, a feedforward pathway from HP to SAP is present as a result of Frank-Starling law and diastolic runoff leading to opposite effects on SAP in response to the same HP variation. However, the abovementioned strong assumption can be relaxed as follows: the open loop hypothesis from SAP to HP holds if the primary direction of causality is from SAP to HP and this situation is preserved in old age. Even the weakened statement might not be verified because the strength of the HP-SAP association along the feedforward pathway might be greater than that in the reverse causal direction especially at REST (36, 41). However, our previous study suggested that the strength of the HP-SAP dependence along the feedback pathway was significant and did not decrease with age (42), thus indicating that the worst case of having a open loop relation from HP to SAP is very unlikely and the HP-SAP

closed loop relation is preserved with age. We advocate experimental protocols opening artificially in healthy individuals the HP-SAP closed loop via pharmacological blockades or carried out over specific groups of subjects with damages to the baroreflex circuit to exclude that the observed changes with age could be attributed to the feedforward pathway.

The study interprets the findings exclusively in terms of cardiac baroreflex control. Possible age-related modifications of additional regulatory circuits, such as the baroreflex control of sympathetic activity and cardiopulmonary reflexes, and even changes of physiological variables, such as the elastic properties of the vascular bed, that have a deep impact on blood pressure control might have contributed to the observed changes. A more comprehensive approach avoiding the sole attribution of the observed modifications to cardiac baroreflex calls for the increase of the number of variables monitored with age compared with the adopted experimental setup.

There are some variables [e.g., $Ph_{HP\text{-}SAP}$(HF) at REST and $K^2_{HP\text{-}SAP}$(LF) during STAND] in which the effects of aging are more visible in the 51–60 decade than in the 61–70 one. This finding seems to be correlated with higher blood pressure values observed in the 51–60 group compared with the 60–71 one both at REST and during STAND. Although the 61–70 group did not take any medication controlling arterial pressure, a possible bias might be related to the fact that the 61–70 group was selected within a retired population who controlled arterial pressure very well, while some subjects in the 51–60 group, although without evident signs of hypertension at the time of recording, might have a tendency to develop in the near future problems in controlling blood pressure and/or might be much more stressed than the 61–70 group due to their active professional life.

Conclusions. The present study stresses that BRS, as derived from cross-spectral analysis of spontaneous HP and SAP variabilities, is not the unique parameter that is worth monitoring during aging. $Ph_{HP\text{-}SAP}$ and $K^2_{HP\text{-}SAP}$ also provide helpful information about the senescence process. Indeed, they suggest the progressive increase of the latency of the baroreflex control and the gradual diminution of its involvement with age, thus reinforcing the notion that elderly people are characterized by an increased difficulty in buffering rising levels of arterial pressure variability associated with sympathetic overactivity. Moreover, $Ph_{HP\text{-}SAP}$ and $K^2_{HP\text{-}SAP}$ show a peculiar gender-related difference supporting the lower orthostatic tolerance with age in women than in men. These indexes are worth being considered, in addition to BRS, as targets of specific countermeasures and/or being utilized to evaluate lifestyles devoted to reduce the impact of senescence on cardiovascular system, limit the likelihood of episodes of hemodynamic instabilities, and improve tolerance to postural challenges. Moreover, these parameters might be fruitfully exploited to improve characterization of the baroreflex control in subjects with orthostatic intolerance of different forms and to favor the understanding of the pathophysiology underlying these highly invalidating phenotypes.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.P., A.C.T., and A.M.C. conceived and designed research; J.C.M.-M., N.M.P., V.M., and P.R.-S. performed experiments; J.C.M.-M., A.P., N.M.P., V.M., and P.R.-S. analyzed data; J.C.M.-M., A.P., N.M.P., V.M., P.R.-S., A.C.T., S.M.M., and A.M.C. interpreted results of experiments; J.C.M.-M. and A.P. prepared figures; J.C.M.-M., A.P., and A.M.C. drafted manuscript; J.C.M.-M., A.P., A.C.T., S.M.M., and A.M.C. edited and revised manuscript; J.C.M.-M., A.P., N.M.P., V.M., P.R.-S., A.C.T., S.M.M., and A.M.C. approved final version of manuscript.

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