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Measuring postural-related changes of spontaneous baroreflex sensitivity after repeated long-duration diving: Frequency domain approaches

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ABSTRACT

Sustained water immersion is thought to modulate orthostatic tolerance to an extent dependent on the duration and repetition over consecutive days of the diving sessions. We tested this hypothesis investigating in ten healthy subjects the potential changes in the cardiovascular response to head-up tilt induced by single and multiple resting air dives. Parametric cross-spectral analysis of spontaneous RR interval and systolic arterial pressure variability was performed in three experimental sessions: before diving (BD), after single 6-hour dive (ASD), and after multiple 6-hour dives (AMD, 5 consecutive days with 18-hour surface interval). From this analysis, baroreflex sensitivity (BRS) was computed as spectral power ratio (αBRS), non-causal transfer function gain (tfBRS) and causal transfer function gain (γBRS) evaluated at low frequency (0.04–0.14 Hz) in the supine position (*su*) as well as in the standing upright position in the early tilt (*et*) and late tilt (*lt*) epochs. We found that, while αBRS decreased significantly in *et* and *lt* compared to *su* during all sessions, tfBRS and γBRS decreased during ASD and AMD but not during BD; moreover γBRS evidenced a progressive decrease from BD to ASD and to AMD in both *et* and *lt* epochs. These results indicate the necessity of following a causal approach for the estimation of BRS in the frequency domain, and suggest a progressive impairment of the baroreflex response to postural stress after single and multiple dives, which may reflect symptoms of increasing orthostatic intolerance.

1. Introduction

Scuba divers are exposed to uncommon environmental conditions, including increased ambient pressure, raised partial pressure of oxygen, increased resistance to movement, cold stress, and others, which produce many physiological effects. While most studies in the literature have focused on physiological changes that occur during immersion (see, e.g., Doubt, 1996), less is known about potential alterations remaining after prolonged water immersion. Some reports have associated long-duration diving with fatigue and/or exercise intolerance (Shykoff, 2008) and with alterations of cardiovascular and autonomic functions which may predispose to orthostatic intolerance (Greenleaf et al., 1988). Characterizing the physiological alterations that follow long-duration immersion is thus an important issue, which may enable interventions aimed at reducing the related impairment of the divers' physiological status.

The adverse consequences of prolonged immersion on autonomic function may be evaluated studying the response of the cardiovascular system to physiological stressors. One of these is the orthostatic stress provoked by passive upright standing, which results in a decrease of venous return and a tachycardic response preventing the arterial pressure drop. Since this physiological perturbation profoundly affects the autonomic neural outflow, prolonged head-up tilt testing is commonly exploited to investigate the combined cardiac and vascular responses to postural stress (Eckberg, 1997). In this context, the short-term cardiovascular regulation can be assessed noninvasively from the spontaneous oscillations of variables such as the systolic arterial pressure (SAP) and the heart period (RR interval of the ECG), measured on a beat-by-beat basis over short temporal scales (up to few minutes). In particular, as postural circulatory stress elicits baroreceptor unloading, evaluation of the baroreflex sensitivity (BRS) from spontaneous RR and SAP variability is of particular interest for the identification of cardiovascular symptoms of orthostatic intolerance (Faes et al., 2006; Westerhof et al., 2006). Among the variety of methods proposed for the estimation of spontaneous BRS (Laude et al., 2004), frequency domain approaches (Robbe et al., 1987; Pagani et al., 1988; Pinna et al., 2002; Faes et al., 2004) allow one to focus the analysis on specific RR and SAP oscillatory components such as the low-frequency rhythm

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(LF: 0.04–0.14 Hz), in order to avoid the confounding effects of other variables (e.g., respiration) operating at different frequencies.

The present study performs a comparative assessment of the capability of different frequency domain BRS estimation methods to detect postural-related changes of baroreflex sensitivity and their potential modification after prolonged water immersion. To this end, three different BRS measures were computed after fitting a bivariate parametric model to the RR and SAP series measured during subsequent epochs of tilt experiments, executed in a group of divers before diving, after a single 6-hour dive, and after multiple 6-hour dives. The comparison of the three measures computed in these different conditions was aimed at (i) relating the methodological assumptions underlying different indexes to the capability of reflecting baroreflex alterations induced by orthostatic stress, and (ii) investigating potential alterations of cardiovascular response to postural stress induced by prolonged water immersion.

2. Methods

2.1. Subjects and experimental protocol

The study population consisted of ten healthy, physically fit US Navy personnel (age: 34 ± 3 years; BMI: 26 ± 0 kg/m^2; mean ± SEM) who underwent three tilt testing experiments: before diving (BD), after a single dive (ASD), and after multiple dives (AMD). Each dive was a resting dive at 135 kPa with air as breathing gas, and lasted 6 h. Multiple dives were performed over five consecutive days (with 18 hour surface interval between dives). BD, ASD and AMD experiments were performed before the first dive, after the first dive, and after the fifth dive, respectively. For each experiment, the protocol consisted in subject stabilization, 8 min of data acquisition in the resting supine position, and a further period (up to 15 min) of data acquisition in the 70° upright position after passive head-up tilting. After recording ECG (lead II) and noninvasive finger arterial pressure (Finometer Pro, Finapres Medical Systems) with a 200 Hz sampling rate, the beat-to-beat variability series of RR interval and SAP were off-line measured on a beat-by-beat basis. Three stationary and artifact-free windows, each of ~5 min duration, were then selected in correspondence with specific epochs of the test: the first in the supine position (*su*); the second in the upright position at early tilt (*et*), i.e., starting around 1 min after the transient phase of head-up tilting; and the third in the upright position at late tilt (*lt*), i.e., starting around 10 min after head-up tilting.

According to the guidelines for cardiovascular variability analysis, which suggest to perform parametric spectral analysis using beat-to-beat sequences despite their unevenly sampled nature (Task Force, 1996), we did not interpolate and resample uniformly the original measured time series. While this choice is consistent with several previous studies (e.g., Pagani et al., 1988; Porta et al., 2002; Faes et al., 2004; Nollo et al., 2005; Faes et al., 2006), it has been shown that spectral estimations performed on beat-to-beat and resampled time series are substantially equivalent (Congi et al., 1998); in our study, keeping the original sequences allowed us also to set causally meaningful zero-lag effects between RR and SAP (see Section 2.2).

2.2. Parametric cross-spectral analysis

Each pair of observed RR interval and SAP variability series, respectively denoted as $r(n)$ and $s(n)$ in the following, was taken as a realization of a bivariate autoregressive (AR) process described as:

$$\mathbf{Y}(n) = \sum_{k=0}^{p} \mathbf{A}(k)\mathbf{Y}(n-k) + \mathbf{U}(n), \tag{1}$$

where $\mathbf{Y}(n) = [s(n),\ r(n)]^{\mathrm{T}}$, $\mathbf{U}(n) = [u_s(n),\ u_r(n)]^{\mathrm{T}}$ is a vector of white and uncorrelated input series (the superscript $^{\mathrm{T}}$ is the matrix transpose operator), and $\mathbf{A}(k) = [\mathbf{a}_1(k)\ \mathbf{a}_2(k)]$ is a 2×2 matrix with $\mathbf{a}_1(k) = [a_{ss}(k)\ \ a_{rs}(k)]^{\mathrm{T}}$, $\mathbf{a}_2(k) = [a_{sr}(k)\ \ a_{rr}(k)]^{\mathrm{T}}$, where $a_{ss}(k)$ and $a_{rr}(k)$ describe self-dependencies (respectively, of $s(n)$ on $s(n–k)$ and of $r(n)$ on $r(n–k)$), while $a_{rs}(k)$ and $a_{sr}(k)$ describe cross-dependencies (respectively, of $r(n)$ on $s(n–k)$ and of $s(n)$ on $r(n–k)$). Identification of the model (1) was performed according to the approach described in Faes et al. (2012). Specifically, we used a vector least-squares approach to estimate the coefficient matrix $\mathbf{A}(k)$ and the covariance matrix of the model inputs. To guarantee model identifiability we set $\mathbf{A}(0) = 0$ except for $a_{rs}(0) \neq 0$, so that nonzero instantaneous effects were allowed from SAP to RR (accounting for fast baroreflex interactions) but not from RR to SAP (discarding non-physiological effects). The model order p was set according to the multivariate version of the Akaike criterion.

After model identification, the estimated coefficients were represented in the frequency domain to describe the variability and interactions between the two series in different spectral bands. Taking the Fourier Transform (FT) of the representation in Eq. (1), we obtained the frequency domain representation $\mathbf{Y}(f) = \mathbf{H}(f)\mathbf{U}(f)$, where $\mathbf{Y}(f)$ and $\mathbf{U}(f)$ are the FTs of $\mathbf{Y}(n)$ and $\mathbf{U}(n)$, and $\mathbf{H}(f)$ is the transfer matrix

$$\mathbf{H}(f) = \begin{bmatrix} H_{ss}(f) & H_{sr}(f) \\ H_{rs}(f) & H_{rr}(f) \end{bmatrix} = \left(\mathbf{I} - \sum_{k=0}^{p} \mathbf{A}(k) e^{-j2\pi fk} \right)^{-1}, \tag{2}$$

where $\mathbf{I}$ is the 2×2 identity matrix. The transfer matrix was exploited to derive the spectral density matrix of the process, $\mathbf{S}(f) = \mathbf{H}(f)\Sigma\mathbf{H}^{*}(f)$, where Σ is the covariance matrix of $\mathbf{U}(n)$ and * stands for Hermitian transpose. The spectral density matrix contains the autospectra of SAP and RR interval, $S_s(f)$ and $S_r(f)$, as diagonal terms, and the cross-spectra between SAP and RR, $S_{sr}(f)$ and $S_{rs}(f) = S_{sr}{}^{*}(f)$, as off-diagonal terms. The elements of the spectral and transfer matrices were then combined to quantify in the frequency domain the coupling between RR and SAP by means of the classical coherence function

$$|\Gamma_{rs}(f)|^2 = \frac{|S_{rs}(f)|^2}{S_s(f)S_r(f)}, \tag{3}$$

and the causal coupling over the feedback baroreflex direction from SAP to RR by means of the causal coherence (Porta et al., 2002; Faes et al., 2004)

$$|\gamma_{rs}(f)|^2 = \frac{\sigma_s^2 |H_{rs}(f)|^2}{S_r(f)}, \tag{4}$$

where $\sigma_s{}^2$ is the variance of $u_s(n)$.

2.3. Frequency domain assessment of baroreflex sensitivity

Exploiting the spectral functions defined in the previous subsection, we followed different approaches to derive frequency domain estimates of the spontaneous BRS. According to the alpha BRS method (αBRS) proposed by Pagani et al. (1988), we estimated the BRS at each frequency f as the ratio of the RR spectral power to the SAP spectral power:

$$G_{\alpha}(f) \equiv \sqrt{\frac{S_r(f)}{S_s(f)}}. \tag{5}$$

According to the so-called transfer function BRS method (tfBRS) first proposed by Robbe et al. (1987), a second estimate of the BRS was taken as the gain of the transfer function from SAP to RR:

$$G_{\mathrm{TF}}(f) \equiv \frac{|S_{rs}(f)|}{S_s(f)} = \sqrt{\frac{S_r(f)|\Gamma_{rs}(f)|^2}{S_s(f)}}, \tag{6}$$

where the last term is obtained by combining the second term with Eq. (3), and contains the quantity $S_{r|s,r}(f) \equiv S_r(f)|\Gamma_{rs}(f)|^2$, that is the part of the RR spectrum $S_r(f)$ which is shared with SAP. As a third estimate of the BRS in the frequency domain we propose the utilization of a different approach, denoted as gamma BRS method (γBRS), whereby BRS is computed at each frequency as

$$G_\gamma(f) \equiv \sqrt{\frac{S_r(f)|\gamma_{rs}(f)|^2}{S_s(f)}}. \quad (7)$$

The difference between tfBRS and γBRS is that in Eq. (7) the RR spectral density is weighted by the causal coherence $|\gamma_{rs}(f)|^2$, while the ordinary coherence $|\Gamma_{rs}(f)|^2$ is used in Eq. (6). In particular, the quantity $S_{r|s}(f) \equiv S_r(f)|\gamma_{rs}(f)|^2$ can be viewed as the part of the RR spectrum $S_r(f)$ causally due to the variability of SAP.

All spectral measures defined in Eqs. (5), (6), and (7) quantify BRS as the gain of the transfer function from SAP to RR interval variability. The difference between the three measures stands in the implicit assumptions which underlie their computation, and consequently pose theoretical constraints to their applicability. Since the αBRS measure defined in Eq. (5) is computed considering only the individual spectral densities of RR and SAP, the underlying assumption is that the whole variability of RR is driven by SAP. As a consequence, the index $G_\alpha(f)$ tends to be higher than the true BRS when part of the measured RR variability is actually originated by sources other than SAP variability. The tfBRS measure defined in Eq. (6) is based on a more general assumption, as its computation provides for possible amounts of RR variability due to sources other than SAP variability; this is achieved by considering the portion of RR variability shared with SAP, instead of the whole RR variability, as the numerator of the gain index $G_{TF}(f)$. Nevertheless, both αBRS and tfBRS measures may be misleading if the two observed variables interact in a closed loop, i.e., if feedforward effects from RR to SAP are present in addition to the feedback effects from SAP to RR which constitute the subject of baroreflex analysis. When this is the case, effects occurring along both pathways contribute to determine the spectral densities appearing at the numerator of Eqs. (5) and (6) and, as a result, baroreflex feedback influences are mixed up with non-baroreflex feedforward influences in the αBRS and tfBRS measures. From this point of view, αBRS and tfBRS are noncausal gain indexes because they cannot extract effects over the causal direction of interest. On the other hand, the γBRS measure can be assumed as causal index of baroreflex gain, because its definition specifically focuses on the pathway from SAP to RR in the computation of BRS. This is possible thanks to the utilization of the causal coherence, which allows considering only the portion of the RR spectrum caused by SAP ($S_{r|s}(f)$ in Eq. (7)) in the definition of the gain index $G_\gamma(f)$. In fact, the γBRS method is the sole method which exploits the causal information contained in the coefficients of the parametric AR model, while the αBRS and tfBRS methods involve only quantities (i.e., the autospectra and the ordinary coherence) that do not bring causal information and –as such– could equally be computed through non-parametric spectral and cross-spectral analyses (see, e.g., Pinna and Maestri, 2001).

The properties illustrated above are reflected in the following relations linking the three BRS measures: $G_{TF}(f) = G_\alpha(f)|\Gamma_{rs}(f)|$; $G_\gamma(f) = G_\alpha(f)|\gamma_{rs}(f)|$; and $G_\gamma(f) = G_{TF}(f)|\gamma_{rs}(f)|/|\Gamma_{rs}(f)|$. Indeed, since coherence and causal coherence are bounded between 0 and 1, it follows that $G_{TF}(f) \le G_\alpha(f)$ and $G_\gamma(f) \le G_\alpha(f)$, indicating that the alpha method tends to overestimate the gain in the absence of full correlation between SAP and RR; moreover, as the causal coherence tends to be lower than the coherence (though the relation does not hold strictly), we expect $G_\gamma(f)$ to take lower values than $G_{TF}(f)$, reflecting the alterations in the gain produced by the possible existence of closed-loop interactions between RR and SAP.

An example of the overall analysis is reported in Fig. 1. In accordance with the whole-band average approach (Pinna et al., 2002), the estimated spectral density functions were integrated to give power values, and the coherence and gain functions averaged to give overall values, within the LF band (0.04–0.14 Hz) of the frequency spectrum. Estimates in the high frequency band of the spectrum (HF, ±0.04 Hz around the respiratory frequency) were not considered because in this band the oscillations of RR and SAP are strongly affected by respiration and thus any bivariate RR–SAP analysis would yield biased estimates of coupling and gain indexes (Porta et al., 2000; Cevese et al., 2001; Faes et al., 2011).

2.4. Statistical analysis

For each computed index (i.e., S_s(LF), S_r(LF), $|\Gamma_{rs}(LF)|^2$, $|\gamma_{rs}(LF)|^2$, G_α(LF), G_{TF}(LF), G_γ(LF)), the statistical analysis was performed as follows. At fixed acquisition time (BD, ASD or AMD), the significance of changes across the three experimental conditions (*su*, *et*, *lt*) was assessed using the Kruskal Wallis ANOVA, followed by paired Wilcoxon sign rank post-hoc test for comparing pairs of distributions. The same statistical procedure was followed to test the significance of changes across the three acquisition times (BD, ASD, AMD) at fixed experimental condition (*su*, *et*, or *lt*). All statistical tests were performed with 5% significance; multiple comparisons in the post-hoc tests were compensated using the Bonferroni correction.

3. Results

Table 1 reports the median (25th–75th percentile) of the distributions of mean and variance of RR and SAP in the considered group of divers. At all acquisition times (BD, ASD, AMD), values documented the response to head-up tilt, showing a decrease of the mean SAP during *et* and *lt* compared with *su*, counteracted by a significant decrease in the mean RR; after multiple dives, the mean SAP decreased significantly also when moving from *et* to *lt*. The hypotension and tachycardia effects induced by tilt were emphasized after a single dive and even more after multiple dives, as documented by the significant reductions in the mean of SAP and RR observed moving from BD to ASD and to AMD during the *et* and *lt* conditions. The tilt transition was characterized also by a significant reduction in the overall RR variability, and by an increase of the SAP variability that was statistically significant at *lt* after one dive and at *et* and *lt* after many dives.

Fig. 2 depicts the results of power spectral density analysis performed at LF in the various conditions. While the RR interval power did not change significantly across the tilt test epochs, the power of the SAP series resulted significantly higher during *et* and *lt* windows compared to the *su* window. These results held for all acquisition times (BD, ASD, AMD). No statistically significant differences were found across the three acquisition times at any fixed experimental condition.

Fig. 3 reports the results of RR–SAP coupling and causal coupling analyses. The coherence $|\Gamma_{rs}(LF)|^2$ displayed high values in all windows, documenting the existence of a significant coupling between SAP and RR interval in all experimental conditions and acquisition times. By contrast, the causal coherence from SAP to RR, $|\gamma_{rs}(LF)|^2$, increased moving from the *su* to the *et* window, and remained at high values during the *lt* window; the increase in the upright position was statistically significant before diving, while it did not reach statistical significance after one dive, nor in the *et* window after multiple dives. Both traditional and causal coherence displayed no statistically significant differences across the three acquisition times at any fixed experimental condition.

Fig. 4 summarizes the results of BRS frequency domain analysis performed in the LF band by means of the three methods described in Section 2.3. When the gain was computed using the αBRS method, the index G_α(LF) decreased significantly moving from *su* to *et*, and remained at low values during *lt*; this trend was observed at all

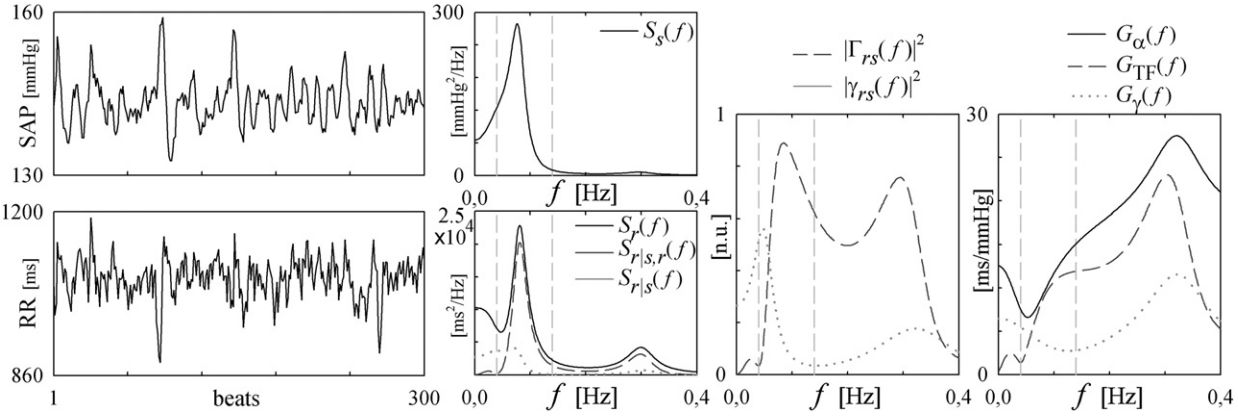

Fig. 1. Example of SAP and RR interval variability series measured for a resting subject in the supine position before diving, and corresponding plots derived from cross-spectral analysis: power spectral density of SAP, $S_s(f)$; power spectral density of RR interval, $S_r(f)$, plotted with its portion shared with SAP, $S_{r|s,r}(f)$, and its portion causally due to SAP, $S_{r|s}(f)$; coherence between SAP and RR interval, $|\Gamma_{sr}(f)|^2$, plotted with the causal coherence from SAP to RR interval, $|\gamma_{rs}(f)|^2$; transfer function gain estimated with the alpha BRS method, $G_\alpha(f)$, with the transfer function BRS method, $G_{TF}(f)$, and with the gamma BRS method, $G_\gamma(f)$. Vertical dashed lines in each spectral plot denote the bounds of the LF band (0.04–0.14 Hz).

acquisition times. In contrast, when estimated by tfBRS and γBRS methods, the baroreflex index did not display statistically significant variations across windows before diving; while the index G_{TF}(LF) displayed just a non-significant tendency to decrease (ANOVA $p = 0.09$), the index G_γ(LF) did not exhibit any significant change (ANOVA $p = 0.44$). After diving, the two indexes showed a significant decrease in the upright position (ANOVA $p < 0.05$). Specifically, after a single dive this decrease with respect to *su* was statistically significant in both *et* and *lt* windows for G_{TF}(LF), and only in the *lt* window for G_γ(LF); after multiple dives, the decrease was statistically significant in both windows for G_{TF}(LF) and G_γ(LF). The gain measures showed also significant variations at varying acquisition times for fixed experimental conditions. Specifically, in the upright position (*et* and *lt* conditions) all measures were significantly lower after multiple dives than before diving. Moreover, the γBRS method evidenced also a reduction in the gain moving from BD to ASD in the *lt* window, and moving from ASD to AMD in the *et* window.

4. Discussion and conclusions

In this paper a bivariate causal cross-spectral analysis of RR interval and SAP variability was applied to characterize potential changes in the cardiovascular response induced by head-up tilt after single and multiple diving sessions. The comparison of causal and non-causal indexes of baroreflex gain under different physiological conditions provided significant methodological and physiological insights. First, with the utilization of the proposed causal approach to BRS estimation a decrease of the gain could be truly considered as a loss of sensitivity, while traditional non-causal approaches tended to misinterpret the tilt-induced sympathetic activation as impaired BRS. Second, we evidenced a progressive impairment of the cardiovascular response to orthostatic stress after diving, characterized by lower arterial pressure values despite increased tachycardia rates, and a significant deterioration of baroreflex coupling and sensitivity.

4.1. Assessment of baroreflex sensitivity

In this work the sensitivity of the spontaneous baroreflex control of heart rate was assessed by spectral techniques. With respect to time-domain and sequence methods, frequency-domain approaches have the advantage to allow the distinction of the oscillatory components that contribute to baroreflex sensitivity. As suggested in previous studies (Robbe et al., 1987; Cevese et al., 2001; Pinna et al., 2002) the analysis of baroreflex gain was thus focused on the LF band, in order to limit the presence of exogenous disturbances (non-baroreflex-mediated) on the RR series, and in particular to exclude spurious RR–SAP coupling arising from respiratory modulation, affecting SAP mechanically and RR centrally.

We compared the performance of three BRS measures, evidencing a different sensitivity of the methods to depict changes induced by tilting maneuver and/or prolonged water immersion. The different behaviors of the three measures can be explained in light of the different assumptions on the underlying physiological system. In this study we observed a significant increase of the SAP LF power with

Table 1
Modification of body weight and cardiovascular variables with prolonged water immersion.

		BD	*ASD*	*AMD*
Weight [kg]		84.9 (82.6–88.1)	83.5 (81.2–86.5)#	83.8 (81.3–85.9)#
μ_{RR} [ms]	*su*	1163 (1075–1277)	1187 (1088–1290)	1114 (985–1183)
	Et	929 (797–1064)*	788 (745–830)*	728 (676–741)*#^
	Lt	920 (761–953)*	730 (673–804)*#	689 (616–766)*#^
μ_{SAP} [mm Hg]	*Su*	131.5 (128.6–139.2)	127.1 (116.9–130.5)#	126.4 (120.6–127.5)#
	Et	120.9 (117.9–123.7)*	106.2 (95.5–116.1)*#	106.3 (99.7–110.2)*#
	Lt	117.5 (114.8–120.8)*	101.9 (95.4–111.9)*#	102.0 (97.0–103.9)*§#
σ^2_{RR} [ms^2]	*Su*	9642 (2529–16,961)	6572 (3571–12,478)	5857 (2865–7088)
	Et	3735 (1901–4127)*	2067 (1914–3419)*	1868 (819–3196)*
	Lt	3169 (2519–3988)*	2338 (1215–4300)*	1985 (618–3904)*
σ^2_{SAP} [mm Hg^2]	*Su*	16.1 (12.0–20.4)	9.8 (8.8–18.4)	9.7 (8.3–11.5)
	Et	19.2 (10.8–26.1)	27.1 (14.13–34.0)	25.9 (21.3–35.3)*
	Lt	19.9 (16.4–27.4)	28.6 (24.4–36.4)*	25.3 (22.9–33.8)*

Values are median (first quartile–third quartile) over subjects of the body weight and of the cardiovascular variables (RR interval mean (μ_{RR}) and variance (σ^2_{RR}), systolic arterial pressure mean (μ_{SAP}) and variance (σ^2_{SAP})) measured in the supine (*su*), early tilt (*et*) and late tilt (*lt*) phases of the testing protocol executed before diving (BD), after a single dive (ASD), and after multiple dives (AMD). Statistically significant differences: *, *et* vs. *su* or *lt* vs. *su*; §, *lt* vs. *et*. #, ASD vs. BD or AMD vs BD; ^, AMD vs. ASD.

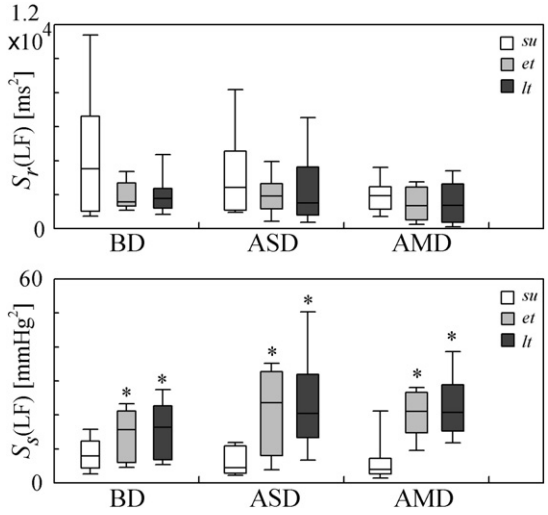

Fig. 2. Distributions over subjects of the spectral power in the LF band computed for RR interval (S_r(LF)) and SAP (S_s(LF)) in the supine (*su*), early tilt (*et*) and late tilt (*lt*) phases of the testing protocol executed before diving (BD), after a single dive (ASD), and after multiple dives (AMD). * statistically significant difference *et* vs. *su* or *lt* vs. *su*. For each distribution, values are median and interquartile range (box), with 10th and 90th percentiles (whiskers).

the transition from supine to upright. Although these trends can be considered as indirect markers of the sympathetic activation usually induced by upright tilt (Akselrod et al., 1985; Cooke et al., 1999; Furlan et al., 2000), they determined a drop of baroreflex gain in the upright position when estimated by the αBRS approach. Indeed, since the increase of SAP LF power was accompanied by unchanged values of the RR interval LF power, the net result according to Eq. (5) was a drop in the αBRS gain index. Therefore, the strict assumption that the whole RR variability is driven by SAP comprised in the αBRS measure led to erroneously interpret the tilt-induced increase of the LF SAP power as a marker of depressed BRS. Utilization of methods computing the part of RR variability that is shared with SAP (tfBRS) or is causally due to SAP (γBRS) helps relaxing the strong assumptions implicit in the computation of the αBRS measure. Indeed, in the present study changes of tfBRS and γBRS indexes moving from supine to upright did not reach statistical significance. In particular, in the computation of γBRS the strong increase of the SAP LF power was compensated by a marked increase of the LF causal coherence, resulting in comparable G_γ values in the supine position and in the two epochs of upright position. These results suggest that the postural stress induced by tilt evokes a larger involvement of the baroreflex, documented in this study by the increased causal coherence, that is not associated with alterations in the magnitude of the reflex RR response to SAP changes, as documented by the absence of significant changes in the γBRS index with tilt.

Moreover, since the αBRS and tfBRS methods tend to mix up baroreflex feedback effects with non-baroreflex feedforward influences, the gain estimates resulting from these approaches may be misleading where the feed-forward mechanical effects of RR on SAP are non-negligible. Previous studies have indeed provided evidence for a major role of non-baroreflex feedforward effects in determining the SAP–RR coupling in the LF band, suggesting that in physiological conditions heart rate and arterial pressure interact in a more complex closed-loop fashion (Di Rienzo et al., 2001; Nollo et al., 2005). To avoid the erroneous estimation of baroreflex gain which may arise from the mixing of feedforward and feedback couplings (Porta et al., 2000), causal coherence (Porta et al., 2002) and transfer function (Faes et al., 2004) have been previously introduced, which allow a selective assessment of coupling on the path from SAP to RR. In a previous study the inclusion of causality in the estimation of baroreflex gain made the index capable to distinguish the tilt-induced autonomic response of control subjects versus patients developing orthostatic syncope, while traditional indexes failed to detect any difference (Faes et al., 2006). Consistently in this study, causal coherence and gain measures displayed higher sensitivity in identifying postural-related cardiovascular changes after prolonged diving. Indeed, while non-causal coherence values displayed no significant changes induced by tilt or diving conditions, causal

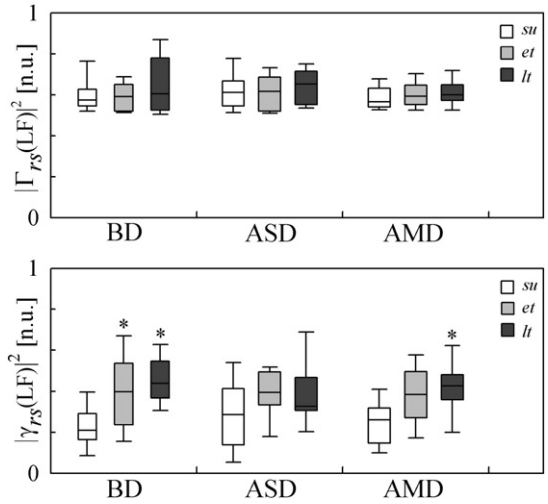

Fig. 3. Distributions over subjects of the coherence between RR and SAP ($|\Gamma_{rs}|^2$) and the causal coherence from SAP to RR ($|\gamma_{rs}|^2$) computed as average over the LF band in the supine (*su*), early tilt (*et*) and late tilt (*lt*) phases of the testing protocol executed before diving (BD), after a single dive (ASD), and after multiple dives (AMD). *, statistically significant difference *et* vs. *su* or *lt* vs. *su*. For each distribution, values are median and interquartile range (box), with 10th and 90th percentiles (whiskers).

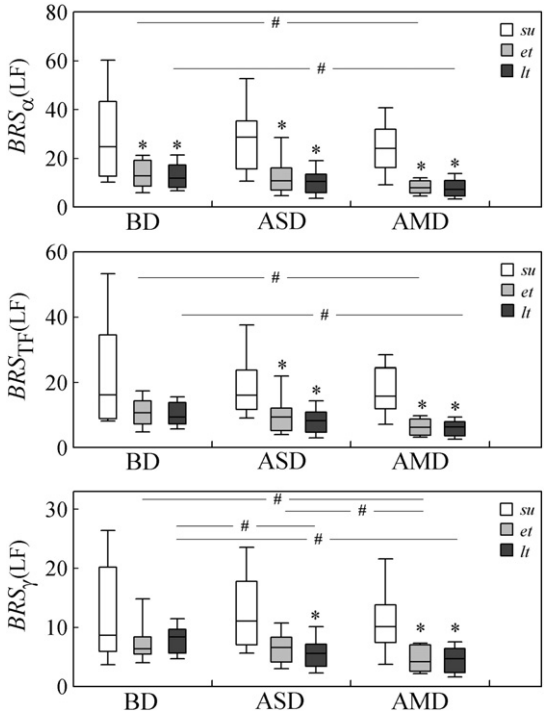

Fig. 4. Distributions over subjects of the gain computed by the alpha BRS method (G_α), the transfer function BRS method (G_{TF}), and the gamma BRS method (G_γ), computed as average over the LF band in the supine (*su*), early tilt (*et*) and late tilt (*lt*) phases of the testing protocol executed before diving (BD), after a single dive (ASD), and after multiple dives (AMD). *, statistically significant difference *et* vs. *su* or *lt* vs. *su*; #, statistically significant difference BD vs. ASD, BD vs. AMD, or ASD vs. AMD. For each distribution, values are median and interquartile range (box), with 10th and 90th percentiles (whiskers).

coherence detected a tilt-induced increase of baroreflex coupling before diving, which was lost after a single diving session (see Fig. 3). Similarly, non-causal baroreflex indexes identified a significant decrease of BRS only after multiple dives, while the causal index was able to evidence a progressive decrease of baroreflex gain after single and multiple diving sessions (see Fig. 4).

4.2. Impairment of cardiovascular control with prolonged diving

The analysis of RR–SAP series provided evidence that long duration diving significantly affected both tonic and oscillatory cardiovascular properties. In particular, after one or several 6-hour immersions the subjects displayed significantly lower systolic pressure values than before diving. This condition of hypotension occurred in all phases of the tilt testing protocol, in spite of the higher tachycardia rates displayed concurrently. Changes in tonic values were accompanied by a progressive decrease of the causal baroreflex gain measured in the upright position, which became more severe after multiple diving sessions. According to these results, we hypothesize that a deterioration of baroreflex coupling and sensitivity, evidenced in this study by causal measures of coherence and gain, may contribute to the impaired hemodynamic response to orthostatic stress after diving. This hypothesis is supported by the results of a previous study showing that proper maintenance of the upright position is reflected, in terms of cardiovascular variability, by a strict coupling among cardiac, vascular and sympathetic nerve discharge oscillations (Furlan et al., 2000). In that study, a common oscillatory pattern was clearly shown to arise between the LF variability of the sympathetic outflow and the RR and SAP target variables in the upright position, which determined increased coherence values between neural and cardiovascular series. In this perspective, the dampened response to upright tilt that we found after diving in terms of coupling and gain from SAP to RR may be interpreted as a loss of the link between cardiac, vascular, and sympathetic components which should underlie a proper reaction to orthostatic stress in physiological conditions.

The paucity of data on cardiovascular responses following diving precludes a direct comparison of our results with other studies. However the observed changes are consistent with previous studies involving surrogate conditions of immersion, such as bed rest and spaceflight, where similar microgravity adaptation processes are active. Reflex control of the circulation has been shown to be altered after cardiovascular adaptation to microgravity leading to orthostatic tachycardia and a reduced blood pressure response, after short-term spaceflights (Buckey et al., 1996), dry immersion (Iwase et al., 2000), and prolonged periods of bed rest (Kamiya et al., 2000; Pagani et al., 2001). In addition, impaired carotid-cardiac baroreflex (Convertino et al., 1990) and spontaneous arterial-cardiac baroreflex function, estimated by either transfer function (Iwasaki et al., 2000) or sequence analysis (Hughson et al., 1994; Kamiya et al., 2000; Pagani et al., 2001), have been consistently observed after spaceflight or bed-rest. Several studies have associated the impairment of baroreflex function with the microgravity-induced reduction in plasma volume (Convertino et al., 1990; Hughson et al., 1994; Buckey et al., 1996; Iwasaki et al., 2000), and the correlation has been further supported by a study demonstrating the recovery of post-bed-rest baroreflex sensitivity with restoration of plasma volume (Iwasaki et al., 2004). Consistently with these studies, we observed, together with the post-dive deterioration of baroreflex gain, a significant decrease in body weight in our subjects (see Table 1), which may be reflective of a reduction in plasma volume. While post-dive baroreflex function alterations may be mainly explained by plasma volume reduction and consequent hypovolemia, the etiology of post-dive orthostatic hypotension may be multifactorial. Previous microgravity studies suggested that, together with blood volume loss and impaired BRS, a reduced orthostatic defense may depend upon alterations in peripheral vasculature and attenuated vasoconstriction (Buckey et al., 1996), reduced adrenergic responsiveness (Convertino et al., 1998), as well as cardiac and vascular smooth muscle atrophy (Perhonen et al., 2001). Further studies are required to assess whether scuba diving may affect these factors and how these may contribute to the development of post-diving hypotension.

4.3. Perspective and conclusions

Scuba diving is one of the most rapidly growing recreational sport activities throughout the world, and involves physiological stresses which are not encountered in other sports; thus, fitness requirements and medical conditions precluding diving should be specifically assessed. Our results, showing diving-induced detrimental changes in cardiovascular and autonomic response, confirm the importance of cardiovascular aspects in the assessment of fitness to dive and may provide indications for the definition of the cardiovascular risk correlated to scuba diving (Wilmshurst, 1998). The diving-induced depression of cardiovascular response to orthostatic stress, potentially leading to the development of orthostatic intolerance, may have significant implications also for the planning, optimization and safety of military diving operations and undersea productive activities (e.g., oil and natural gas exploitation), where personnel may be required to be immersed for extended periods of time, either swimming or at rest (Pendergast and Lundgren, 2009). The induced cardiovascular alterations may indeed limit performance and result in an increased incidence of fatigue and/or exercise intolerance (Greenleaf et al., 1988; Shykoff, 2008). Further studies, involving specific tests for performance evaluation as well as different immersion conditions, are needed to depict a thorough description of diving-induced alterations and assess their implications on performance. In this context, the causal approach used in the present work may represent a sensitive and reliable tool for the quantitative assessment of cardiovascular regulatory mechanisms under different experimental conditions.

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