Cardiac baroreflex hysteresis is one of the determinants of the heart period variability asymmetry

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Abstract

In heart period (HP) variability (HPV) recordings the percentage of negative HP variations tends to be greater than that of positive ones and this pattern is referred to as HPV asymmetry (HPVA). HPVA has been studied in several experimental conditions in healthy and pathological populations, but its origin is unclear. The baroreflex (BR) exhibits an asymmetric behavior as well given that it reacts more importantly to positive than negative arterial pressure (AP) variations. We tested the hypothesis that the BR asymmetry (BRA) is a HPVA determinant over spontaneous fluctuations of HP and systolic AP (SAP).

We studied 100 healthy subjects (age from 21 to 70 yrs, 54 males) comprising 20 subjects in each age decade. Electrocardiogram and noninvasive AP were recorded for 15 minutes at rest in supine position (REST) and during active standing (STAND). The HPVA was evaluated via Porta's index and Guzik's index, while the BRA was assessed as the difference, and normalized difference, between BR sensitivities computed over positive and negative SAP variations via the sequence method applied to HP and SAP variability.

HPVA significantly increased during STAND and decreased progressively with age. BRA was not significantly detected both at REST and during STAND. However, we found a significant positive association between BRA and HPVA markers during STAND persisting even within the age groups.

This study supports the use of HPVA indexes as descriptors of BRA and identified a challenge soliciting the BR response like STAND to maximize the association between HPVA and BRA markers.

Keywords: heart rate variability, baroreflex sensitivity, cardiovascular control, autonomic nervous system, aging, postural challenge.

Introduction

Heart period (HP) fluctuations are characterized by an asymmetric behavior under time reversal suggesting that the contribution of positive and negative HP variations to the sum of square successive differences is not equal. In particular, the heart decelerates faster than it accelerates, thus resulting in HP variability (HPV) features with the upward side steeper than the downward one and the percentage of negative HP variations greater than that of positive HP changes. This phenomenon is usually termed as HPV asymmetry (HPVA) (34, 69). The HPVA was assessed via different metrics applied to the difference between two successive HP values such as the sum of square positive differences (61), the percentage of negative differences (10, 65, 67), the balance between the Shannon entropy of positive and negative differences (14) and skewness of the differences (25).

In physiological conditions HPVA is known to be influenced by autonomic function state and aging (10, 12, 14, 63, 65, 67, 69). Sympathetic activation and vagal withdrawal induced by a postural challenge increase HPVA (10, 12, 63, 65, 69), while aging process decreases progressively HPVA (14). In pathological conditions HPVA was found to be reduced in heart failure patients (67, 70), in patients with type 1 diabetes (33) and in individuals with obstructive sleep apnea (32). Recent studies demonstrated that HPVA is influenced by emotional and mental state and altered in mood and mental disorders such as depression and attention deficit hyperactivity disorder (42, 75, 76, 78). Although some factors influencing the HPVA have been identified, the mechanism underlying HPVA has not been fully elucidated yet.

A portion of HPV is due to the cardiac arm of baroreflex (BR) given that BR buffers arterial pressure (AP) changes with suitable HP variations (44, 60). The BR response is known to be influenced by the sign of the AP variations: indeed, BR compensates more efficiently systolic AP (SAP) raises than drops given that the BR sensitivity (BRS) is larger when SAP increases than decreases (60). This specific feature of the BR control, originally observed in (60), was termed BR hysteresis (23, 71, 74) or BR asymmetry (BRA) (19, 20, 37, 81). BRA was supported by a vagal reflex being abolished by atropine and left unmodified by propranolol (23). BRA was indicated as one of the plausible mechanisms involved in producing HPVA (34) because the larger BRS in response to SAP raises than drops would result in larger absolute HP variations when SAP increases than decreases and, therefore, in making decelerations faster than accelerations. However, this hypothesis was never tested systematically. This lack is mainly due to the missing joint assessment of BRA and HPVA markers and to the weak confidence in assessing BRA from spontaneous fluctuations of HP and SAP. BRA exploration requires methods allowing the separate quantification

of the HP response evoked by positive and negative SAP changes from spontaneous HP and SAP variability (19, 20) such as the sequence (SEQ) technique (5, 59) and the phase rectified signal averaging method (3, 53). Methods for BRA quantification from spontaneous HP and SAP variability have been recently compared and it has been suggested that the SEQ method is the most powerful in describing BRA (19, 20).

In this study we hypothesize that the BRA could be one of the determinants of HPVA. If HPVA was a reflection of BRA, HPVA metrics could be exploited as a proxy of BRA markers with a practical advantage in the estimation of BRA especially in those populations that might exhibit a differential deficit in coping with AP drops than rises (31, 54, 55, 81). Therefore, the aim of the study is to test the association between HPVA and BRA indexes in a database of 100 healthy subjects with different ages (from 21 to 70 years, 5 gender-balanced groups with 20 individuals in each decade) undergoing recordings at rest in supine position (REST) and during active standing (STAND). Since STAND is known to increase HPVA (10, 12, 63, 65, 69) and soliciting a BR response (13, 20, 66), this experimental condition is expected to make more manifest the association between HPVA and BRA, while the reduction of BRS and sinus node responsiveness with age (46, 47, 51, 68) is expected to limit the strength of this association.

Materials and methods

Experimental protocol and data acquisition

The experimental protocol was fully described in (11, 68). Briefly, we studied 100 nonsmoking healthy subjects, 54 males, aged from 21 to 70 yrs, median = 45 yrs). According to the age they were divided into five gender-balanced groups, each composed by 20 individuals: the 21-30 group (10 males, age from 21 to 30 yrs, median age = 26 yrs), the 31-40 group (11 males, age from 31 to 40 yrs, median age = 34 yrs), the 41-50 group (10 males, age from 41 to 50 yrs, median age = 45 yrs), the 51-60 group (10 males, age from 51 to 60 yrs, median age = 55 yrs), and the 61-70 group (13 males, age from 61 to 70 yrs, median age = 65 yrs). Each enrolled subject underwent a detailed clinical and physical examination to verify that he/she had neither history nor clinical evidence of any disease. Enrolled subjects were non-smokers, non-habitual drinkers and non-obese (BMI < 30 $Kg \cdot m^{-2}$) and did not take any medicine influencing cardiovascular system. Only women without contraceptive medication or without hormone replacement therapy were included. Cardiovascular variability of premenopausal females was recorded during their follicular phase. All women in the groups 51-60 and 61-70 were in the menopausal phase. Peak oxygen uptake (peak VO_2) was evaluated during an incremental cardiopulmonary exercise test on a treadmill on the basis

of a subject-specific ramp protocol. The protocol consisted of a 4 min warm-up over the treadmill at 1.4 mph with 0% inclination. Then, the velocity of the treadmill was incremented every 30 s in proportion to the maximum walking velocity of the subject assessed in a previous separate session. When the maximal walking velocity was reached, the inclination of the treadmill was increased 0.5% every 15 s until volitional exhaustion. Gas analysis was performed on a breath-by-breath basis (CPX-D, Med-Graphics, St Paul, MN, USA). Peak VO_2 was operationally defined as the highest VO_2 observed during the last 30 s of exercise. Peak VO_2 assessment was performed the week before the experimental session planned for the acquisition of cardiovascular variability. All subjects were evaluated in the afternoon in a temperature- and humidity-controlled room. Subjects were instructed to avoid caffeinated and alcoholic beverages as well as strenuous exercise during the day before the experiment. They all had a light meal at least 2 hours prior to the experimental session. The subjects were instrumented and maintained at REST for 10 minutes before starting recording. Then, electrocardiogram (ECG), from a modified lead I, continuous plethysmographic AP (Finometer PRO, Finapress Medical System, The Netherlands) and respiratory movements via a thoracic belt (Marazza, Monza, Italy) were recorded (Power Lab 8/35, ADInstruments, Australia) for 15 minute at REST and for 15 minutes during STAND. Signals were sampled at 400 Hz. The STAND session followed always the REST one. The subjects were instructed to breathe spontaneously but they were not allowed to talk. All subjects completed STAND without experiencing any sign of presyncope.

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 (n.173/2011). Each participant signed a written informed consent before entering the protocol.

Beat-to-beat variability series extraction

The temporal distance between two consecutive R-wave peaks detected on the ECG was taken as an approximation of the HP. The delineation of the R-wave peaks was based on a threshold on the first derivative of the ECG and on parabolic interpolation to fix the R-wave apex. The maximum of the AP signal inside each HP was taken as the SAP value associated to the current HP. The detections of the R-wave peaks and SAP values were visually checked and corrected in case of misidentifications. HP and SAP values resulting from ectopic beats were corrected via linear interpolation using the most adjacent HP and SAP values unaffected by ectopies. Corrections never exceed 5% of the frame length utilized for the analysis. In agreement with the standard for the

assessment of HPVA and BRS analysis from spontaneous cardiovascular variability (48, 62, 65), series lasting 256 beats were randomly selected within REST and STAND sessions. Selection during STAND was carried out starting three minutes after the posture change to avoid the early response to the challenge. Time domain indexes such as mean and variance of HP and SAP series were calculated, labeled respectively μ_{HP}, σ^2_{HP}, μ_{SAP}, and σ^2_{SAP}, and expressed respectively in ms, ms^2, mmHg, and $mmHg^2$. The respiratory frequency, indicated as f_R and expressed in Hz, was derived from the respiratory movement signal. The markers were expressed also as absolute and percent variations during STAND with respect to REST condition.

BRA assessment

According to (19, 20) among the possible BR analysis methods for the quantification of BRA from HP and SAP variability we selected the SEQ method (5, 59) as implemented in (62, 64). The SEQ method is based on the search for positive (SEQ+) and negative (SEQ-) joint HP-SAP patterns of BR origin. The SEQ+ pattern features three consecutive and contemporaneous HP and SAP increases (i.e. positive HP and SAP ramps), while the SEQ- pattern presents three consecutive and contemporaneous HP and SAP decreases (i.e. negative HP and SAP ramps). The selection of contemporaneous HP and SAP ramps (i.e. with latency equal to 0 beats) allowed us the focalization of the fast vagal arm of the cardiac BR compatible with HP responses occurring within the current HP where SAP was measured (1, 66), namely with latencies as short as 240 ms (22). The use of strict monotonic criteria allowed the sole inclusion of HP-SAP patterns free from noise and more likely of cardiac BR origin. All SEQ+ and SEQ- joint schemes were considered of BR origin regardless of the magnitude of total, or partial, SAP and HP variations and the strength of the linear association between HP and SAP values (62). The BRS driven by positive SAP variations, termed BRS_{SEQ+}, was estimated as the mean of the slopes of the regression lines of HP on SAP over all SEQ+ patterns. The BRS driven by negative SAP variations, labelled BRS_{SEQ-}, was estimated as the mean of the slopes of the regression lines of HP on SAP over all SEQ- patterns. BRS_{SEQ+} and BRS_{SEQ-} were positive by definition and expressed in $ms \cdot mmHg^{-1}$.

BRA markers were obtained as the difference between BRS driven by positive and negative SAP variations, namely $BRS_{SEQ+} - BRS_{SEQ-}$, and as the previously defined difference normalized by BRS_{SEQ-}, namely $(BRS_{SEQ+} - BRS_{SEQ-}) / BRS_{SEQ-}$. The larger the absolute value of the indexes, the more relevant the BRA. $BRS_{SEQ+} - BRS_{SEQ-}$ was expressed in $ms \cdot mmHg^{-1}$, while $(BRS_{SEQ+} - BRS_{SEQ-}) / BRS_{SEQ-}$ was dimensionless.

200 **HPVA evaluation**

201 The HPVA was quantified via two frequently exploited indexes (65): Porta's index (PI_{HP}) and
202 Guzik's index (GI_{HP}). The PI_{HP} (69) evaluates the percentage of negative HP variations with respect
203 to the total amount of HP changes. It is expressed in % and ranges from 0 to 100. Values of PI_{HP}
204 larger than 50 indicate that the number of negative HP variations is larger than that of positive HP
205 changes. The GI_{HP} (34) is computed as the percent sum of the square positive HP variations with
206 respect to the overall sum of square HP changes. Like PI_{HP}, also GI_{HP} is expressed in % and ranges
207 from 0 to 100. Values of GI_{HP} larger than 50 indicate that the averaged magnitude of square positive
208 HP variations is larger than that of the square negative HP changes.
209
210 **Statistical analysis**

211 BRS parameters considering separately positive and negative AP variations as well as HPVA
212 markers were pooled together regardless of age. The unpaired t-test, or Mann-Whitney rank sum
213 test when appropriate, and the paired t-test, or Wilcoxon signed rank test when appropriate, were
214 applied to BRS and HPVA markers respectively. These analyses were separately carried out at
215 REST and during STAND.

216 Two-way analysis of variance (one factor repetition, Holm-Sidak test for multiple
217 comparisons) was utilized to assess the difference between BRS parameters computed by separately
218 considering positive and negative SAP variations within the same age group (i.e. 21-30, 31-40, 41-
219 50, 51-60 or 61-70) and changes compared to the 21-30 group assigned the type of BRS marker (i.e.
220 BRS_{SEQ+} or BRS_{SEQ-}). This analysis was separately performed at REST and during STAND. Two-
221 way repeated measures analysis of variance (one factor repetition, Holm-Sidak test for multiple
222 comparisons) was applied to test the difference between μ_{HP}, σ^2_{HP}, μ_{SAP}, σ^2_{SAP}, f_R, and HPVA
223 markers computed at REST and during STAND within the same age group (i.e. 21-30, 31-40, 41-
224 50, 51-60 or 61-70) and changes compared to the 21-30 group assigned the experimental condition
225 (i.e. REST or STAND). One-way analysis of variance (Holm-Sidak test for multiple comparisons),
226 or Kruskal-Wallis one-way analysis of variance on ranks (Dunnett test for multiple comparisons)
227 when appropriate, was utilized to assess the significance of the percent variation of μ_{HP}, σ^2_{HP}, μ_{SAP},
228 σ^2_{SAP}, and f_R during STAND with respect to REST and the significance of age, body mass index
229 (BMI) and peak VO_2 changes compared to the 21-30 group.

230 Pearson correlation analysis was carried out to assess the significance of the correlation of
231 BRS estimates and HPVA markers on age. The same analysis was carried out to assess the
232 correlation between BRA and HPVA indexes. This analysis was separately carried out at REST and

during STAND regardless of age and even within each age group. Pearson product moment correlation coefficient r and type I error probability p were calculated. Statistical analysis was carried out using a commercial statistical program (Sigmaplot, Systat Software, Inc., Chicago, IL, version 11.0). A $p<0.05$ was always deemed as significant.

Results

Table 1 summarizes age, BMI, and peak VO_2 of all groups. The groups had different ages compared to the 21-30 one, while they were homogeneous for BMI. The groups had comparable levels of fitness with the notable exception of the oldest group that had a significantly smaller peak VO_2 compared to the 21-30 group.

Time domain HP and SAP markers and f_R at REST and during STAND were summarized in Tab.2. STAND shortened μ_{HP} in all groups. The effect of aging was evident during STAND. Indeed, at REST μ_{HP} remained stable with age, while during STAND μ_{HP} lengthened in 41-50, 51-60 and 61-70 groups compared to the 21-30 group. STAND reduced σ^2_{HP} only in the youngest group. The reduction of σ^2_{HP} with age compared to the 21-30 group was visible at REST in 41-50, 51-60 and 61-70 group and during STAND in the oldest cluster. The μ_{SAP} increased in response to the orthostatic challenge in all the groups with the exception of the youngest and oldest groups. An increase of μ_{SAP} with age was detectable and this result was more evident during STAND. Indeed, at REST solely the μ_{SAP} of the 51-60 cluster was higher than that of 21-30 group, while during STAND μ_{SAP} was increased in the 31-40. 41-50, 51-60 and 61-70 groups. The expected increase of σ^2_{SAP} in response to STAND was found in the 21-30 and 31-40 clusters. Remarkably, in the oldest bin of age STAND induced a decrement of σ^2_{SAP}. Aging increased σ^2_{SAP} and this rise was more evident at REST. Indeed, σ^2_{SAP} was larger in 51-60 and 61-70 groups compared to the youngestr cluster. Remarkably, f_R was not influenced either by STAND or aging.

Table 3 summarizes the absolute and percent variations of time domain HP and SAP markers (i.e. $\Delta\mu_{HP}$, $\Delta\sigma^2_{HP}$, $\Delta\mu_{SAP}$, and $\Delta\sigma^2_{SAP}$) and f_R (i.e. Δf_R) during STAND with respect to REST as a function of age group (i.e. 21-30, 31-40, 41-50, 51-60, and 61-70). Compared to 21-30 group, the tachycardic response to STAND was less relevant in 41-50, 51-60, and 61-70 groups and the hypertensive response to STAND was more important in 41-50 and 51-60 groups. In the oldest group the modification of σ^2_{SAP} during STAND was significantly smaller. $\Delta\sigma^2_{HP}$ and Δf_R remained stable with age. These results were evident when data were expressed in both absolute and percent variations.

The upper panels of Fig.1 compare the BRS estimates computed over positive and negative SAP variations at REST (Fig.1a) and during STAND (Fig.1b). Data were pooled together regardless of age. Assigned the experimental condition, BRS estimates computed over positive and negative SAP changes were similar. The results of the BRS evaluation as a function of age are shown in the lower panels of Fig.1. BRS estimates driven by positive (black bars) or negative (white bars) SAP variations are reported at REST (Fig.1c) and during STAND (Fig.1d) in 21-30, 31-40, 41-50, 51-60, and 61-70 groups. At REST BRS_{SEQ} decreased in 51-60 and 61-70 groups compared to 21-30 group and this result held regardless of the sign of SAP variation. At REST BRS_{SEQ} was smaller in the 41-50 group compared to the 21-30 one as well but this result was evident only in BRS_{SEQ+}. During STAND BRS_{SEQ} decreased in the 51-60 and 61-70 group compared to 21-30 group but this reduction was significant only for the BRS_{SEQ+} index. BRS estimates computed over positive and negative SAP changes were similar even when the difference between BR slopes was tested within the same age group.

Figure 2 shows the results of the linear correlation analysis of BRS estimates on age at REST (Figs.2a,c) and during STAND (Figs.2b,d). BRS estimates were computed over positive (Figs.2a,b) and negative (Figs.2c,d) SAP changes. At REST BRS_{SEQ+} and BRS_{SEQ-} were significantly and negatively correlated with age (r=-0.465, p=7.31×10^{-6} and r=-0.590, p=1.66×10^{-8} in Figs.2a,c respectively). When BRS_{SEQ+} was considered, the significant negative correlation with age was confirmed during STAND as well (Fig.2b: r=-0.385, p=1.68×10^{-4}), while no correlation was found between BRS_{SEQ-} and age (Fig.2d: r=-0.169, p=1.02×10^{-1}). However, when markers of BRA were computed (i.e. $BRS_{SEQ+} - BRS_{SEQ-}$ and $(BRS_{SEQ+} - BRS_{SEQ-}) / BRS_{SEQ-}$), no significant linear relationship with age was detected both at REST and during STAND.

The upper panels of Fig.3 show PI_{HP} (Fig.3a) and GI_{HP} (Fig.3b) as a function of the experimental condition (i.e. REST and STAND). Data were pooled together regardless of age. Both PI_{HP} and GI_{HP} increased significantly during STAND. The course of PI_{HP} and GI_{HP} with age is shown in Figs.3c,d respectively. HPVA markers are given at REST (black bars) and during STAND (white bars) in the 21-30, 31-40, 41-50, 51-60, and 61-70 groups. The general tendency toward an increase of both PI_{HP} and GI_{HP} during STAND was significant in the youngest groups (i.e. 21-30). During STAND in the 21-30 group PI_{HP} and GI_{HP} was above 50 (i.e. the dotted line) in 85% and 90% of the subjects respectively. At REST no significant changes with age was observed regardless of the type of the HPVA marker. During STAND PI_{HP} decreased in 41-50, 51-60, and 61-70 groups compared to 21-30 subjects, while GI_{HP} was smaller in 41-50 and 61-70 groups with respect to 21-30 individuals.

Figure 4 shows the results of the linear correlation analysis of HPVA indexes, namely PI_{HP} (Figs.4a,b) and GI_{HP} (Figs.4c,d) on age. Analysis was carried out at REST (Figs.4a,c) and during STAND (Figs.4b,d). At REST GI_{HP} was significantly and negatively correlated with age (i.e. r=-0.221, p=2.74×10^{-2}), while PI_{HP} was not associated with age (i.e. r=-0.089, p=3.81×10^{-1}). During STAND both PI_{HP} and GI_{HP} were significantly and negatively correlated with age (i.e. r=-0.276, p=5.48×10^{-3} and, r=-0.238, p=1.69×10^{-2} respectively).

Figure 5 shows the results of the linear correlation analysis of BRA indexes on HPVA markers at REST. Data are pooled together regardless of age. The analyses in the planes [PI_{HP}, BRS_{SEQ+} - BRS_{SEQ-}], [GI_{HP}, BRS_{SEQ+} - BRS_{SEQ-}], [PI_{HP}, (BRS_{SEQ+} - BRS_{SEQ-}) / BRS_{SEQ-}], and [GI_{HP}, (BRS_{SEQ+} - BRS_{SEQ-}) / BRS_{SEQ-}] are shown in Figs.5a,b,c,d respectively. No association was detected between BRA and HPVA markers. Figure 6 has the same structure as Fig.5 but it shows the results of the linear correlation analysis of BRA indexes on HPVA markers during STAND. During STAND BRA indexes were significantly and positively correlated with both PI_{HP} and GI_{HP}. Pearson correlation coefficient r and type I error probability p computed over data shown in Figs.6a,b,c,d were r=0.606 and p=2.38×10^{-10}, r=0.489 and p=1.01×10^{-6}, r=0.551 and p=1.82×10^{-8}, r=0.438 and p=1.58×10^{-5} respectively.

Table 4 summarizes the results of correlation analysis of BRA indexes on HPVA markers during STAND (i.e. during the experimental condition in which the strength of the correlation between BRA and HPVA markers was significant) as a function of age group. PI_{HP} was significantly and positively associated with both BRA parameters within all age groups with the notable exception of the 51-60 one. Conversely, GI_{HP} was significantly and positively associated with both BRA parameters just within the 41-50 group.

Discussion

The main findings of this study can be summarized as follows: i) BRA was not detectable using spontaneous variability of HP and SAP and this result held regardless of experimental condition and age group; ii) HPVA was more evident during STAND and decreased with age; iii) BRA markers were significantly and positively correlated with HPVA indexes during STAND, while they were uncorrelated at REST.

BRA is not detectable from HP and SAP variability

BRA, identified via the difference between the absolute amplitudes of the HP response per unit AP rise and fall, was proven through a pharmacological approach (60, 71, 74, 81) and

mechanical suction/pressure stimulation of the barosensitive areas of the carotid arteries performed via neck chamber (23). It was found that BR slopes are larger when AP is rising than falling (23, 60, 71, 74, 81). BRA was found to be enlarged via the decrease of the BRS to falling AP after prolonged physical exercise, thus accounting for hypotension episodes after intense training (80). The practical consequence of BRA is that HP is longer after two consecutive AP changes of equal absolute magnitude but opposite sign (74). Among the methods for the BR characterization based on spontaneous variability of HP and SAP (48), a restricted set of them (3, 5, 53) has the possibility to measure BRA without administering vasoactive drug and performing two separate experimental sessions for the computation of the BRS in response to positive and negative SAP variations. Among the techniques suitable for the assessment of BRA, the SEQ method was identified as the most powerful one given that it allows a sufficient degree of uncorrelation between HP responses to SAP changes of opposite sign (19, 20). Thus, this method was exploited in this study. When the SEQ method was applied and BRS estimates were pooled together regardless of age, no significant BRA was detected. Indeed, BRS estimates computed over positive SAP variations were similar to those calculated over negative SAP changes and no systematic difference between BR slopes was detected within the same age group. This finding held regardless of the experimental condition (i.e. REST or STAND). The limited ability of the BRS markers derived from spontaneous HP and SAP patterns in detecting BRA was stressed also by the trends of BRS estimates with age at REST. Indeed, the rates of decrease of BRS with age were similar regardless of whether the BRS was estimated over positive or negative SAP variations, thus leading to the uncorrelation of BRA markers to age. This finding suggests that both types of BRS estimates contribute equally to the well-known decrease of BRS during senescence observed via spontaneous HP and SAP variability analysis (27, 41, 46, 51, 71). Our result is in agreement with Rudas *et al* (71) who reported similar BRS_{SEQ+} and BRS_{SEQ-} values just after vasoactive drug injections utilized to probe BRA via the pharmacological method (60). A similar inability of the methods based on spontaneous HP and SAP variability was highlighted in several studies (16, 17, 19, 20, 50). The original additional finding is that the inability of detecting BRA from spontaneous BRS estimates is confirmed even in old subjects. This inability cannot be ascribed to pool data regardless of the gender because, in disagreement with the literature (46), our data did not support gender-related differences in the sensitivity of the vagal arm of the cardiac BR either at REST or during STAND (51). The unique sign that might suggest a different behavior of BRS markers computed over positive and negative SAP variations is that, during STAND, the BRS was significantly correlated with age solely when computed over positive SAP variations. This result might suggest that during STAND BRS_{SEQ-}

values, especially in the youngest group, were smaller than BRS_{SEQ+} estimates and this situation in association with of the low value of BRS during STAND and high dispersion, makes impossible to observe the trend of BRS_{SEQ-} with age that was, conversely, evident in the case of BRS_{SEQ+}.

There are several reasons that might explain the difficulty of characterizing BRA from spontaneous HP and SAP fluctuations. First, if BRA was present, HP would be longer just after two consecutive SAP changes featuring the same absolute value but opposite sign and, consequently, BR resetting, leading to no HP variation while varying SAP, would occur to complete the hysteresis loop in the (SAP,HP) plane (74). BR resetting might operate more frequently and over smaller AP changes in physiological closed loop conditions than during pharmacological challenges (71) or mechanical stimulation (23), thus making impossible to differentiate the BRS computed over positive and negative SAP variations in relation to the physiological dispersion of the BRS estimates. Second, the HP-SAP relation is composed by two parts, namely the portion describing the mechanical transduction of SAP changes into variations of barosensory vessel diameter and the portion accounting for the neural afferent and efferent pathways linking barosensory vessel diameter modifications to HP fluctuations (36). The steeper slope of the AP-diameter relation when AP rises than falls (6, 58) can explain BRA (74). However, it is well-known that the neural pathway functioning can mask BRA induced by the mechanical portion of the HP-SAP relation (74). The complexity of the interactions among these two components might be increased in close loop physiological conditions, thus resulting in the inability to observe BRA in our experimental setting. Third, the inability of BRS estimates obtained from spontaneous HP and SAP fluctuations to highlight BRA might be the result of the smallness of the physiological SAP variations leading to the exploration of a limited region of the HP-SAP sigmoidal relation and/or to the limited activation of the BR (21, 57), as demonstrated by the small amount of HP-SAP joint patterns of BR origin (49). Conversely, under a pharmacological challenge the SAP variations are relevant and this relevance assures the engagement of BR and the exploration of a larger portion of the HP-SAP sigmoidal curve (60, 71, 74).

HPVA is detectable during STAND and decreases progressively with age

HPVA takes the form of bradycardic runs lasting less than tachycardic ones or, equivalently, heart decelerates much faster than it accelerates. As a consequence, simple metrics, such as the percentage of negative variations (65, 69) or the percent contribution of square positive variations to the whole sum of square differences (34, 61), detect HPVA simply by checking their departure from the situation of perfect HPV symmetry (i.e. 50%). The presence of this pattern makes some

statistical properties of HPV different when the flow of time is reversed (14, 67, 69) and this feature is incompatible with linear dynamics that are perfectly symmetric under time reversal (79). Therefore, HPVA was indicated as one of the patterns responsible for the nonlinear characteristic of short-term HPV (10, 63, 65, 67). The origin of this nonlinear feature is unclear. Since the ratio of inspiratory to expiratory time (i.e. the I:E ratio) is usually 1:2 in healthy population (73) and respiration produces the HP shortening during inspiration and the HP lengthening during expiration, usually referred to as respiratory sinus arrhythmia (RSA) (35), it would be expected that, in presence of a negligible RSA, the percentage of negative HP variations is smaller than that of positive HP changes with PI_{HP} significantly smaller than 50%. While increasing RSA with an unmodified I:E ratio, the percentage of negative HP variations should increase, while that of positive HP changes should decrease, thus leading to values of PI_{HP} closer to 50% or even significantly above 50%. This trend might suggest to a positive association between HPVA and RSA amplitude and might explain the increase of HPVA in experimental conditions evoking a relevant increase of RSA such as paced breathing at slow respiratory rate (69). Remarkably, in the present study HPVA markers increased significantly during STAND compared to REST (10, 12) and this rise was significant especially in the youngest group. The HPVA increase was observed during STAND despite the decrease of RSA (13, 52). This finding suggests that the RSA cannot be seen as a unique determinant of HPVA. The same consideration holds for any experimental condition featuring a dominant sympathetic drive and vagal withdrawal, such as during daytime and head-up tilt, in which an HPVA rise was observed (10, 65, 67).

The reduction of RSA with age (4, 11, 45, 46, 72) might account for the progressive reduction of HPVA with age as proven by the negative trend of HPVA markers with age. This finding suggests that aging makes the contribution of accelerations and decelerations more balanced by reducing the fastness of decelerations and/or by increasing the quickness of accelerations. The reduction of HPVA with age is in agreement with (14), who interpreted the loss of nonlinearity associated to the migration of HPVA markers towards 50% as the breakdown of complexity of the cardiac control and as a hallmark of its degraded functioning during senescence (28). This trend cannot be the mere effect of the progressive sympathetic activation and vagal withdrawal with age (4, 11, 45, 46, 72), because, if that was the case, HPVA would be expected to increase (65, 67). Therefore, we suggest that the state of autonomic function is not the unique determinant of the level of HPVA and this observation, in connection with the BRA characteristic, prompts for considering the possible impact of BRA in contributing to the HPVA.

BRA markers are positively correlated with HPVA indexes during STAND

BRA can in principle explain HPVA. Indeed, given that the BR slope of the HP response to a SAP increase is steeper than that to a SAP decrease and the association between HP and SAP changes imposed by BR is positive (23, 60, 71, 74, 81), the upward side of the HP pattern is expected to be faster than the downward side when HP variations are fully driven by SAP changes. The characterization of BRA was originally performed via interventional approaches (23, 60, 71, 74, 81). Unfortunately, BRA was not detected in our study using BRS estimated via HP and SAP spontaneous variations both at REST and during STAND. This finding suggests that even the association between BRA and HPVA could be undetectable because the information contained in BRS_{SEQ+} and BRS_{SEQ-} about BRA was insufficient or blurred by noise. This expectation was confirmed just at REST. On the contrary, during STAND a significant positive association between BRA, quantified via the SEQ technique, and HPVA markers was detected. This finding stresses the cardiac BR origin of HPVA. Indeed, during STAND the cardiac arm of BR is more involved in regulating AP than at REST (26, 40, 56, 66). The stimulation of the cardiac arm of the BR induced by STAND makes it possible to reveal the association between BRA and HPVA from HP and SAP spontaneous fluctuations. Also the reduced variance of the BRS estimates associated with the decrease towards 0 of both BRS_{SEQ+} and BRS_{SEQ-} during STAND might have contributed to make possible the detection of the significant association between BRA and HPVA markers. The cardiac BR origin of HPVA might account for the increased HPVA during head-up tilt (12, 63, 65, 69) and during slow paced breathing (69). Indeed, the dominant low frequency rhythm at 0.1 Hz in healthy subjects observed during postural challenges is the likely expression of the BR control (1, 18, 30) and the large RSA observed during controlled respiration at slow breathing rate is, at least partially, the effect of the perturbation of the BR control (2, 24) driven by modifications of the venous return and stroke volume (77). The observed link between BRA and HPVA during STAND suggests that the loss of HPVA with age might be the consequence of the reduced BRA with age (71). However, since the trend of BRA with age was not observed in our study, we assume that, on the one hand, additional mechanisms of HPVA generation should be considered and, on the other hand, BRA is weakly measured from HP and SAP spontaneous fluctuations. Among the possible mechanisms alternative to BRA and to a relevant RSA associated with a physiological I:E ratio, the asymmetric shape of the activity of central sympathetic and respiratory rhythm generators governing HP variability in the low and high frequency bands might play a role in producing HPVA. We also remark that during STAND the HPVA should not be a simple mirroring of SAP variability asymmetry via the BR because HPVA significantly increased compared to REST, while SAP

463 variability asymmetry remained unvaried (12), thus suggesting that HPVA is genuinely generated
464 by the HP-SAP relation.
465
466 **Possible dependences of BRA on age**
467 It is well-known that aging reduced BRS (27, 41, 46, 51, 71) and this result was confirmed in
468 this study by the trends of BRS_{SEQ+} and BRS_{SEQ-} with age. This finding was explained as a
469 consequence of the progressive reduction of gain of the vascular mechanical component (38) due to
470 the increased stiffness of the barosensory vessels (43) and, more importantly, as a consequence of
471 the progressive decrease of the gain of the neural component (38) due to the impairment of
472 autonomic central integration network (39), sympathetic overactivity and vagal withdrawal (72),
473 and the reduced sinus node responsiveness to neural inputs and stressors with age (7, 15, 47). The
474 negative trends of BRS with age do not imply automatically that BRA is modified with age. It
475 depends on the slope of the decrement of BRS_{SEQ+} and BRS_{SEQ-} with age. Since we observed that
476 the slopes of BRS_{SEQ+} and BRS_{SEQ-} with age were similar at REST, we suggested that the BRA was
477 not modified with age at REST. However, since the significant and negative association of BRS
478 with age during STAND was detected only over BRS_{SEQ+}, while BRS_{SEQ-} did not vary with age,
479 BRA could decrease with age during STAND, even though this observation was not directly
480 supported by the BRA markers derived from SEQ analysis. Remarkably, this tendency was
481 mirrored by the negative trend of PI_{HP} and GI_{HP} with age and by the significant association of PI_{HP}
482 and GI_{HP} with BRA markers during STAND. The presence of BRA results from the fact that
483 BRS_{SEQ+} and BRS_{SEQ-} reflect the gain of the cardiac BR to different baroreceptor firing. Bonyhay
484 and colleagues (6) showed that increases of carotid artery diameter are steeper after phenylephrine
485 administration than decreases after nitroprusside and Burke and colleagues (8) reported different
486 carotid sinus nerve firing in response to AP rises and falls. According to these observations BRA
487 might be seen as the sole consequence of the mechanical hysteresis of barosensory arteries. Even
488 though it is possible that aging could disrupt this vascular response pattern because the process of
489 gradual increase of the stiffness of the barosensory vessels (43) might act differently on the
490 mechanical transduction of falling and rising AP into barosensory vessel stretch, it is more likely
491 that aging disrupts the autonomic integration at the central level that contributes importantly to the
492 cardiac BR hysteresis (38, 74). Moreover, since there might be a relation between the basal level of
493 autonomic nerve activity and BRS, especially when sympathetic activity is high (9, 29), the
494 disruption of the BRA with age might be the result of the diverse impact of an increase of
495 sympathetic activity and vagal withdrawal with age over BRS_{SEQ+} and BRS_{SEQ-}. The fact that the

trends of BRA with age was more evident during STAND and correlation of BRA on HPVA markers was significant solely during STAND might be the sole effect that during STAND the dominant causal direction is from SAP to HP along the cardiac BR control (26, 40, 56, 66), thus making more powerful the estimate of BRA markers based on cardiovascular variability and their relation with HPVA. Remarkably, the association between BRA and HPVA was generally preserved during STAND in the various age clusters (only in the 51-60 group the association was not significant), thus stressing that STAND provides a favorable experimental condition for using HPVA as a BRA proxy in spite of the possible decrement of BRA with age.

BR hysteresis and BRA

BR hysteresis (23, 71, 74) is often equated to BRA (19, 20, 37, 81). However, the two concepts are not fully equivalent. BR hysteresis might occur without BRA as a result, for example, of a delayed response of HP to SAP changes. Conversely, BRA requires a temporal element (e.g. the BR resetting implying no HP change in presence of SAP variation) to close the loop in the (SAP,HP) plane (74). This temporal element should be present in BR control because otherwise HP would become rapidly incompatible with life and this situation might happen, for example, as soon as a series of SAP variations of opposite sign but with the same absolute values occur consecutively over time. This inevitable presence explains why BRA and BR hysteresis are inextricably linked in the literature. The choice of exploring the fast responses of HP to SAP changes by setting the delay between HP and SAP ramps to 0 beats in the SEQ method (1, 66), on the one hand, limits the results, and the interpretation, to the fast vagal component of cardiac BR and, on the other hand, allows the more direct equivalence between BR hysteresis and BRA because trivial hysteresis phenomena just due to non-null HP-SAP latency are excluded from this analysis.

Limitations and future developments

Even though our BRS estimates did not support gender-related differences at REST or during STAND (51), subtle gender-related differences within the same age group might be present (46) especially when cardiac BR responses to positive and negative SAP changes are separated. A robust analysis of this additional factor requires the enlargement of the population size to increase appropriately the statistical power of the study. One of the major limitations of the study is that the impact of the breathing pattern is unknown. Indeed, our subjects breathed spontaneously without controlling respiratory rate, deepness or I:E ratio. Only the breathing frequency was monitored. Since the respiratory rate did not vary with age and experimental condition, we can exclude that

breathing frequency played a role in the conclusions of this study. However, the impact of respiratory deepness, breathing rate and I:E ratio remained unexplored. We advocate future studies more specifically designed to elucidate the impact of the I:E ratio, breathing deepness and rate on HPVA and its association with BRA. Also the potential asymmetry of neural patterns generated by the activity of central rhythm generators deserves to be evaluated because it might play an important role in originating the HPVA according to a central hypothesis of the genesis of HPVA.

Since in this experimental protocol BRS was not evaluated using interventional approaches, it remains to be ascertained whether interventional estimates of BRA based on pharmacological challenges or mechanical pressure/suction stimulation via neck chamber device could be related to BRA estimated from spontaneous HP and SAP fluctuations. This more complex protocol might provide more support to the possibility of inferring BRA from HPVA markers whether the interventional and spontaneous estimates of BRA were associated with HPVA and new suggestions could be derived from checking which of the two assessments of BRA would be more strongly related to HPVA.

Perspectives and significance

The hypothesis that the HPVA, namely the greater likelihood of finding HP decreases than increases, can be explained by the different response of BR to AP ups and downs was tested over spontaneous fluctuations of HP and SAP. The hypothesis was accepted as BRA and HPVA indexes were significantly and positively correlated in an experimental condition activating the BR control (i.e. STAND). However, BRA, as measured from HP and SAP variability, explained only partially HPVA given that the association between BRA and HPVA was found to be significant only when BR was solicited.

Given that this report indicates a possible mechanism supporting the presence of an asymmetric behavior of the HPV, it sheds light into a specific nonlinear pattern robustly detected in HPV. Moreover, this study supports the use of HPVA markers to surrogate BRA indexes when the BR is solicited as it occurs during an orthostatic challenge. Since subjects undergoing episodes of hypotension might feature the exclusive impairment of the BR response to SAP drops and a greater BRA (81), the possibility to measure the BRA based on HPVA might help the identification of subjects at risk. This tool might be particularly useful in studying postural hypotension (31). Since an increased BRA due to a greater reduction of the BRS_{SEQ-} compared to BRS_{SEQ+} has been documented after prolonged exercise (80), thus possibly explaining episodes of post-exercise orthostatic intolerance (54, 55), HPVA markers might be exploited in monitoring the return to pre-

562 exercise BR control via simple commercial HPV devices that are becoming more and more popular
563 in fitness and sport centers. However, given that HPVA cannot be fully explained by BRA, it is
564 necessary to investigate the asymmetry of neural variability patterns acting on the sinus node but
565 unrelated to AP changes and their potential contribution to HPVA. These components could be
566 studied by means of direct neural recordings in pathological populations featuring an impairment of
567 the BR control or in animal models in which the BR control was opened surgically.
568

569 ## Authors' contributions

570 A.P., conception and design of research; N.M.P., J.M.-M., P.R.-S., and V.M. performed
571 experiments; B.D.M. analyzed the data; B.D.M. and A.P. drafted the manuscript; B.D.M. and A.P.
572 prepared the figures; B.D.M., V.B., B.C., E.V., R.M.A., N.M.P., J.M.-M., P.R.-S., V.M., A.M.C.,
573 L.A.D.V. and A.P. interpreted the results; B.D.M., V.B., B.C., E.V., R.M.A., N.M.P., J.M.-M.,
574 P.R.-S., V.M., A.M.C., L.A.D.V. and A.P. edited and revised the manuscript; B.D.M., V.B., B.C.,
575 E.V., R.M.A., N.M.P., J.M.-M., P.R.-S., V.M., A.M.C., L.A.D.V. and A.P. approved the final
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577

578 ## Disclosures

579 No conflicts of interest are declared by the authors.
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Figure captions

Fig.1. The simple error bar graphs on the top panels show the BRS calculated as a function of the sign of the SAP variations at REST (a) and during STAND (b). Data are pooled together regardless of the age group (i.e. 21-30, 31-40, 41-50, 51-60 and 61-70). The grouped error bar graphs on the bottom panels show the BRS computed over positive (black bars) and negative (white bars) SAP variations as a function of age group (i.e. 21-30, 31-40, 41-50, 51-60, and 61-70) at REST (c) and during STAND (d). Data are given as mean plus standard deviation. The symbol * indicates $p<0.05$ versus the 21-30 group within the same type of BRS estimate.

Fig.2. The scatter plots show the results of the linear correlation analysis of BRS_{SEQ+} (a,b) and BRS_{SEQ-} (c,d) on age at REST (a,c) and during STAND (b,d). Each open circle corresponds to a pair of BRS versus age in an assigned individual. The linear regression line (solid line) and its 95% confidence interval (dotted lines) are plotted when Pearson correlation coefficient is different from 0 with $p<0.05$.

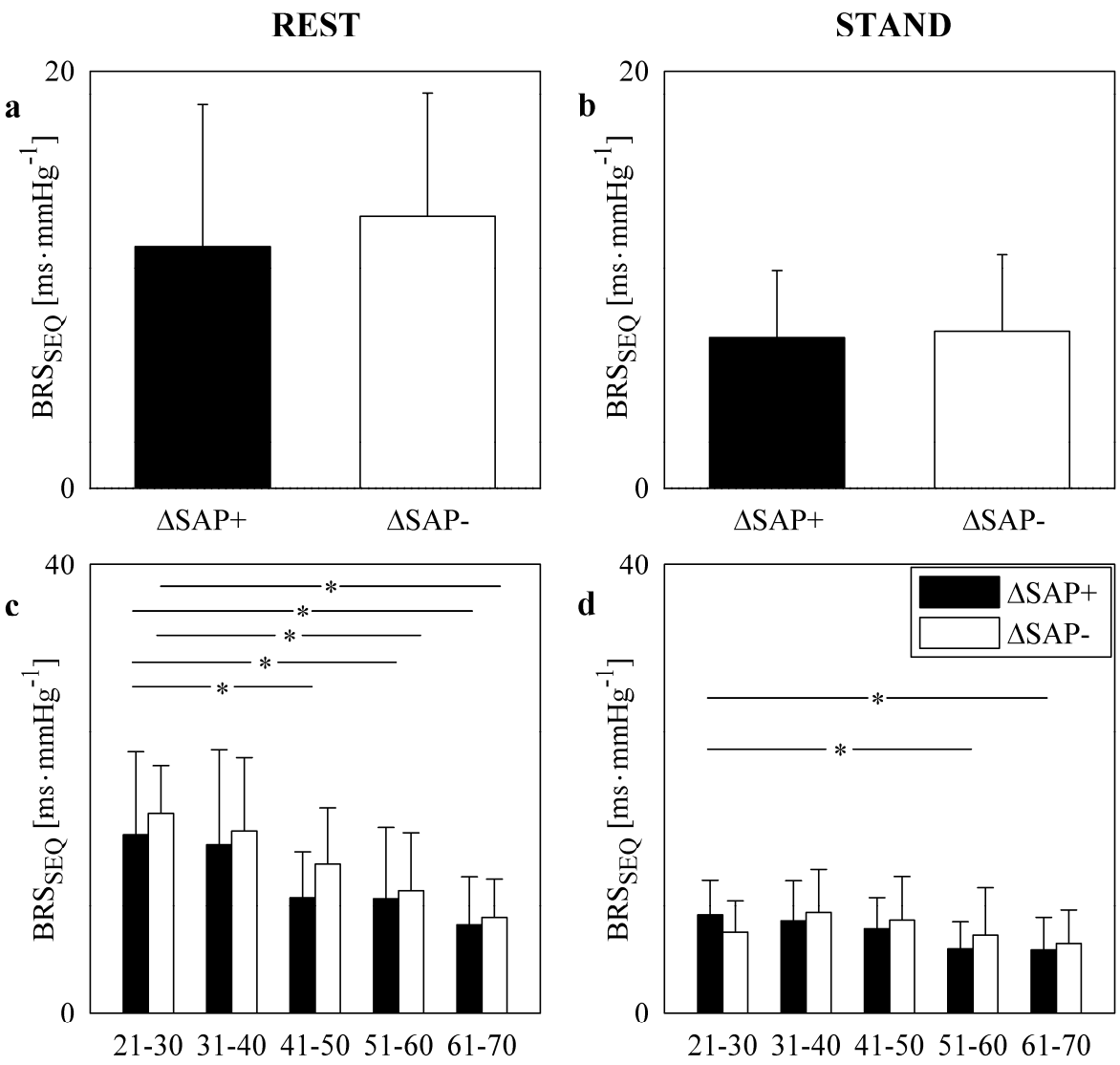
Fig.3. The simple error bar graphs on the top panels show PI_{HP} (a) and GI_{HP} (b) as a function of the experimental condition (i.e. REST and STAND). Data are pooled together regardless of the age group (i.e. 21-30, 31-40, 41-50, 51-60 and 61-70). The grouped error bar graphs on the bottom panels show PI_{HP} (c) and GI_{HP} (d) as a function of age group (i.e. 21-30, 31-40, 41-50, 51-60, and 61-70) at REST (black bars) and during STAND (white bars). Data are reported as mean plus standard deviation. The horizontal dotted line denotes $PI_{HP}=GI_{HP}=50$, namely the situation of no HPVA. The symbol * indicates $p<0.05$ versus the 21-30 group within the same experimental condition, while the symbol § indicates $p<0.05$ versus REST with the same group of subjects.

Fig.4. The scatter plots show the results of the linear correlation analysis of HPVA markers, namely PI_{HP} (a,b) and GI_{HP} (c,d), on age at REST (a,c) and during STAND (b,d). Each open circle corresponds to a pair of HPVA index versus age in an assigned individual. The linear regression line (solid line) and its 95% confidence interval (dotted lines) are plotted when Pearson correlation coefficient is different from 0 with $p<0.05$.

Fig.5. The scatter plots show the results of the linear correlation analysis of BRA indexes on HPVA markers at REST. The scatter plots are drawn in the planes $[PI_{HP}, BRS_{SEQ+} - BRS_{SEQ-}]$ (a), $[GI_{HP}, BRS_{SEQ+} - BRS_{SEQ-}]$ (b), $[PI_{HP}, (BRS_{SEQ+} - BRS_{SEQ-}) / BRS_{SEQ-}]$ (c), $[GI_{HP}, (BRS_{SEQ+} - BRS_{SEQ-}) / BRS_{SEQ-}]$ (d). Each open circle corresponds to a pair of BRA marker versus HPVA index in an assigned individual. Pairs are pooled together regardless of age. The

linear regression line (solid line) and its 95% confidence interval (dotted lines) are plotted when Pearson correlation coefficient is different from 0 with $p<0.05$.

Fig.6. The scatter plots show the results of the linear correlation analysis of BRA indexes on HPVA markers during STAND. The scatter plots are drawn in the planes [PI_{HP}, BRS_{SEQ+} - BRS_{SEQ-}] (a), [GI_{HP}, BRS_{SEQ+} - BRS_{SEQ-}] (b), [PI_{HP}, (BRS_{SEQ+} - BRS_{SEQ-}) / BRS_{SEQ-}] (c), [GI_{HP}, (BRS_{SEQ+} - BRS_{SEQ-}) / BRS_{SEQ-}] (d). Each open circle corresponds to a pair of BRA marker versus HPVA index in an assigned individual. Pairs are pooled together regardless of age. The linear regression line (solid line) and its 95% confidence interval (dotted lines) are plotted when Pearson correlation coefficient is different from 0 with $p<0.05$.


REST
STAND
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b
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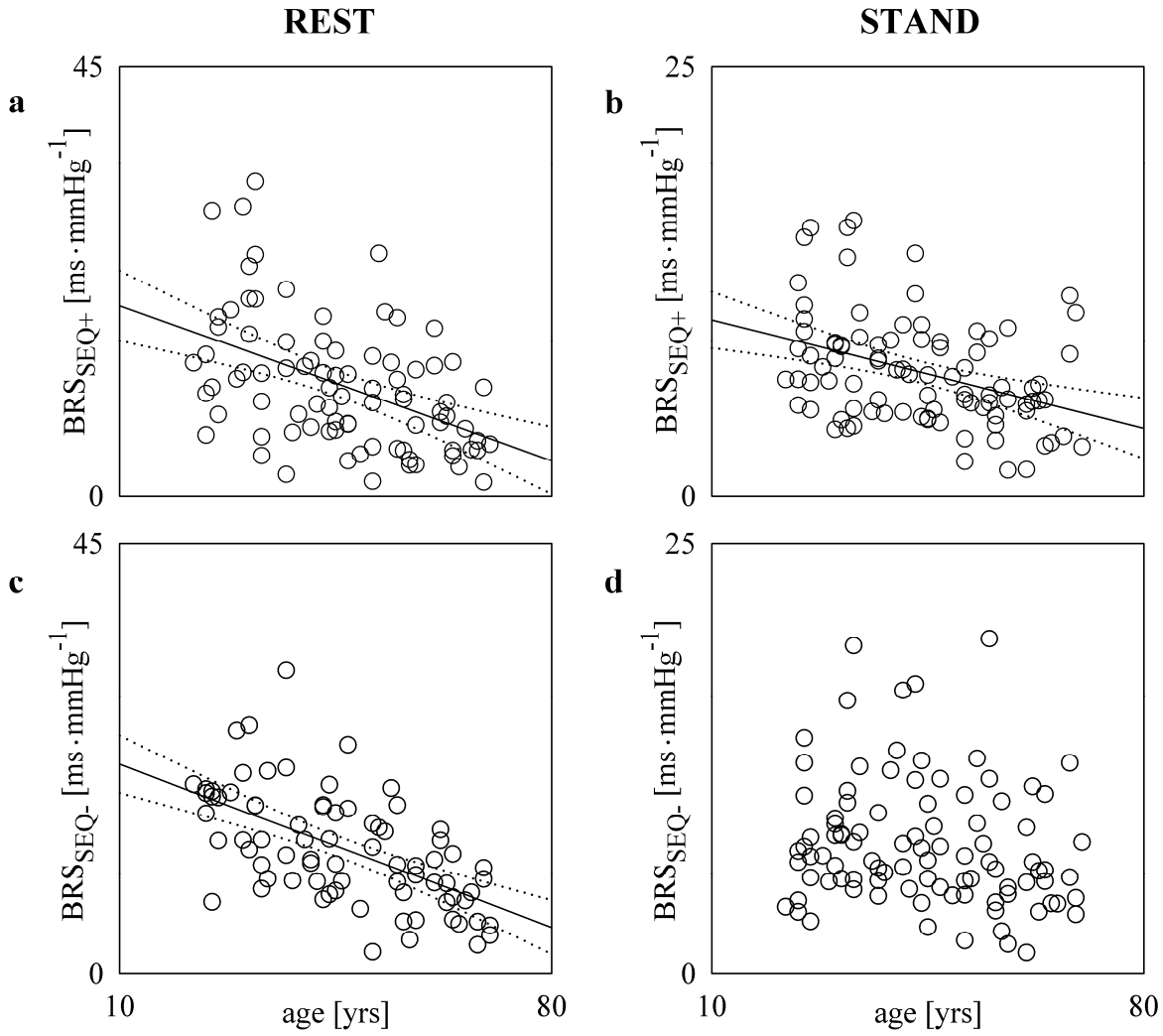
20
0
40
BRS_SEQ [ms·mmHg^-1]
ΔSAP+
ΔSAP-
21-30
31-40
41-50
51-60
61-70
*

Fig.1


REST
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d
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age [yrs]
BRS_SEQ+ [ms·mmHg^-1]
BRS_SEQ- [ms·mmHg^-1]

Fig.2

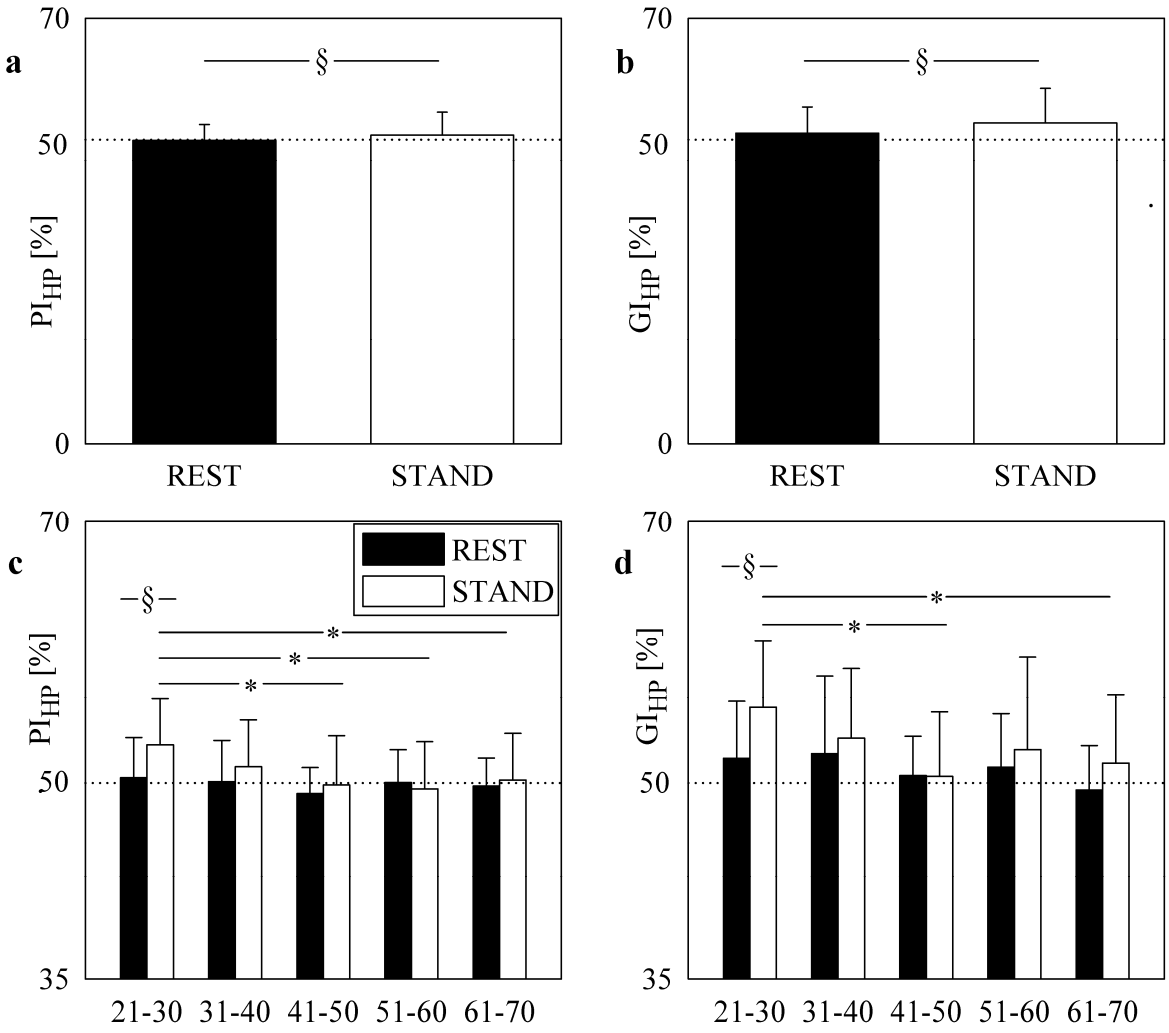


Fig.3

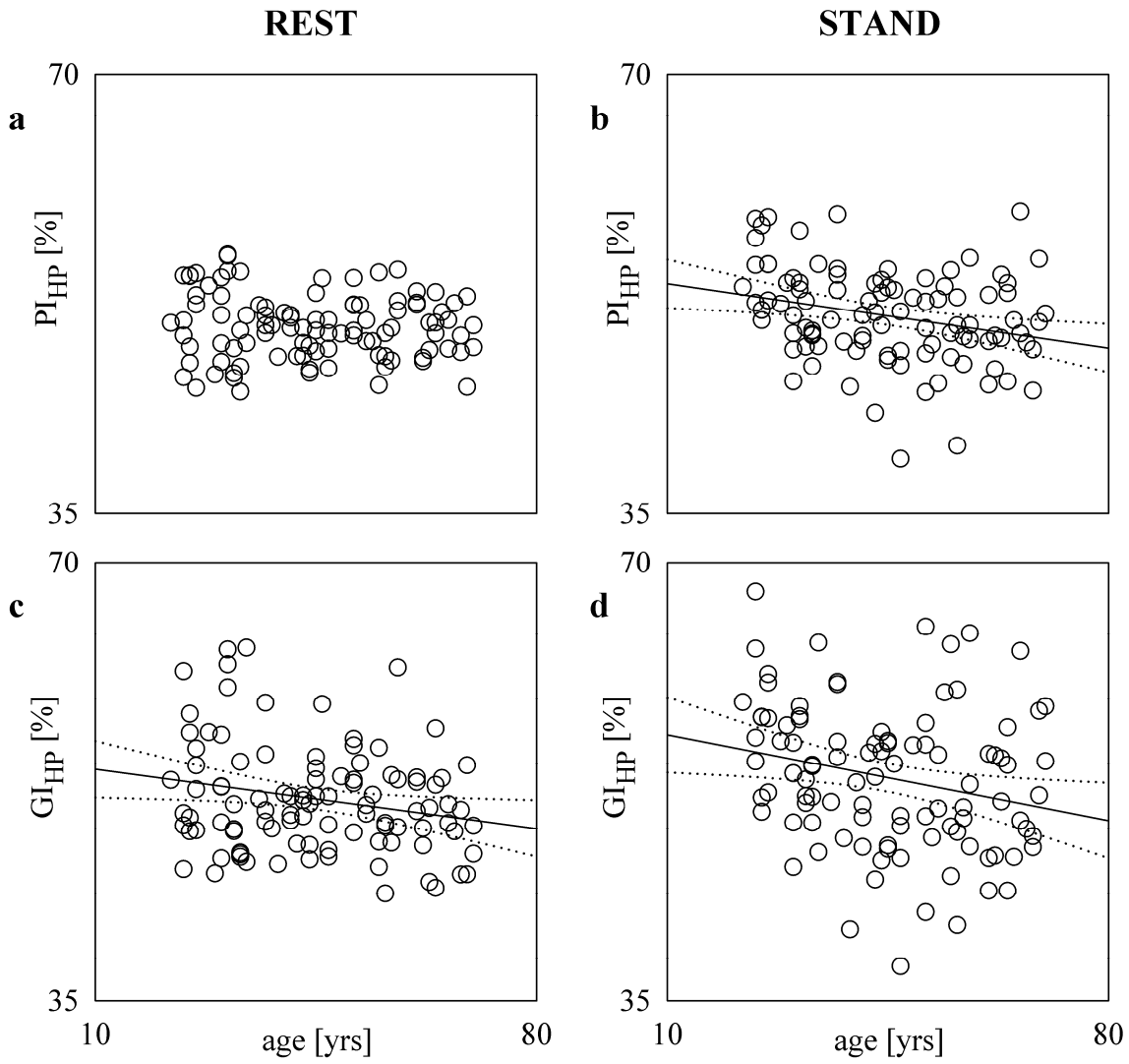
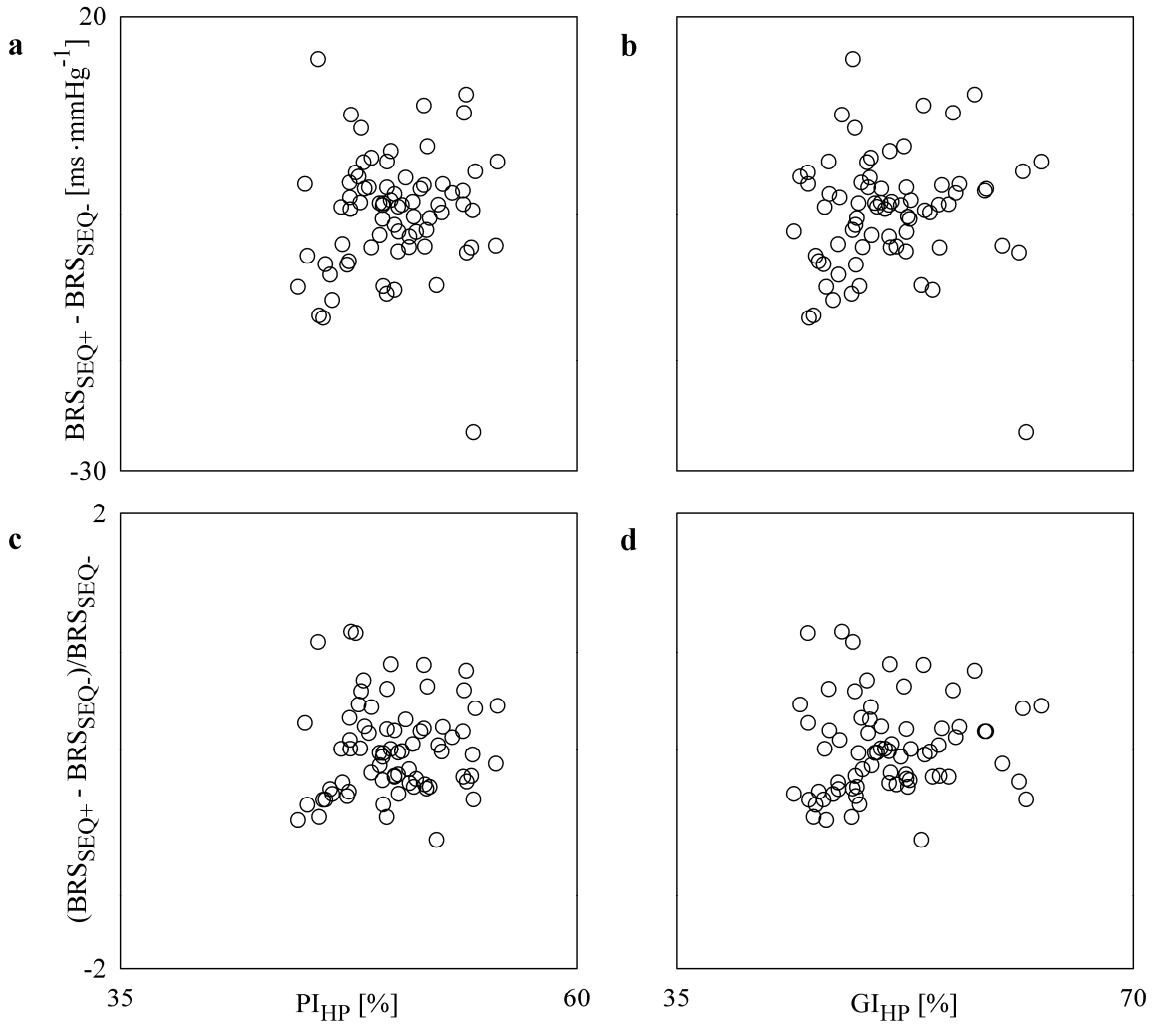


Fig.4


a
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-30
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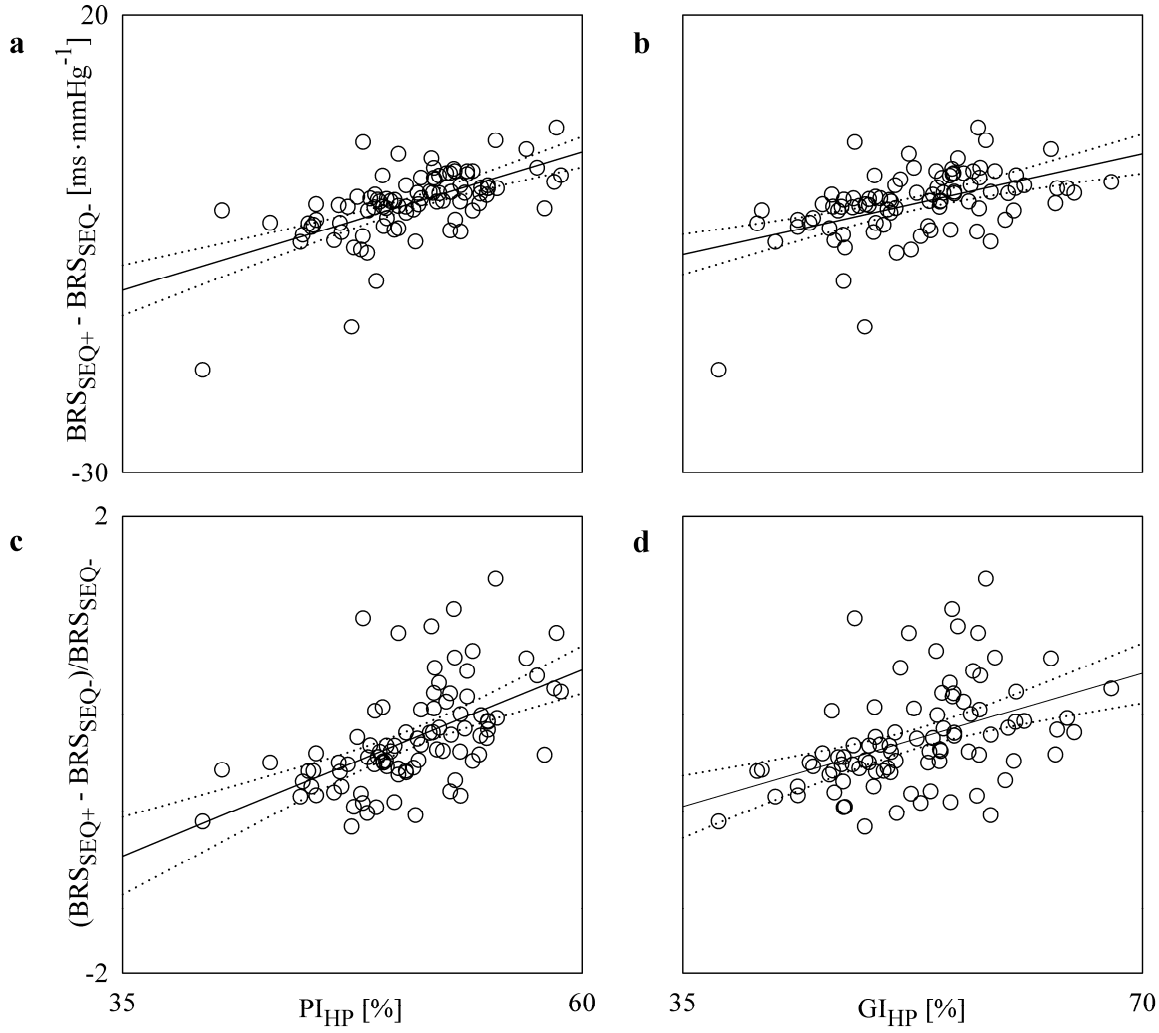
BRS_{SEQ+} - BRS_{SEQ-} [ms ·mmHg^{-1}]
(BRS_{SEQ+} - BRS_{SEQ-})/BRS_{SEQ-}
PI_{HP} [%]
GI_{HP} [%]

Fig.5



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868

869 Fig.6

870

871 **Table 1.** Characteristics of the population.

Index	21-30	31-40	41-50	51-60	61-70
Age [yrs]	26.18±2.54	34.27±3.03*	44.41±2.34*	54.91±3.19*	64.68±2.68*
BMI [$kg \cdot m^{-2}$]	23.81±2.26	23.72±2.47	25.42±2.52	25.04±2.18	25.70±3.12
Peak VO_2 [$ml \cdot min^{-1} \cdot kg^{-1}$]	33.49±7.10	36.45±8.32	31.23±8.98	28.25±7.42	24.47±6.40*

872 21-30, 31-40, 41-50, 51-60, 61-70: min-max range expressed in yrs; BMI: body mass index; VO_2: oxygen uptake. Data are presented as mean±standard deviation. The symbol *
873 indicates $p<0.05$ versus 21-30 group.
874
875

876 **Table 2.** Time domain HP and SAP markers and respiratory rate as a function of the age.

Index	21-30		31-40		41-50		51-60		61-70	
	REST	STAND	REST	STAND	REST	STAND	REST	STAND	REST	STAND
μ_{HP} [ms]	878±137	695±96§	929±104	769±103§	914±104	786±95§*	887±108	767±96§*	922±108	809±99§*
σ^2_{HP} [ms^2]	2543±1880	1821±1284§	1949±1782	1916±1129	1452±986*	1437±1186	1255±1246*	1069±692	949±561*	681±439*
μ_{SAP} [mmHg]	113.3±8.2	112.5±11.3	118.8±11.0	123.5±15.4§*	115.7±10.9	123.5±11.1§*	127.9±12.7*	137.1±14.2§*	118.8±12.2	122.0±14.1*
σ^2_{SAP} [$mmHg^2$]	17.7±12.1	31.0±16.0§	20.0±11.3	35.1±17.4§	26.4±17.7	32.2±21.9	33.2±27.4*	41.2±20.7	42.0±32.1*	30.0±15.6§
f_R [Hz]	0.31±0.05	0.30±0.04	0.30±0.05	0.28±0.06	0.28±0.04	0.28±0.05	0.28±0.04	0.28±0.03	0.29±0.04	0.30±0.05

877 21-30, 31-40, 41-50, 51-60, 61-70: min-max range expressed in yrs; REST: at rest in supine position; STAND: active standing; HP: heart period; SAP: systolic arterial pressure;
878 μ_{HP}: HP mean; σ^2_{HP}: HP variance; μ_{SAP}: SAP mean; σ^2_{SAP}: SAP variance; f_R: breathing rate. Data are presented as mean±standard deviation. The symbol § indicates $p<0.05$ vs
879 REST within the same age group. The symbol * indicates $p<0.05$ versus 21-30 group within the same experimental condition.

880

881

882 **Table 3.** Percent variation of time domain HP and SAP markers and respiratory rate in response to STAND.

Index	21-30	31-40	41-50	51-60	61-70
$\Delta\mu_{HP}$ [ms]	-183.48±93.84	-160.59±91.90	-127.62±67.35*	-119.68±74.02*	-113.08±60.84*
$\Delta\mu_{HP}$ [%]	-20.05±10.07	-17.01±8.67	-13.79±6.53*	-13.19±7.56*	-12.11±5.79*
$\Delta\sigma^2_{HP}$ [ms^2]	-722.13±1597.57	-32.74±1908.59	-14.85±1366.67	-185.60±1200.75	-267.68±445.25
$\Delta\sigma^2_{HP}$ [%]	-4.53±74.67	44.21±118.74	20.40±103.93	16.18±83.45	-21.48±44.73
$\Delta\mu_{SAP}$ [mmHg]	-0.75±6.23	4.76±7.80	7.80±6.04*	9.15±10.65*	3.18±7.86
$\Delta\mu_{SAP}$ [%]	-0.75±5.48	3.86±6.53	6.92±5.50*	7.42±8.25*	2.76±6.84
$\Delta\sigma^2_{SAP}$ [$mmHg^2$]	13.27±14.51	15.03±13.29	5.82±17.78	7.95±26.02	-11.96±31.48*
$\Delta\sigma^2_{SAP}$ [%]	134.59±143.68	119.15±132.95	51.08±94.31	69.82±107.51	-1.19±61.80*
Δf_R [Hz]	-0.009±0.046	-0.016±0.056	-0.001±0.054	0.001±0.037	0.013±0.043
Δf_R [%]	-1.28±16.96	-4.22±19.30	0.81±18.78	1.69±12.47	5.12±15.98

883 21-30, 31-40, 41-50, 51-60, 61-70: min-max range expressed in yrs; REST: at rest in supine position; STAND: active standing; HP: heart period; SAP: systolic arterial pressure;
884 $\Delta\mu_{HP}$: percent variation of the HP mean during STAND with respect to REST; $\Delta\sigma^2_{HP}$: percent variation of the HP variance during STAND with respect to REST; $\Delta\mu_{SAP}$: percent
885 variation of the SAP mean during STAND with respect to REST; $\Delta\sigma^2_{SAP}$: percent variation of the SAP variance during STAND with respect to REST. Δf_R: percent variation of
886 the breathing rate during STAND with respect to REST. Data are presented as mean±standard deviation. The symbol * indicates $p<0.05$ versus 21-30 group.
887
888

889 **Table 4.** Results of the correlation of BRA markers on HPVA indexes during STAND within each age group.

Correlation	21-30		31-40		41-50		51-60		61-70	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
BRS_{SEQ+} - BRS_{SEQ-} vs PI_{HP}	0.527	2.0×10^{-2}\$	0.639	2.4×10^{-3}\$	0.754	3.0×10^{-4}\$	0.382	1.1×10^{-1}	0.563	3.6×10^{-2}\$
(BRS_{SEQ+} - BRS_{SEQ-}) / BRS_{SEQ-} vs PI_{HP}	0.471	4.2×10^{-2}\$	0.700	5.9×10^{-4}\$	0.565	1.5×10^{-2}\$	0.417	7.5×10^{-2}	0.545	4.4×10^{-2}\$
BRS_{SEQ+} - BRS_{SEQ-} vs GI_{HP}	0.406	8.5×10^{-2}	0.384	9.4×10^{-2}	0.823	2.7×10^{-5}\$	0.324	1.8×10^{-1}	0.294	3.1×10^{-1}
(BRS_{SEQ+} - BRS_{SEQ-}) / BRS_{SEQ-} vs GI_{HP}	0.425	6.9×10^{-2}	0.422	6.4×10^{-2}	0.701	1.2×10^{-3}\$	0.343	1.5×10^{-1}	0.303	2.9×10^{-1}

890 21-30, 31-40, 41-50, 51-60, 61-70: min-max range expressed in yrs; BR: baroreflex; BRS: BR sensitivity; BRA: BR asymmetry; SEQ: sequence method; BRS_{SEQ+}: BRS computed
891 via the SEQ method over positive SAP variations; BRS_{SEQ-}: BRS computed via the SEQ method over negative SAP variations; PI_{HP}: Porta's index computed over HP series; GI_{HP}:
892 Guzik's index computed over HP series; *r*: Pearson product moment correlation coefficient; *p*: type I error probability. The symbol \$ indicates a significant association with $p<0.05$.
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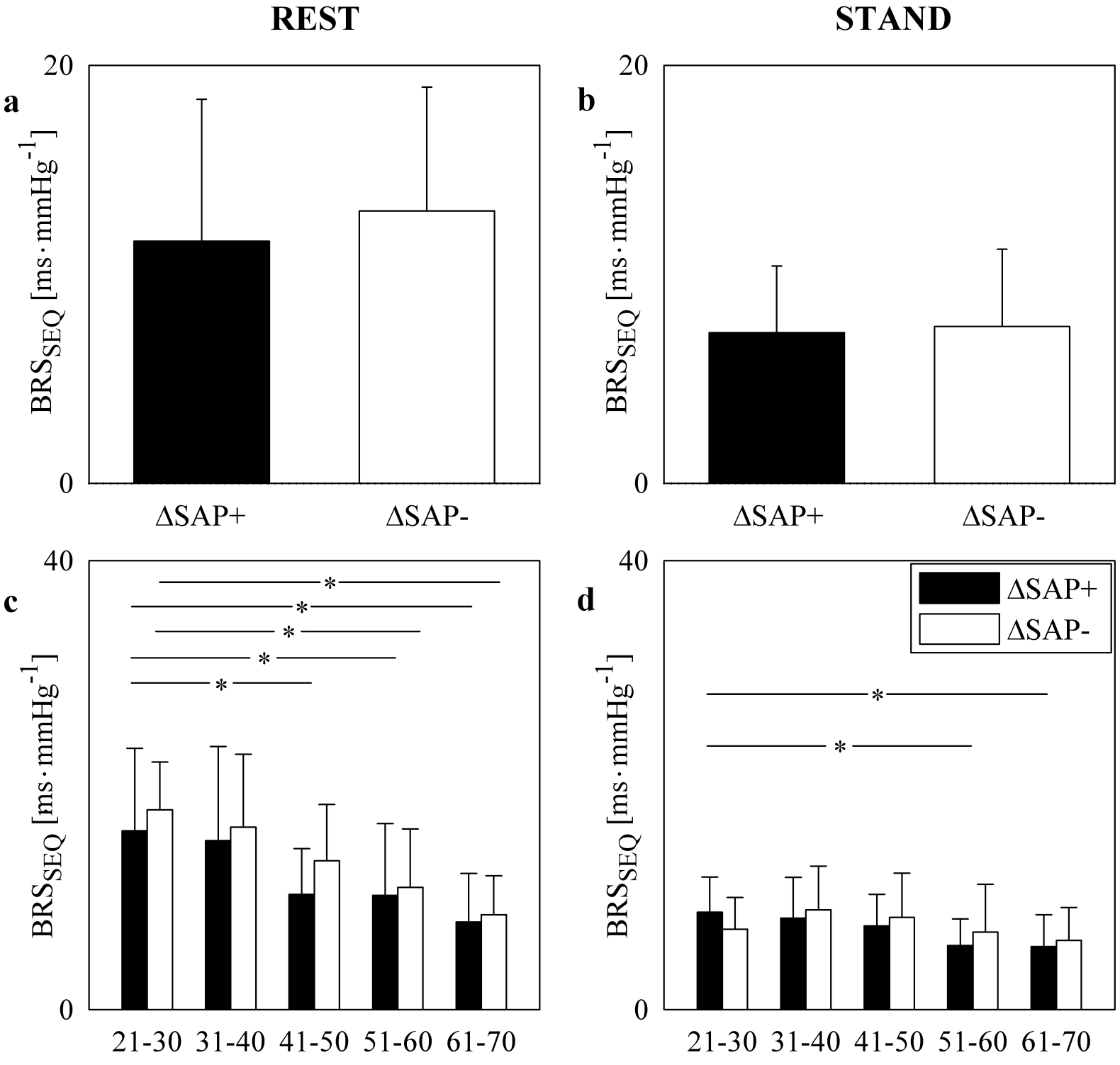
REST
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BRS_SEQ [ms · mmHg^-1]
ΔSAP+
ΔSAP-
21-30
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41-50
51-60
61-70
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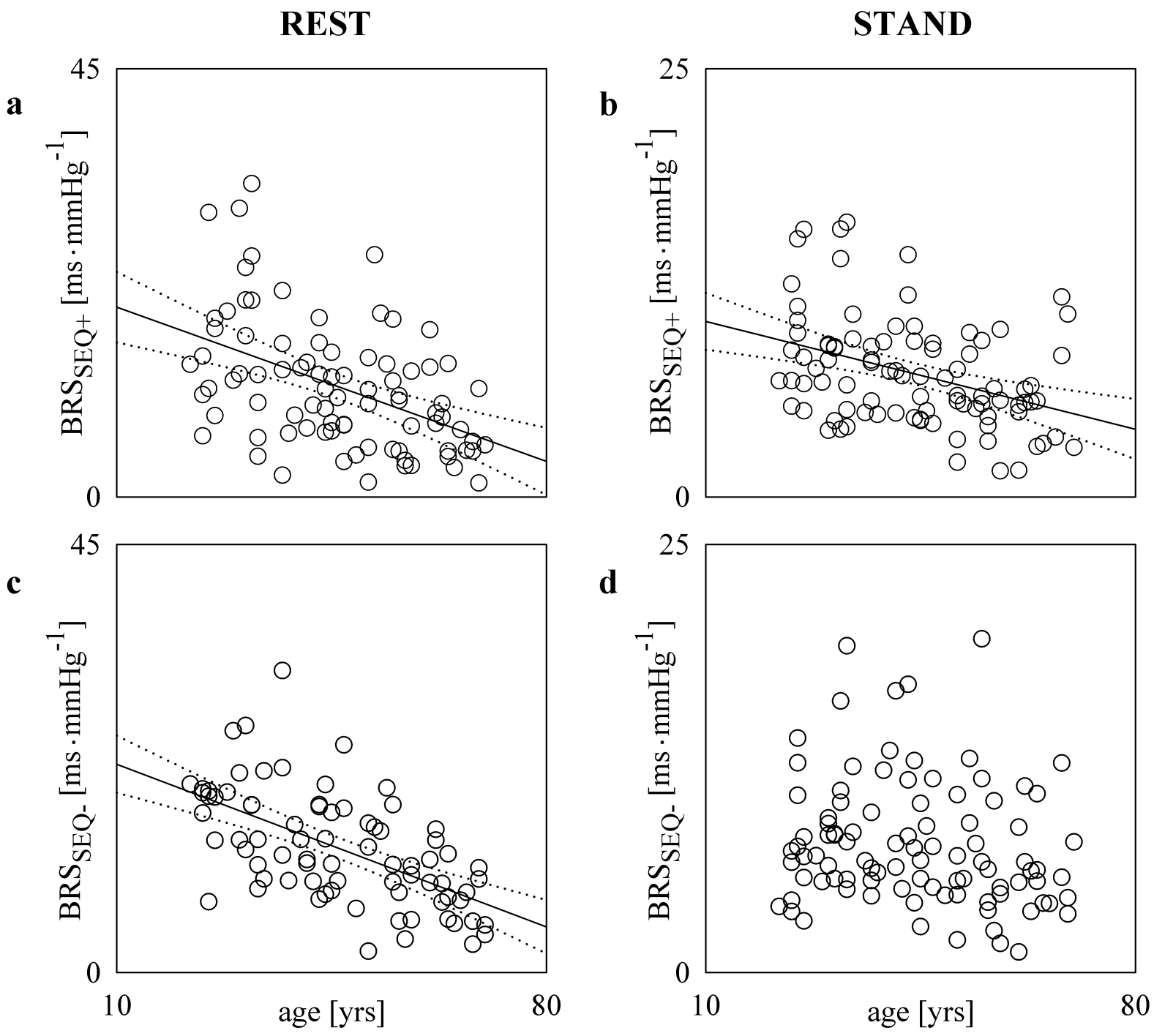
REST
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age [yrs]
BRS_SEQ+ [ms·mmHg^-1]
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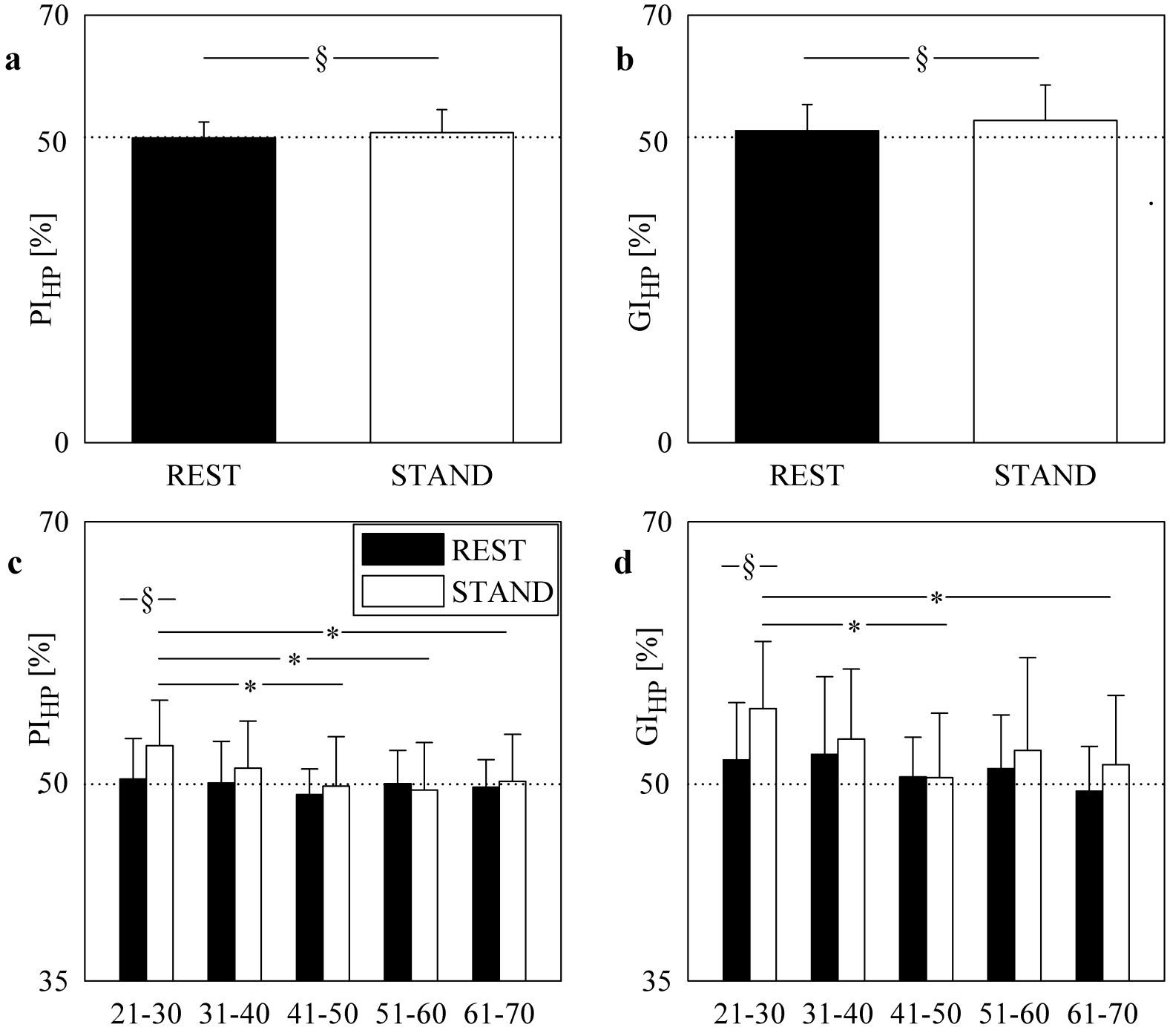
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REST
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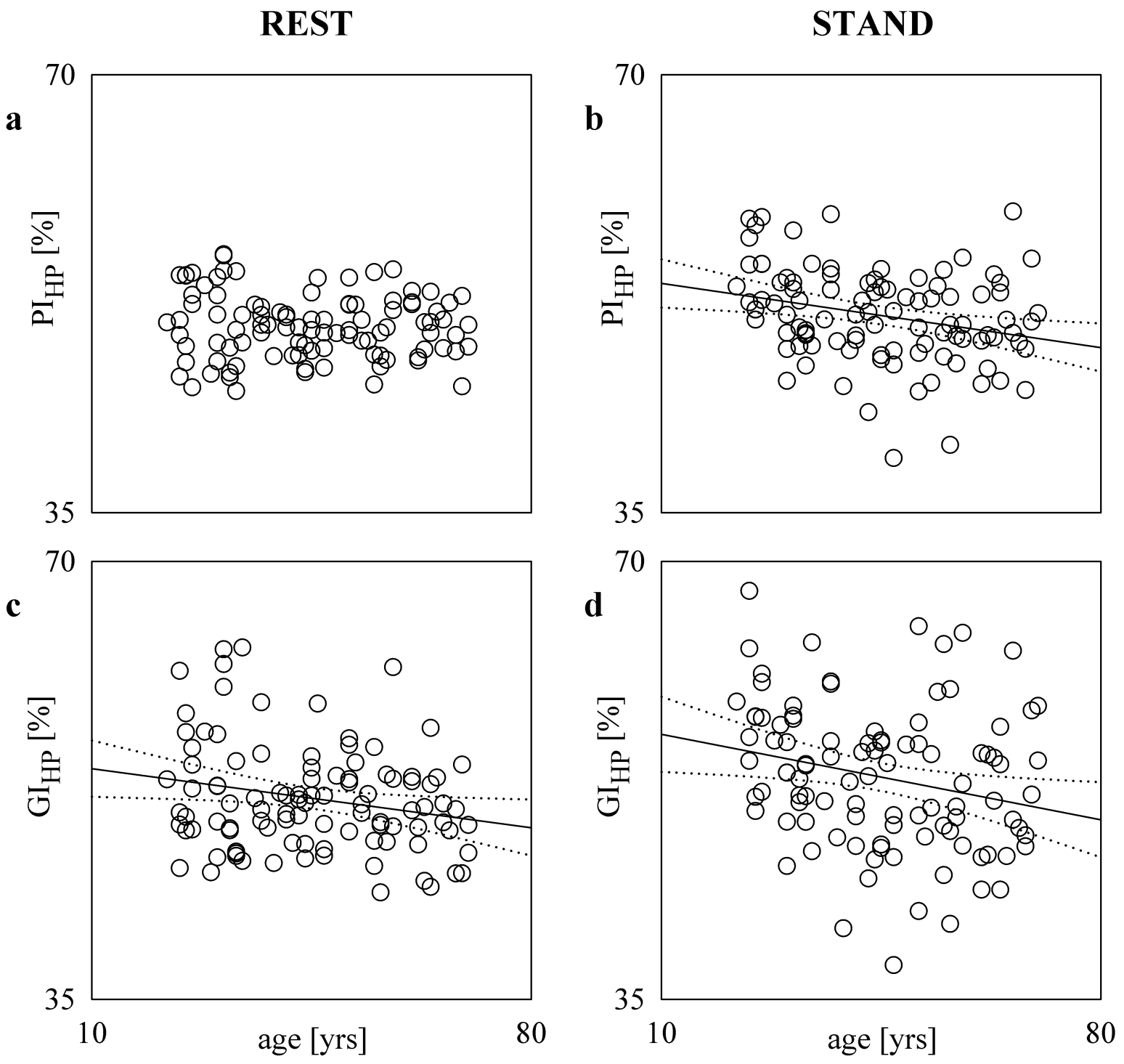
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age [yrs]
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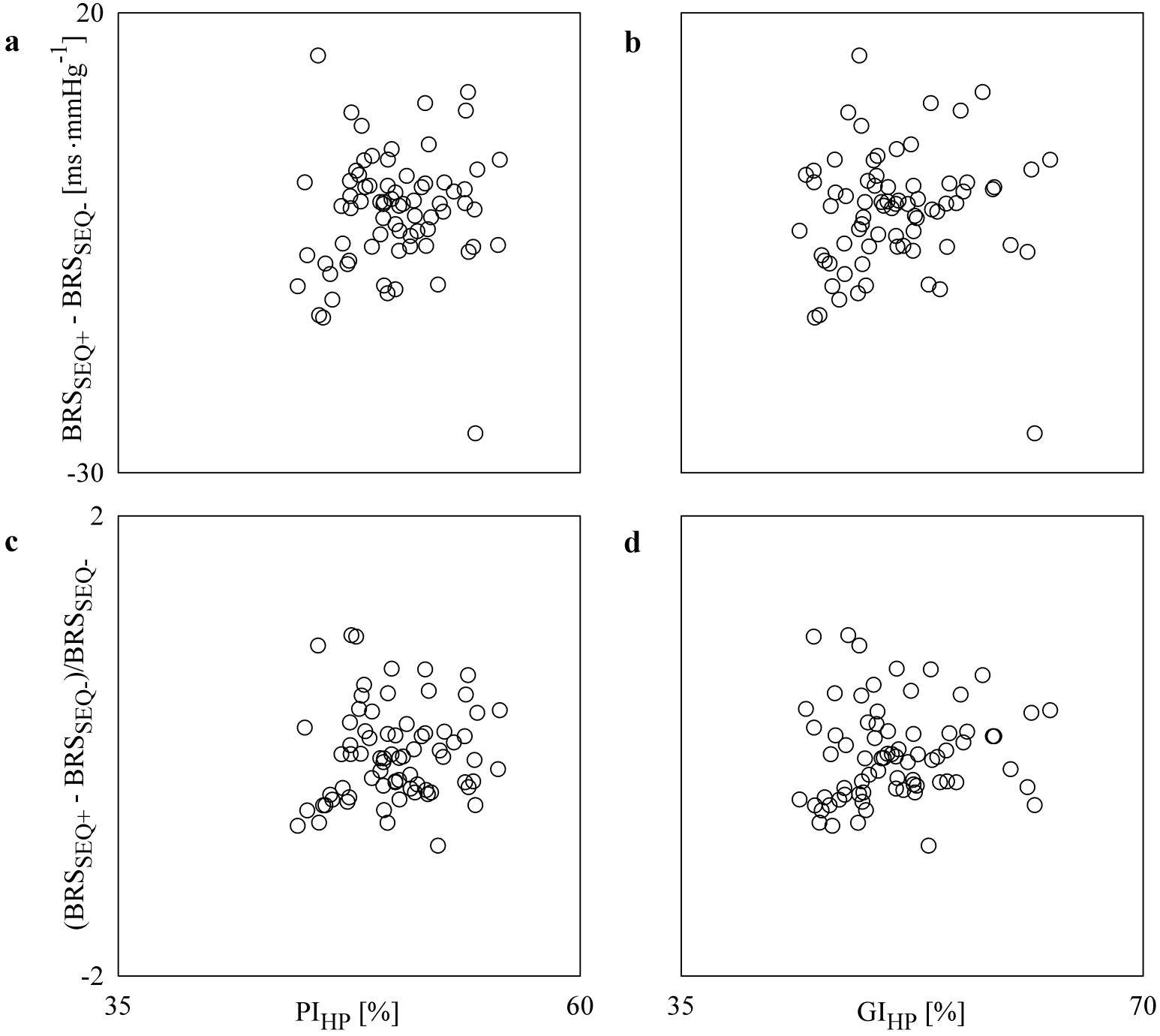
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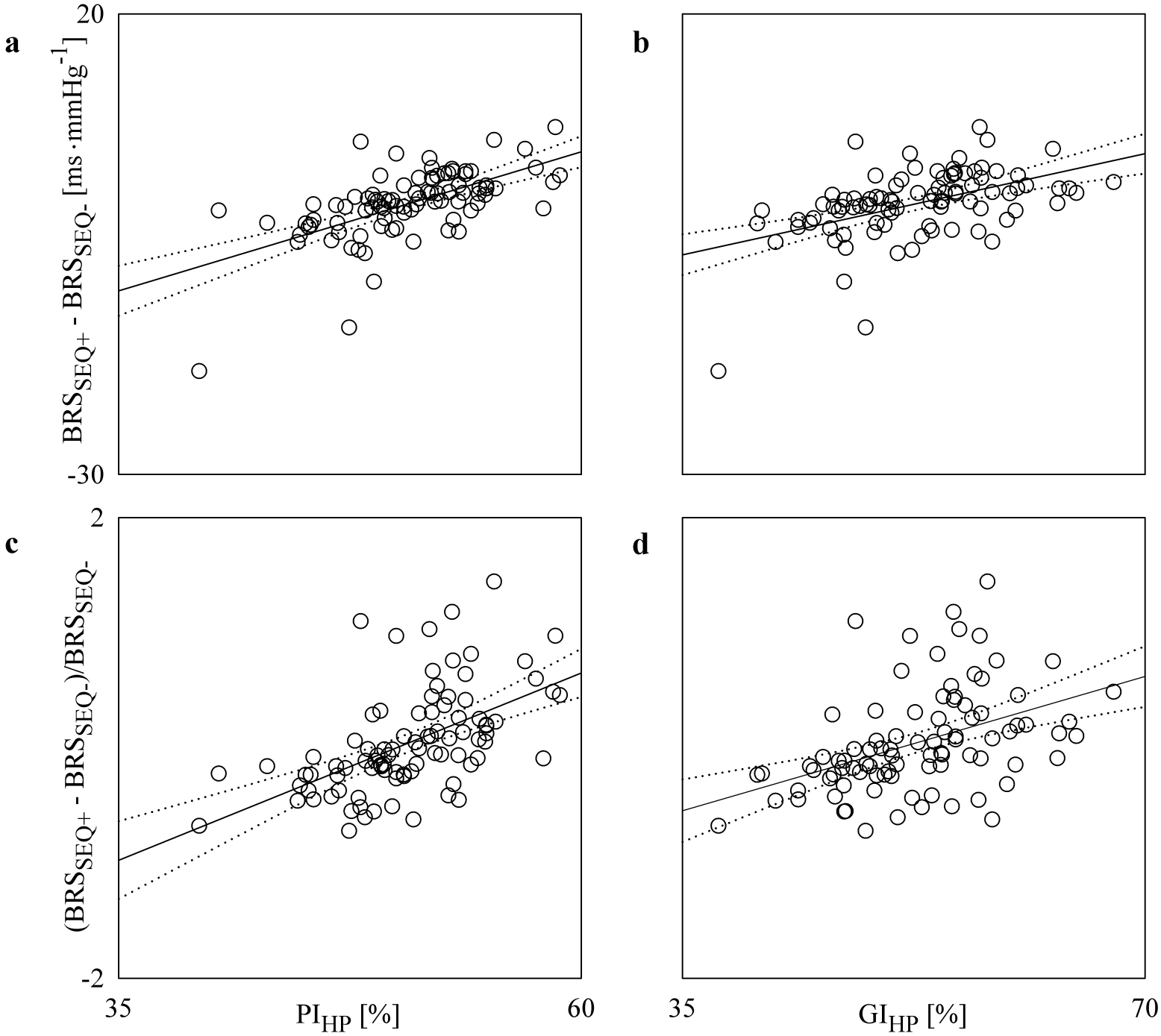
BRS_{SEQ+} - BRS_{SEQ-} [ms · mmHg^{-1}]
(BRS_{SEQ+} - BRS_{SEQ-})/BRS_{SEQ-}
PI_{HP} [%]
GI_{HP} [%]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BRS_{SEQ+} - BRS_{SEQ-} [ms·mmHg^{-1}]
(BRS_{SEQ+} - BRS_{SEQ-})/BRS_{SEQ-}
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PI_{HP} [%]
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GI_{HP} [%]