# RESPIRATION-RELATED CEREBRAL BLOOD FLOW VARIABILITY INCREASES DURING CONTROL-MODE NON-INVASIVE VENTILATION IN NORMOVOLEMIA AND HYPOVOLEMIA

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**Running head:** CBF variability increases during controlled ventilation

## Abstract

**Purpose** Increased variability in cerebral blood flow (CBF) predisposes to adverse cerebrovascular events. Oscillations in arterial blood pressure and $PaCO_2$ induce CBF variability. Less is known about how heart rate (HR) variability affects CBF. We experimentally reduced respiration-induced HR variability in healthy subjects, hypothesizing that CBF variability would increase.

**Methods** Internal carotid artery (ICA) blood velocity was recorded by Doppler ultrasound in ten healthy subjects during baseline, control-mode, non-invasive mechanical ventilation (NIV), i.e. with fixed respiratory rate, hypovolemia induced by lower body negative pressure, and combinations of these. ICA beat volume (ICABV) and ICA blood flow (ICABF) were calculated. HR, mean arterial blood pressure (MAP), respiratory frequency (RF), and end-tidal $CO_2$ were recorded. Integrals of power spectra at each subject's RF±0.03Hz were used to measure variability. Phase angle/coherence measured coupling between cardiovascular variables.

**Results** Control-mode NIV reduced HR variability (-56%, p=0.002) and ICABV variability (-64%, p=0.006) and increased ICABF variability (+140%, p=0.002) around RF. NIV+hypovolemia reduced variability in HR and ICABV by 70–80% (p=0.002) and doubled ICABF variability (p=0.03). MAP variability was unchanged in either condition. Respiration-induced HR and ICABV oscillations were in inverse phase and highly coherent (coherence>0.9) during baseline, but this coherence decreased during NIV, in normovolemia and hypovolemia (p= 0.01).

**Conclusion** Controlling respiration in awake healthy humans reduced HR variability and increased CBF variability in hypovolemia and normovolemia. We suggest respiration-induced HR variability to be a mechanism in CBF regulation. Maintaining spontaneous respiration in patients receiving ventilatory support may be beneficial also for cerebral circulatory purposes.


**Key words:** internal carotid artery**,** cerebral blood flow, respiration, heart rate variability, positive pressure ventilation, hypovolemia.

**Abbreviation list**

| | |
|---|---|
| CBF | cerebral blood flow |
| $ETCO_2$ | end-tidal carbon dioxide |
| HF | high frequency |
| HR | heart rate |
| HRV | heart rate variability |
| ICA | internal carotid artery |
| ICABF | internal carotid artery blood flow |
| ICABV | internal carotid artery beat volume |
| MAP | mean arterial pressure |
| NIV | non-invasive ventilation |
| $PaCO_2$ | arterial partial pressure of carbon dioxide |
| PEEP | positive end expiratory pressure |
| RF | respiratory frequency |
| RSA | respiratory sinus arrhythmia |
| 95% CI | 95% confidence intervals |

## Introduction

Cerebral blood flow (CBF) preservation is an important clinical target in critically ill patients. Traditionally, preserving patient mean arterial blood pressure (MAP) in the plateau region of the CBF autoregulation curve and maintaining normocapnia has been a strategy for ensuring adequate CBF. However, as both arterial blood pressure and the arterial partial pressure of $CO_2$ ($P_aCO_2$) in humans are highly dynamic, there is an increasing interest in CBF variability and its potential clinical significance. CBF variability has been extensively studied in relation to arterial blood pressure variability, in order to assess the dynamic cerebral autoregulation. Blood pressure variations have been connected to increased stroke incidence, brain damage, and poor health outcomes (Jung and Kim 2013; Shimbo et al. 2012; Tatasciore et al. 2007). Increased CBF variability is one of the mechanisms thought to underlie the relationship between elevated blood pressure variability and brain dysfunction (Tzeng and MacRae 2013), and thus to mediate stroke complications in patients (Jung and Kim 2013).

Cerebral autoregulation is less effective above 0.08 Hz (Zhang et al. 1998) and most effective below 0.05 Hz (Claassen et al. 2016). High-frequency (HF, 0.15–0.4 Hz) oscillations in MAP, resulting from HF oscillations in cardiac output, are thus considered to be transmitted to the brain vasculature. However, little CBF variability is observed in the HF range in spontaneously breathing healthy individuals (Rickards and Tzeng 2014). Kuo et al report a HF component in middle cerebral artery velocity variability, however constituting only 16% of its total variability (Kuo et al. 1998); the HF arterial blood pressure variability being approximately 21%. Spontaneous respiration contributes significantly to haemodynamic variability in the HF range (0.15–0.4 Hz), inducing fluctuations in heart rate (HR), left cardiac stroke volume, cardiac output and MAP (Toska and Eriksen 1993). The observed low CBF variability, contrasted by the significant respiration-induced hemodynamic variability of the central circulation, leads to the speculation that a regulatory mechanism minimizes CBF variations in the HF interval. Respiratory sinus arrhythmia (RSA), the HR variability (HRV) in the HF interval, has been shown to stabilize cardiac output and thus MAP in the HF range (Elstad et al. 2015; Toska and Eriksen 1993).

Several modelling methods have been employed in order to study the effect of beat-to-beat MAP and breath-to-breath end-tidal $CO_2$ ($ETCO_2$) fluctuations on CBF variability (Mitsis et al. 2004; Panerai et al. 2000). The effect of respiration and HRV on CBF variability however is not well documented. Reduced short-term HRV has been observed in patients with

cardiovascular and cerebrovascular pathological conditions (Akil et al. 2015; Nagata et al. 2006). Reduced HRV is an independent prospective marker of stroke risk in healthy individuals (Binici et al. 2011). Experimentally, HRV can be reduced by control-mode non-invasive ventilatory support (NIV) (Elstad et al. 2015). The HF spectral power calculated from power spectral analysis is an important tool for measurement of HRV (Larsen et al. 2010).

The purpose of this study was to examine the effect of spontaneous respiration and HRV on CBF variability during normovolemia and mild hypovolemia. Internal carotid artery blood flow (ICABF) variability at each subject's respiratory frequency (RF) was assessed in relation to the respiration-induced variations in HR and MAP, using frequency domain analysis. Since HRV in the HF-range (HF-HRV) during spontaneous breathing has been shown to reduce variations in cardiac output (Elstad et al. 2015), which directly influences CBF (Ogoh et al. 2005), we hypothesized that respiratory-induced HRV (RF-HRV) may reduce ICABF variability as well. To test our hypothesis, we trained subjects to accept low-pressure control-mode NIV (i.e., with fixed tidal volume and respiratory rate) to prevent spontaneous initiation of breathing and reduce HRV. The effects of reduced HRV on ICABF were studied both in normovolemia and in hypovolemia induced by lower body negative pressure (LBNP). Hypovolemia and controlled ventilation is a commonly encountered clinical situation e.g. in the operating theatre; increased CBF variability under these circumstances may affect patient outcome and result in adverse cerebrovascular effects. To the best of our knowledge, the effects of hypovolemia and mechanical ventilation on CBF variability have only been studied separately previously. In this protocol we investigated their combined effect on ICABF variability.

## Materials and methods

Steady-state data from these experiments have been published (Skytioti et al. 2016). In this study, we investigated the oscillatory responses of the cerebrovascular variables. Fifteen young healthy volunteers were recruited and gave written informed consent to participate in the study. All procedures were performed according to the Declaration of Helsinki. The regional ethics committee approved the protocol and the procedures (NO: 2014/2228, December 2014).

None of the subjects was a smoker or taking any medication. The subjects were instructed to abstain from caffeinated beverages and strenuous physical activity for 12 hours before the experiment. They also avoided food and drink for two hours and alcohol for 24 hours before the experiment.

We analysed recordings from each subject in four different experimental conditions: spontaneous breathing+normovolemia (baseline), spontaneous breathing+hypovolemia (hypovolemia), control-mode NIV+normovolemia (NIV), and control-mode NIV+hypovolemia. Five subjects were excluded because we did not obtain technically successful recordings in all four experimental conditions, i.e., at least 2 minutes of continuous, artefact-free beat-by-beat recordings of all cardiovascular variables. Thus, the analyses presented here are from 10 subjects (4 males, 6 females), median age 22 years (range 20–30 years).

*Experimental protocol*

The experimental protocol is described in detail in a previous paper (Skytioti et al. 2016). The experimental challenges were control-mode (i.e no spontaneous breaths), pressure-regulated, volume-controlled NIV (VIVO50, Diacor a/s, Norway) and LBNP (Hisdal et al. 2003), alone or in combination. The subjects lay supine in an LBNP chamber wearing a facemask throughout the procedure. During parts of the experiment NIV was applied with mandatory respiratory frequency, tidal volume, positive end-expiratory pressure (PEEP), and maximal inspiratory pressure set to match each subject's natural respiratory pattern. Spontaneous inspiration was prevented by training the subjects in a preparatory session not to initiate inspiration, but to accept the frequency and tidal volume administered by the ventilator. Ventilator settings (median (range)) were: RF: 14 breaths per min (11–16); target tidal volume: 650mL (500–850). Maximum and minimum inspiratory pressures were set to 14 $cmH_2O$ and 4.5 $cmH_2O$ respectively for all subjects. PEEP values of 1.3 $cmH_2O$ (0.9-1.4) were recorded

during normovolemia and 1.2 (0.8-1.4) during hypovolemia. The periods of LBNP (-30 mmHg, induced over 0.3 sec (Hisdal et al. 2003)) generated an acute central blood volume shift corresponding to a depletion of 10–20% of the total blood volume (500–1000 mL) and resulting in mild to moderate central hypovolemia (Hisdal et al. 2003).

An initial 10-min baseline period of normovolemia was followed by 10-min of simulated central hypovolemia. A 10-minute recovery period of normovolemia followed. During each interval, the subjects breathed spontaneously for 5 minutes and were subjected to control-mode NIV for 5 minutes (Fig.1). Each subject underwent the procedure twice, with a few minutes' pause between rounds. The first 30-min round was randomized to start with either NIV or spontaneous breathing; in the second round the sequence was reversed.

*Recordings*

Mean blood velocity in the ICA (5 MHz probe, insonation angle: 45°, SD-100, Vingmed Sound, Horten, Norway) was recorded beat-by-beat by a trained operator using Doppler ultrasound. ICA was chosen over the widely used middle cerebral artery because the diameter of the vessel was needed for blood flow calculations. The diameter of the subject's right ICA was measured approximately 2 cm above the bifurcation of the common carotid artery (Willie et al. 2012) before the beginning of the recordings using B-mode Ultrasound (10 MHz and 2.5 MHz, System Five, GE Vingmed Sound, Norway). Non-invasive finger arterial pressure was recorded continuously (Finometer, Finapres Medical System, Netherlands), and beat-by-beat MAP was calculated by numerical integration. Respiration was recorded breath-by-breath with an elastic belt around the abdomen (Respiration and Body position Amplifier, Scan-Med a/s, Norway). The signal from the belt resulting from the stretch is referred to as respiration. The RF at maximal power was used in the calculations. HR was calculated from the R-R interval in a three-lead ECG sampled at 300Hz. $ETCO_2$ in the facemask was recorded by a capnograph (Artema MM201, Artema Medical AB, Sweden). Blood velocity wave, respiration band signal, arterial blood pressure curve, stroke volume (Finometer), LBNP, $ETCO_2$ and room temperature were sampled at 100 Hz. Mean ICA blood velocity, HR (ECG) and MAP were also sampled beat-by-beat. The recordings were transferred on-line to a recording computer running dedicated data collection and analysis software (Program for real-time data acquisition, Morten Eriksen, Norway). ICA beat volume (ICABV) was calculated beat-by-beat from blood velocity and the diameter of the ICA. ICABF was calculated beat-by-beat from ICABV multiplied by instantaneous HR.

*Mathematical and statistical analyses*

All data was resampled at 4Hz employing an interpolation scheme. For each experimental condition, we selected a continuous sequence of 2 minutes after manual inspection for artefacts. The power spectrum, which is a plot of the variance of a time series as a function of frequency, was computed by the Fast Fourier Transform algorithm. The frequency resolution was kept the same in all conditions (~0.0083Hz). Coherence analysis, which provides a description of the relationship between two fluctuating variables in a specified range of frequencies, was used to examine the association between cardiovascular variables. Using cross-spectral analysis, it is possible to examine whether the variability of two distinct time series is interrelated in the frequency domain.

The integrals (area under the curve) of the power spectra were calculated in the subsequent three frequency intervals as estimates of variability in each frequency band. First, respiration-induced variability in cardiovascular variables was calculated at each subject's peak RF ± 0.03 Hz (RF-variability). Since spontaneous RF varied between subjects from 0.14 Hz to 0.3 Hz, we moved the RF-interval (RF±0.03Hz) accordingly. The percentage of the total respiration power included in the RF±0.03Hz interval was calculated on the signal from the Respiration and Body position Amplifier. In spontaneous breathing states this interval included almost 80% of the respiration power (normovolemia: 77% (56%-85%), hypovolemia: 78% (65%–85%)). During control-mode NIV all respiration power was included in the RF±0.03Hz interval.

Integrals were also calculated for the HF interval (0.15–0.4 Hz, HF-variability), which is widely used for the study of respiratory variability. Third, the total variability in the interval 0.05–0.5 Hz was calculated in order to quantify the changes in respiratory-induced variability of the cardiovascular variables relative to the overall variability. The relative RF-variability of each cardiovascular variable was then calculated as the fraction of the RF-variability (RF ± 0.03Hz) to the total variability.

Interaction between cardiovascular variables was examined by computing phase angles and coherence from the cross-spectra at peak RF (RF ± 0.02 Hz), for the following pairs of variables: Respiration–HR, Respiration–ICABV, Respiration–ICABF, HR–ICABV, and MAP–ICABF. The phase angle is the time delay between two waves that oscillate at the same frequency. Coherence provides a measure of the coupling between two signals over the range of frequencies examined.

Averaged phase angles were calculated by weighting the phase angles with their squared coherence. Two oscillating variables were considered to be in phase when the absolute phase difference between them was less than 45° (0.79 rad) and in inverse phase when the absolute phase difference was more than 135° (2.35 rad). Waves that meet in antiphase weaken each other, a phenomenon called destructive interference. This has been observed for HR and cardiac stroke volume at the RF, in effect diminishing cardiac output oscillations (Toska and Eriksen 1993).

Numbers are medians and 95% confidence intervals (95% CI) calculated by Hodges-Lehmann's estimate if not otherwise specified. For illustration purposes, integrals calculated for each experimental condition were also normalized with respect to the median value during baseline. Wilcoxon matched-pairs signed-rank test against a two-sided alternative (Hollander and Wolfe 1999) was used to test the differences in ICABF variability between conditions (StatXact, Cytel Studio 10, Cytel Inc., Cambridge, MA, USA) and the level of significance was set at p=0.05 before analysis. In addition we report the p-values (calculated after analysis) for the change in variability of the rest of the cardiovascular variables for informative purposes.

## Results

All ten subjects completed the protocol successfully and tolerated the abruptly induced central hypovolemia without signs of presyncope. The ten subjects complied successfully with the ventilator; they did not initiate inspiration but accepted passively the fixed respiratory rate and tidal volume administered by the ventilator. $ETCO_2$ was decreased during control mode NIV due to the slight hyperventilation which was necessary in order to diminish spontaneous inspiratory effort. However, $ETCO_2$ and RF were stable during each of the 2-min time intervals selected for frequency analysis in each experimental condition.

Raw data from one subject (recordings of ICABF, HR and ICABV) during the procedure are presented in Fig.2.

*Cerebrovascular variability during baseline*

Table 1 shows steady state values of the studied variables during the four different experimental states. Synchronous high-amplitude oscillations in HR and ICABV were observed during baseline whereas little variability was observed in ICABF over the respiratory cycle (Fig. 2, Fig. 3a). Inspiration coincided with an increase in HR and a decrease in ICABV, and expiration with a decrease in HR and an increase in ICABV (Fig 3a).

The total, HF, and RF variability in HR and ICABV were maximal during spontaneous breathing+normovolemia (Table 2, Fig 4a). The relative RF-HRV was 39% (23%–45%) of the total variability (measured over the entire 0.05–0.5 Hz interval). Thus, respiration-centered HRV is an important part of HRV. In contrast, the relative RF-ICABF variability during baseline constituted only 13% (11%–15%) of the total ICABF variability.

Table 3 shows the changes in phase angle and coherence between the conditions for the chosen pairs of variables. Respiration and HR oscillations were in phase and highly coherent (median coherence: 0.93). Respiration was in inverse phase and highly coupled with ICABV oscillations (median coherence: 0.94). Similarly, HRV and ICABV oscillations were in inverse phase and highly coherent (median coherence: 0.90), the increases in HR coinciding with drops in ICABV (Fig. 2a). These effects were also demonstrated by the power spectra, where maximum power was observed for HR and ICABV at the RF during baseline. In contrast, small variability in MAP and ICABF was observed (Fig 5a).

Coherence between MAP and ICABF was very low during spontaneous breathing+normovolemia (Table 3), the condition when both these variables reached their

lowest variability. Low coherence may indicate a nonlinear relationship, an absence of a relationship between two signals (Zhang et al. 1998), or a low signal-to-noise ratio, which is common with spontaneous oscillations (Claassen et al. 2009).

*Effect of control-mode NIV on cerebrovascular variability*

A significant drop in HRV in all frequency bands, in HF-ICABV variability and in RF-ICABV variability occurred during control-mode NIV (Table 2, Fig. 4b). Relative RF-HRV was 33% (12%–40%), similar to during baseline. The decrease in the respiratory-induced oscillations of HR and ICABV is also depicted in Fig. 3b.

RF-ICABF variability increased during control-mode NIV by about 140% (Table 2, Fig. 4b). In eight out of ten subjects, a peak in the power spectrum of ICABF was observed at the set RF (Fig. 5b); this peak was absent during spontaneous breathing. A similar peak at the set RF was observed for MAP variability in six of the ten subjects (Fig. 5b). The relative RF-ICABF variability was increased to 33% (24%–37%, p=0.002), compared to 13% at baseline.

Despite the reduction in RSA magnitude during control-mode NIV, respiration and HR oscillations remained in phase and were highly coherent (median coherence: 0.91). In contrast, coherence between respiration and ICABV as well as between HR and ICABV decreased (Table 3) compared to during baseline.

*Effect of hypovolemia on cerebrovascular variability*

Both HF-ICABV variability and RF-ICABV variability decreased during hypovolemia (Table 2, Fig. 4c). The relative RF-HRV was 36% (19%–44%). ICABF variability did not change with hypovolemia in either frequency interval. The relative RF-ICABF variability was 18% (14%–21%), slightly increased from baseline (p=0.05).

RSA was maintained during hypovolemia (HR in phase with respiration and highly coherent, Table 3). ICABV oscillations was in inverse phase and highly coherent with both respiration and HR oscillations (Table 3).

*Effect of combined control-mode NIV and hypovolemia on cerebrovascular variability*

HF-HRV and RF-HRV were about 70–80% lower during control-mode NIV+hypovolemia than during baseline, as was HF- and RF-ICABV variability (Table 2, Fig. 4d). In contrast, RF-ICABF variability increased by about 100%. The relative RF-HRV was 22% (10%–37%)

300 of the total, i.e. lower than during baseline, and the relative RF-ICABF variability was 24%
301 (6%–30%).

302 During combined control-mode NIV and hypovolemia, an increased respiratory component
303 in ICABF variability appeared around the set RF in eight out of ten subjects (Fig. 5d), similar
304 to during NIV in normovolemia. No such peak in ICABF variability was present during
305 baseline or during hypovolemia alone, i.e. in conditions with spontaneous breathing. A similar
306 peak in MAP variability at the set RF was observed in seven of the ten subjects (Fig. 5d), but
307 on a group level, MAP variability did not differ significantly between experimental conditions
308 in either frequency interval (Table 2, Fig. 4d).

309 Coherence and phase angle between respiration and HR did not change, indicating the
310 presence of RSA though with a low magnitude. In contrast, the coherence between respiration
311 and ICABV, HR and ICABV and respiration and ICABF reached their lowest values in this
312 state (Table 3).

## Discussion

In the present study we evaluated respiratory-induced HRV as a possible regulatory mechanism of CBF and examined the effect of respiratory variations on CBF during combined control-mode NIV and hypovolemia. We found that respiratory-induced ICABF variability in spontaneously breathing subjects was minimal, constituting only a small fraction of total ICABF variability both during normovolemia and hypovolemia. Control-mode NIV reduced respiratory HRV and increased respiratory variability in ICABF, during normovolemia and hypovolemia. We suggest that respiratory HRV during spontaneous breathing minimizes CBF variability. This finding might be of clinical importance as increased CBF variability has been connected to adverse cerebrovascular events.

During spontaneous breathing, HRV counteracted ICABV fluctuations, as drops in ICABV coincided with increases in HR during inspiration, and vice versa during expiration, thus minimizing fluctuations in ICABF. In contrast, during control-mode NIV with mandatory set RF and tidal volume, respiration-induced variability in ICABF was increased by 140% during NIV+normovolemia and 100% during NIV+hypovolemia. The coupling between respiration-induced oscillations in ICABV and HR decreased markedly during control-mode NIV (both in normovolemia and hypovolemia), from the situation of highly-coupled, inverse-phase oscillations during spontaneous breathing+normovolemia. Our study thus demonstrates a connection between decreased respiration-induced HRV and increased ICABF variability that may be of clinical relevance.

Both cardiac stroke volume and cardiac output have been shown to be linearly related to middle cerebral artery blood velocity (Bronzwaer et al. 2014; Ogoh et al. 2005). Increased oscillations in these variables could therefore be linearly transmitted to middle cerebral artery blood velocity and thus to CBF. We demonstrated that despite respiration-synchronous oscillations in ICABV during spontaneous breathing, ICABF variability was minimal due to buffering of the ICABV variability by HRV, which stabilized ICABF over the respiratory cycle.

Possible physiological roles of RSA have been investigated both in humans and animal models. Artificially induced RSA in a dog model optimized gas exchange and oxygen transport and reduced intrapulmonary shunt (Hayano et al. 1996), but this could not be demonstrated in humans (Tzeng et al. 2009). RSA has also been shown to buffer respiratory variability in left cardiac output, thus stabilizing MAP and systemic flow (Elstad 2012; Elstad

et al. 2015). Minimization of cardiac work has been proposed as another function of RSA (Ben-Tal et al. 2012). We here suggest minimization of respiration-related CBF variability as an additional physiological role of RSA.

In the present study, control-mode NIV reduced RSA substantially compared to the physiologic setting of spontaneous breathing. The main mechanism for this decrease in RSA was probably the reduction in the central feed-forward drive due to the elimination of spontaneous inspiratory effort (Beda et al. 2012; Elstad et al. 2015). The positive intrathoracic pressure during NIV may have affected the pulmonary and cardiac stretch receptors. In pigs, controlled mechanical ventilation reduced RSA amplitude and cardioventilatory coupling compared to assisted mechanical ventilation (Beda et al. 2012). Our study thus, in line with previous reports, shows that controlled mechanical ventilation reduces RSA, making control-mode NIV a good protocol to explore the effects of RSA on ICABF variability in human subjects.

Hypovolemia exaggerates the effects of positive pressure ventilation on the circulation. The combination of hypovolemia and control-mode mechanical ventilation is frequent in the operating theatre and not uncommon among critical care patients; thus our findings of increased variability in cerebrovascular flow under such circumstances are of interest. Because HRV decreases during hypovolemia (Elstad and Walloe 2015), the combination of controlled ventilation and hypovolemia was hypothesized to induce a larger drop in RSA. This hypothesis was verified (Table 2). However, despite a greater reduction in RSA during control-mode NIV+hypovolemia than during NIV alone, CBF variability in our study was similar in these two states.

Positive pressure ventilation and PEEP may affect cardiac stroke volume by impeding right ventricular filling. Our study employed very low PEEP settings that did not likely impact stroke volume much. In contrast hypovolemia induced by LBNP reduced stroke volume markedly (Skytioti et al. 2016).

It has been argued that increased CBF oscillations are not necessarily harmful for the brain (Rickards and Tzeng 2014). Increased middle cerebral artery velocity oscillations have been suggested to delay presyncopal symptoms during progressive hypovolemia in subjects breathing through an inspiratory threshold device (Rickards et al. 2007). Amplification of the respiratory pump with the inspiratory threshold device produced increased oscillations in CBF velocity in their study, resembling the high-amplitude respiration-induced ICABV oscillations

we observed during baseline (Fig. 1, Fig. 2a). In contrast, the use of control-mode NIV in the present study prevented spontaneous respiration and reduced the spontaneous RF- and HF-ICABV variability. Concurrently, an increased respiratory variability component appeared in ICABF.

During control-mode NIV, RF-ICABF variability increased, and a decrease in coupling between HR and ICABV was observed. The relative RF-ICABF variability was also higher during controlled than spontaneous breathing, indicating that the respiratory-induced CBF variations became a more important component of total CBF variability during controlled ventilation. Both the decreased coupling between HRV and ICABV variability and the cyclic changes in intrathoracic pressure during NIV may have been responsible for the increase in RF-ICABF variability. The effects of mechanical ventilation on the circulation are more pronounced the higher the mean airway pressure, as higher intrathoracic pressure impedes venous return and decreases right ventricular filling. Hypovolemia will exaggerate these effects (Cheifetz 2014). However, while each cyclic increase in intrathoracic pressure increases right ventricular afterload, it also improves left ventricular filling and decreases left ventricular afterload. Thus, during hypovolemia, mechanical ventilation induces larger respiratory oscillations in cardiac output (Michard 2005; Rimehaug et al. 2016), and these may be transferred to the CBF. We demonstrated an increase in RF-ICABF variability during control-mode NIV. Increased variability in MAP and cardiac output may have contributed though we were unable to demonstrate this in our study.

*Clinical implications*

HRV is affected by several pathophysiological conditions. Reductions in HRV have been associated with poor clinical outcomes after acute myocardial infarction (Balanescu et al. 2004) and have been reported to be a predictor of post stroke mortality (Makikallio et al. 2004). Several studies have linked lower HRV with brain dysfunction (Biswas et al. 2000; Katz-Leurer et al. 2014; Kholod et al. 2013). A decrease in HRV was associated with cerebrovascular pathology in normotensive diabetic patients (Nagata et al. 2006).

In healthy spontaneously breathing subjects, minimal variability is observed in CBF (Kuo et al. 1998; Rickards et al. 2007). We found that ICABF oscillations increased around the set RF during control-mode NIV, both in normovolemia and in hypovolemia. This observation could be of clinical importance as it may indicate that CBF regulation might be disturbed by the applied therapy in patients treated with controlled mechanical ventilation for extended periods

409 of time. Elderly patients in particular may be more vulnerable, also due to a higher prevalence
410 of medical comorbidities. A recent study indicated an association between impaired
411 intraoperative CBF autoregulation and postoperative cognitive dysfunction in patients over 65
412 years old (Goettel et al. 2017).

413 Associations between cerebral pathology and exaggerated blood pressure variations have been
414 reported and possible underlying mechanisms are larger CBF fluctuations, neurohumoral
415 activation, endothelial dysfunction, inflammatory mediators, and oxidative stress (Jung and
416 Kim 2013; Shimbo et al. 2012; Tatasciore et al. 2007; Tatasciore et al. 2008). Optimizing
417 intravascular volume, avoiding excess sedation, and using lung-protective ventilation
418 strategies with supported rather than controlled ventilation may contribute to improved
419 cerebral outcomes in critical care patients.

420 *Considerations*

421 CBF is highly dependent on $P_aCO_2$ (Panerai et al. 2010) and any kind of physical stimulation.
422 The experiments were performed in a quiet room, in resting subjects who had familiarized
423 themselves with the equipment and procedures. To minimize confounding factors, we
424 recorded $ETCO_2$ as an estimate of the $P_aCO_2$ and ensured that there were no significant
425 changes during each 2-min time interval selected for frequency analysis in each experimental
426 state. However there was a decrease in $ETCO_2$ from baseline to NIV and to
427 NIV+hypovolemia. Since $ETCO_2$ necessarily can be determined only once per breath, we
428 could not study the effect of $ETCO_2$ fluctuations on ICABF variability in the HF range. To
429 evaluate whether cyclical changes in arterial partial pressure of $CO_2$ actually affect CBF
430 would require continuous invasive measurements. It has been shown that the effect of breath-
431 by-breath $ETCO_2$ fluctuations on the middle cerebral artery blood flow velocity are
432 considerable in the very low frequencies and in the low frequencies, implying that slow
433 variations in $ETCO_2$ have a larger impact on middle cerebral artery blood flow velocity
434 (Mitsis et al. 2006). Mitsis et al showed that $ETCO_2$ variations have a considerable effect on
435 CBF at frequencies below 0.04 Hz (Mitsis et al. 2004). Taking into account the negligible
436 effect of $ETCO_2$ in the HF range, we assumed that $ETCO_2$ fluctuations did not affect
437 cerebrovascular respiratory variability.

438 Another methodological concern was whether the diameter of the ICA, which was measured
439 once in the beginning of each experiment, remained stable throughout the experiment or
440 changed in response to changes in $ETCO_2$ and LBNP. $PaCO_2$ change <1.3 kPa is considered

441 unlikely to cause ICA diameter changes (Sato et al. 2012; Willie et al. 2012), and no change
442 in ICA diameter was reported between baseline and -35mmHg LBNP (Ogoh et al. 2015). As
443 the largest change in $ETCO_2$ in our experiments was 1.1 kPa and the LBNP level was -30
444 mmHg we assumed that ICA diameter remained the same.

445 We calculated RF variability at subjects' peak RF±0.03Hz in addition to in the HF interval. In
446 spontaneously breathing subjects, the RF varies with time, and therefore spontaneous
447 respiratory variability may be widely distributed. To take this into account, we calculated the
448 percentage of the total respiration power (respiration signal) included in the RF±0.03Hz
449 interval. Almost 80% of the respiration power was included. During control-mode NIV with
450 or without hypovolemia, all respiratory variability was included in the RF±0.03Hz interval.
451 The measured change in RF-HRV would probably have been larger if it was calculated over a
452 wider interval during spontaneous breathing.

453 Our findings during NIV directly apply only if conditions are similar, i.e. healthy subjects, no
454 sedation or paralytics, marginal PEEP, controlled respiratory rate, and low-grade pressures.
455 The subjects did not initiate inspiration, but our study design did not hinder diaphragmatic
456 muscle activity participating in the respiratory cycle. Thus, the applied interventions were
457 milder than those often used in clinical settings, where more pronounced effects of controlled
458 mechanical ventilation would be expected. This is suggested also by animal studies (Beda et
459 al. 2012).

460 *Conclusion*

461 Respiratory and cardiovascular variability may introduce changes in cerebral hemodynamics
462 despite several counteracting mechanisms, such as the cerebral autoregulation of CBF. We
463 suggest that respiration-induced HRV could be an additional mechanism that contributes to
464 stabilization of short-term CBF variability in spontaneously breathing, healthy individuals. A
465 reduction in HRV, experimentally induced in our study by control-mode NIV, was
466 accompanied by a significant increase in CBF variability in both normovolemia and mild
467 hypovolemia.

468

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## Disclosures

The authors declare that there is no conflict of interest.

## Ethical approval

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and /or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

## Authors' contributions

All authors have contributed to the design of the study as well as the acquisition, analysis and interpretation of data. M. S. drafted the manuscript and all authors have revised it critically and approved the version to be published.

487

488 **References**


489 Akil E, Tamam Y, Akil MA, Kaplan I, Bilik MZ, Acar A, Tamam B (2015) Identifying autonomic nervous
490 system dysfunction in acute cerebrovascular attack by assessments of heart rate variability and
491 catecholamine levels. J Neurosci Rural Pract 6:145-150 doi:10.4103/0976-3147.153216
492 Balanescu S, Corlan AD, Dorobantu M, Gherasim L (2004) Prognostic value of heart rate variability after acute
493 myocardial infarction Med Sci Monit 10:Cr307-315
494 Beda A et al. (2012) Effects of assisted and variable mechanical ventilation on cardiorespiratory interactions in
495 anesthetized pigs. Physiol Meas 33:503-519 doi:10.1088/0967-3334/33/3/503
496 Ben-Tal A, Shamailov SS, Paton JF (2012) Evaluating the physiological significance of respiratory sinus
497 arrhythmia: looking beyond ventilation-perfusion efficiency. J physiol 590:1989-2008
498 doi:10.1113/jphysiol.2011.222422
499 Binici Z, Mouridsen MR, Kober L, Sajadieh A (2011) Decreased nighttime heart rate variability is associated
500 with increased stroke risk. Stroke 42:3196-3201 doi:10.1161/strokeaha.110.607697
501 Biswas AK, Scott WA, Sommerauer JF, Luckett PM (2000) Heart rate variability after acute traumatic brain
502 injury in children Crit Care Med 28:3907-3912
503 Bronzwaer AS, Stok WJ, Westerhof BE, van Lieshout JJ (2014) Arterial pressure variations as parameters of
504 brain perfusion in response to central blood volume depletion and repletion Front Physiol 5:157
505 doi:10.3389/fphys.2014.00157
506 Cheifetz IM (2014) Cardiorespiratory Interactions: The Relationship Between Mechanical Ventilation and
507 Hemodynamics Respir Care doi:10.4187/respcare.03486
508 Claassen JA, Levine BD, Zhang R (2009) Dynamic cerebral autoregulation during repeated squat-stand
509 maneuvers J Appl Physiol (Bethesda, Md : 1985) 106:153-160 doi:10.1152/japplphysiol.90822.2008
510 Claassen JA, Meel-van den Abeelen AS, Simpson DM, Panerai RB (2016) Transfer function analysis of
511 dynamic cerebral autoregulation: A white paper from the International Cerebral Autoregulation
512 Research Network. J Cereb Blood Flow Metab 36:665-680 doi:10.1177/0271678x15626425
513 Elstad M (2012) Respiratory variations in pulmonary and systemic blood flow in healthy humans Acta
514 physiologica (Oxford, England) 205:341-348 doi:10.1111/j.1748-1716.2012.02419.x
515 Elstad M, Walloe L (2015) Heart rate variability and stroke volume variability to detect central hypovolemia
516 during spontaneous breathing and supported ventilation in young, healthy volunteers Physiol Meas
517 36:671-681 doi:10.1088/0967-3334/36/4/671
518 Elstad M, Walloe L, Holme NL, Maes E, Thoresen M (2015) Respiratory sinus arrhythmia stabilizes mean
519 arterial blood pressure at high-frequency interval in healthy humans Eur J Appl Physiol 115:521-530
520 doi:10.1007/s00421-014-3042-3
521 Goettel N et al. (2017) Associations Between Impaired Cerebral Blood Flow Autoregulation, Cerebral
522 Oxygenation, and Biomarkers of Brain Injury and Postoperative Cognitive Dysfunction in Elderly
523 Patients After Major Noncardiac Surgery Anesthesia and analgesia 124:934-942
524 doi:10.1213/ane.0000000000001803
525 Hayano J, Yasuma F, Okada A, Mukai S, Fujinami T (1996) Respiratory sinus arrhythmia. A phenomenon
526 improving pulmonary gas exchange and circulatory efficiency Circulation 94:842-847
527 Hisdal J, Toska K, Walloe L (2003) Design of a chamber for lower body negative pressure with controlled onset
528 rate Aviat Space Environ Med 74:874-878
529 Hollander M, Wolfe DA (1999) Nonparametric statistical methods. Wiley series in probability and statistics :
530 text and references section, 2nd ed. edn. Wiley, New York
531 Jung HW, Kim KI (2013) Blood Pressure Variability and Cognitive Function in the Elderly Pulse (Basel,
532 Switzerland) 1:29-34 doi:10.1159/000348622
533 Katz-Leurer M, Zohar N, Boum A, Keren O (2014) Monitoring changes in heart rate, as an indicator of the
534 cardiovascular autonomic nervous function, among patients at the sub-acute phase post-brain damage
535 during a physiotherapy session: a preliminary investigation Brain Inj 28:127-131
536 doi:10.3109/02699052.2013.848381
537 Kholod H, Jamil A, Katz-Leurer M (2013) The associations between motor ability, walking activity and heart
538 rate and heart rate variability parameters among children with cerebral palsy and typically developed
539 controls NeuroRehabilitation 33:113-119 doi:10.3233/nre-130934
540 Kuo TB, Chern CM, Sheng WY, Wong WJ, Hu HH (1998) Frequency domain analysis of cerebral blood flow
541 velocity and its correlation with arterial blood pressure J cereb Blood Flow Metab 18:311-318
542 doi:10.1097/00004647-199803000-00010

543 Larsen PD, Tzeng YC, Sin PY, Galletly DC (2010) Respiratory sinus arrhythmia in conscious humans during
544 spontaneous respiration Respir Physiol Neurobiol 174:111-118 doi:10.1016/j.resp.2010.04.021
545 Makikallio AM, Makikallio TH, Korpelainen JT, Sotaniemi KA, Huikuri HV, Myllyla VV (2004) Heart rate
546 dynamics predict poststroke mortality Neurology 62:1822-1826
547 Michard F (2005) Changes in arterial pressure during mechanical ventilation Anesthesiology 103:419-428; quiz
548 449-415
549 Mitsis GD, Poulin MJ, Robbins PA, Marmarelis VZ (2004) Nonlinear modeling of the dynamic effects of
550 arterial pressure and CO2 variations on cerebral blood flow in healthy humans IEEE transactions on
551 bio-medical engineering 51:1932-1943 doi:10.1109/tbme.2004.834272
552 Mitsis GD, Zhang R, Levine BD, Marmarelis VZ (2006) Cerebral hemodynamics during orthostatic stress
553 assessed by nonlinear modeling J Appl Physiol (Bethesda, Md : 1985) 101:354-366
554 doi:10.1152/japplphysiol.00548.2005
555 Nagata K et al. (2006) Differences in heart rate variability in non-hypertensive diabetic patients correlate with
556 the presence of underlying cerebrovascular disease Clin Physiol Funct imaging 26:92-98
557 doi:10.1111/j.1475-097X.2006.00654.x
558 Ogoh S et al. (2005) The effect of changes in cardiac output on middle cerebral artery mean blood velocity at
559 rest and during exercise J Physiol 569:697-704 doi:10.1113/jphysiol.2005.095836
560 Ogoh S, Sato K, Okazaki K, Miyamoto T, Hirasawa A, Sadamoto T, Shibasaki M (2015) Blood flow in internal
561 carotid and vertebral arteries during graded lower body negative pressure in humans Exp Physiol
562 doi:10.1113/expphysiol.2014.083964
563 Panerai RB, Dineen NE, Brodie FG, Robinson TG (2010) Spontaneous fluctuations in cerebral blood flow
564 regulation: contribution of PaCO2. J Appli Physiol 109(6): 1868-1869.
565 doi:10.1152/japplphysiol.00857.2010
566 Panerai RB, Simpson DM, Deverson ST, Mahony P, Hayes P, Evans DH (2000) Multivariate dynamic analysis
567 of cerebral blood flow regulation in humans IEEE transactions on bio-medical engineering 47:419-423
568 doi:10.1109/10.827312
569 Rickards CA, Ryan KL, Cooke WH, Lurie KG, Convertino VA (2007) Inspiratory resistance delays the
570 reporting of symptoms with central hypovolemia: association with cerebral blood flow Am J Physiol
571 Regul Integr Comp Physiol 293:R243-250 doi:10.1152/ajpregu.00087.2007
572 Rickards CA, Tzeng YC (2014) Arterial pressure and cerebral blood flow variability: friend or foe? A review
573 Front Physiol 5:120 doi:10.3389/fphys.2014.00120
574 Rimehaug AE, Hoff IE, Hoiseth LO, Hisdal J, Aadahl P, Kirkeby-Garstad I (2016) Cardiac power parameters
575 during hypovolemia, induced by the lower body negative pressure technique, in healthy volunteers
576 BMC anesthesiology 16:31 doi:10.1186/s12871-016-0195-0
577 Sato K, Sadamoto T, Hirasawa A, Oue A, Subudhi AW, Miyazawa T, Ogoh S (2012) Differential blood flow
578 responses to CO2 in human internal and external carotid and vertebral arteries. J Physiol 590:3277-3290
579 doi:10.1113/jphysiol.2012.230425
580 Shimbo D et al. (2012) Association between annual visit-to-visit blood pressure variability and stroke in
581 postmenopausal women: data from the Women's Health Initiative Hypertension 60:625-630
582 doi:10.1161/hypertensionaha.112.193094
583 Skytioti M, Sovik S, Elstad M (2016) Internal carotid artery blood flow in healthy awake subjects is reduced by
584 simulated hypovolemia and noninvasive mechanical ventilation Physiol Rep 4
585 doi:10.14814/phy2.12969
586 Tatasciore A et al. (2007) Awake systolic blood pressure variability correlates with target-organ damage in
587 hypertensive subjects Hypertension 50:325-332 doi:10.1161/hypertensionaha.107.090084
588 Tatasciore A et al. (2008) Awake blood pressure variability, inflammatory markers and target organ damage in
589 newly diagnosed hypertension Hypertens res 31:2137-2146 doi:10.1291/hypres.31.2137
590 Toska K, Eriksen M (1993) Respiration-synchronous fluctuations in stroke volume, heart rate and arterial
591 pressure in humans J Physiol 472:501-512
592 Tzeng YC, MacRae BA (2013) Interindividual relationships between blood pressure and cerebral blood flow
593 variability with intact and blunted cerebrovascular control J Appl Physiol (Bethesda, Md : 1985)
594 114:888-895 doi:10.1152/japplphysiol.01388.2012
595 Tzeng YC, Sin PY, Galletly DC (2009) Human sinus arrhythmia: inconsistencies of a teleological hypothesis
596 Am J Physiol Heart Circ Physiol 296:H65-70 doi:10.1152/ajpheart.00716.2008
597 Willie CK et al. (2012) Regional brain blood flow in man during acute changes in arterial blood gases J Physiol
598 590:3261-3275 doi:10.1113/jphysiol.2012.228551
599 Zhang R, Zuckerman JH, Giller CA, Levine BD (1998) Transfer function analysis of dynamic cerebral
600 autoregulation in humans Am J PhysioL 274:H233-241

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**Table 1.** Cardiovascular and respiratory variables in the four different conditions: Normovolemia with and without control-mode NIV, hypovolemia with and without control-mode NIV. Healthy human subjects, N=10.

| | **Normovolemia** | | **Hypovolemia** | |
|---|---|---|---|---|
| | **Spontaneous breathing** | **Control-mode NIV** | **Spontaneous breathing** | **Control-mode NIV** |
| **ICABF** (ml $min^{-1}$) | 251 (190–285) | 226** (163–253) | 228 (179–285) | 210** (149–245) |
| **ICABV** (ml) | 4.8 (3.1–5.4) | 4.2* (2.8–5.2) | 3.8** (2.7–4.4) | 3.3** (2.2–3.9) |
| **HR** (bpm) | 56.6 (46.6–59.6) | 54.7 (45.5–59.4) | 64.0** (55.2–67.4) | 65.1** (55.9–68.0) |
| **MAP** (mmHg) | 78.3 (69.9–81.2) | 77.4 (71.2–80.7) | 80.3 (72.9–83.3) | 78.8 (72.2–81.7) |
| **$ETCO_2$** (kPa) | 4.9 (4.4–5.1) | 4.3** (4.0–4.6) | 4.7** (4.2–4.9) | 4.0** (3.5–4.2) |

Data are medians and 95% Confidence Intervals calculated by Hodges Lehmann estimate. NIV: non-invasive ventilatory support**,** ICABF: internal carotid artery blood flow, ICABV**:** internal carotid artery beat volume, HR: heart rate, MAP: mean arterial pressure, ETCO2: end-tidal $CO_2$. Significance level compared to baseline: * $p<0.05$, ** $p\leq0.01$.

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**Table 2.** Cardiovascular variability estimated from integrals of the power spectra at the respiratory frequency +/- 0.03 Hz, in the high-frequency interval (0.15–0.4 Hz) and total variability (0.05–0.5Hz). Healthy human subjects, N=10.

| | **Normovolemia** | | **Hypovolemia** | |
|---|---|---|---|---|
| | **Spontaneous breathing** | **Control-mode NIV** | **Spontaneous breathing** | **Control-mode NIV** |
| **Variability at respiratory frequency ±0.03 Hz** | | | | |
| **HR** ($bpm^2$) | 4.1 (2.0–5.9) | 1.8** (0.6–2.2) | 2.6 (1.2–3.7) | 1.1** (0.4–1.5) |
| **MAP** ($mmHg^2$) | 1.0 (0.6–1.4) | 1.1 (0.5–2.0) | 1.9 (0.7–4.6) | 1.7 (0.6–2.5) |
| **ICABV** ($ml^2$) | 0.042 (0.013–0.062) | 0.015** (0.006–0.021) | 0.013** (0.005–0.021) | 0.009** (0.004–0.012) |
| **ICABF** $(ml\ min^{-1})^2$ | 14.0 (5.1–19.4) | 33.7** (12.4–47.4) | 25.6 (6.2–32.3) | 29.1* (7.6–43.4) |
| **Variability in the high-frequency interval** | | | | |
| **HR** ($bpm^2$) | 7.4 (3.1–10.9) | 2.8** (1.3–3.4) | 4.1* (1.7–4.6) | 1.8** (0.9–2.5) |
| **MAP** ($mmHg^2$) | 2.3 (1.4–2.7) | 2.3 (1.2–6.4) | 3.9* (1.8–7.1) | 4.0 (1.7–9.4) |
| **ICABV** ($ml^2$) | 0.065 (0.024–0.094) | 0.025* (0.013–0.033) | 0.023** (0.01–0.034) | 0.015* (0.009–0.021) |
| **ICABF** $(ml\ min^{-1})^2$ | 44.9 (14.4–65.5) | 68.0 (22.0–86.4) | 48.0 (18.1–75.8) | 47.5 (21.2–85.3) |
| **Total Variability** | | | | |
| **HR** ($bpm^2$) | 12.7 (5.1–17.4) | 4.5** (2.6–5.6) | 7.7 (4.7–9.3) | 7.2 (2.5–9.2) |
| **MAP** ($mmHg^2$) | 9.0 (3.8–11.7) | 5.0 (3.9–16.9) | 12.0 (3.9–24.0) | 12.7 (5.2–25.7) |
| **ICABV** ($ml^2$) | 0.100 (0.048–0.135) | 0.045 (0.02–0.058) | 0.046 (0.02–0.061) | 0.034* (0.017–0.047) |
| **ICABF** $(ml\ min^{-1})^2$ | 100.5 (37.1–158.7) | 123.3 (39.9–148.5) | 128.8 (37.0–160.1) | 97.8 (52.7–322.7) |
| **RF** (Hz) | 0.23 (0.2–0.24) | 0.23 (0.21–0.25) | 0.21** (0.18–0.22) | 0.23 (0.21–0.25) |

Values are medians and 95% confidence intervals calculated by Hodges-Lehmann estimates. NIV: non-invasive ventilatory support, HR: heart rate, MAP: mean arterial pressure, ICABV: internal carotid artery beat volume, ICABF: internal carotid artery blood flow, RF: respiratory frequency. Significance level compared to baseline: * $p<0.05$, ** $p<0.01$.

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**Table 3.** Phase angles and coherences between cardiorespiratory variables during normovolemia and hypovolemia, with and without NIV.

| | Normovolemia | | Hypovolemia | |
|---|---|---|---|---|
| | **Spontaneous Breathing** | **Control-mode NIV** | **Spontaneous Breathing** | **Control-mode NIV** |
| **RESP-HR** | | | | |
| Phase angle | 0.46 (0.7,0.3) | 0.76 (1.27,0.47) | 0.59 (1.06,0.36) | 0.79 (1.37,0.63) |
| **Coherence** | **0.93 (0.87–0.97)** | **0.91 (0.84–0.94)** | **0.91 (0.85–0.95)** | **0.92 (0.88–0.94)** |
| **RESP–ICABV** | | | | |
| Phase angle | 2.89 (2.70–3.21) | 2.79 (2.32–3.23) | 3.16 (2.76–3.42) | 3.66 (2.55–3.83) |
| **Coherence** | **0.94 (0.85–0.96)** | **0.78 (0.48–0.84)*** | **0.84 (0.70–0.88)** | **0.62 (0.30–0.77)*** |
| **RESP–ICABF** | | | | |
| Phase angle | 3.19 (1.87–3.70) | 4.67 (3.58–5.02) | 4.36 (4.15–4.53) | 5.03 (4.26–5.47) |
| **Coherence** | **0.77 (0.64–0.84)** | **0.87 (0.69–0.89)** | **0.70 (0.52–0.73)** | **0.66 (0.43–0.78)** |
| **HR–ICABV** | | | | |
| Phase angle | 3.28 (3.16–3.50) | 3.77 (3.45–4.12) | 3.62 (3.46–4.04) | 3.77 (3.15–4.71) |
| **Coherence** | **0.90 (0.80–0.97)** | **0.82 (0.53–0.89)*** | **0.90 (0.76–0.92)** | **0.65 (0.30–0.76)*** |
| **MAP–ICABF** | | | | |
| Phase angle | 6.23 (1.54–5.84) | 5.30 (4.31–6.09) | 0.71 (6.02–1.47) | 5.93 (4.77–6.28) |
| **Coherence** | **0.36 (0.20–0.45)** | **0.49 (0.31–0.59)** | **0.45 (0.25–0.54)** | **0.49 (0.18–0.63)** |

Number of subjects =10. Coherence-weighted phase angles in rad (on a unit circle from 0 to 2π). Data are medians and 95% confidence intervals calculated by Hodges-Lehmann estimates. NIV: non-invasive ventilatory support, RESP: respiration, HR: heart rate, ICABV: Internal carotid artery beat volume, ICABF: Internal carotid artery blood flow. Significance level compared to baseline: *p=0.01.

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# Figure Captions

**Fig. 1** Study protocol displaying subjects' breathing conditions and lower body chamber pressure during 10 min of normovolemia, 10 min of simulated hypovolemia and 10 min of normovolemia–recovery. This sequence was run twice in each subject, once starting with controlled non-invasive ventilation, i.e. with set respiratory frequency and tidal volume (NIV), and once starting with spontaneous breathing. LBNP: lower body negative pressure

**Fig. 2** Recordings (raw data) from one subject during 10 min of normovolemia and 10 min of simulated hypovolemia induced by lower body negative pressure, with and without non-invasive control-mode ventilatory support (NIV). During spontaneous breathing+normovolemia there were large synchronous fluctuations in heart rate (HR) and internal carotid artery beat volume (ICABV) but minimal oscillations in internal carotid artery blood flow (ICABF). Controlled mechanical ventilation and hypovolemia, separately and combined, reduced HR and ICABV oscillations

**Fig. 3** Oscillations in HR, ICABV, ICABF and MAP during 30 sec (6-8 respiratory cycles) in four states (a. Baseline: spontaneous breathing+normovolemia, b. NIV: control-mode NIV+normovolemia, c. Hypovolemia: spontaneous breathing+hypovolemia, and d. Control-mode NIV+hypovolemia), in the same subject as Fig. 2. Top graph shows respiration (upward stroke: inspiration, downward stroke: expiration). Inspiration (dotted lines) coincided with increases in HR and decreases in ICABV. Expiration coincided with decreases in HR and increases in ICABV. HR: heart rate, ICABV: internal carotid artery beat volume, ICABF: internal carotid artery blood flow, MAP: Mean arterial pressure, NIV: controlled non-invasive ventilation

**Fig. 4** Respiratory variability (integrals of power spectra) changes in heart rate (HR), mean arterial pressure (MAP), internal carotid artery beat volume (ICABV) and internal carotid artery blood flow (ICABF) at peak respiratory frequency in four states (a. Baseline: spontaneous breathing+normovolemia, b. NIV: control-mode NIV+normovolemia, c. Hypovolemia: spontaneous breathing+hypovolemia, and d. Control-mode NIV+hypovolemia). Variability was normalized with respect to the median value during Baseline. Data from 10 healthy humans 20–30 years old. Values are medians and 95% confidence intervals calculated by Hodges-Lehmann estimates. *Significant difference from spontaneous breathing+normovolemia (Wilcoxon matched-pairs signed-rank test)

637 **Fig. 5** Power spectra (HF-interval: 0.15-0.4Hz) of cardiovascular variables during four states
638 (a. Baseline: spontaneous breathing+normovolemia, b. NIV: control-mode
639 NIV+normovolemia, c. Hypovolemia: spontaneous breathing+hypovolemia, and d. control-
640 mode NIV+hypovolemia), in the same subject as Fig. 2 and Fig. 3. The RF-interval (0.24–0.3
641 Hz) is noted. HR: heart rate, ICABV: internal carotid artery beat volume, ICABF: internal
642 carotid artery blood flow, MAP: Mean arterial pressure, RF-SPONT: spontaneous respiratory
643 frequency, RF-NIV: set respiratory frequency during controlled non-invasive ventilation

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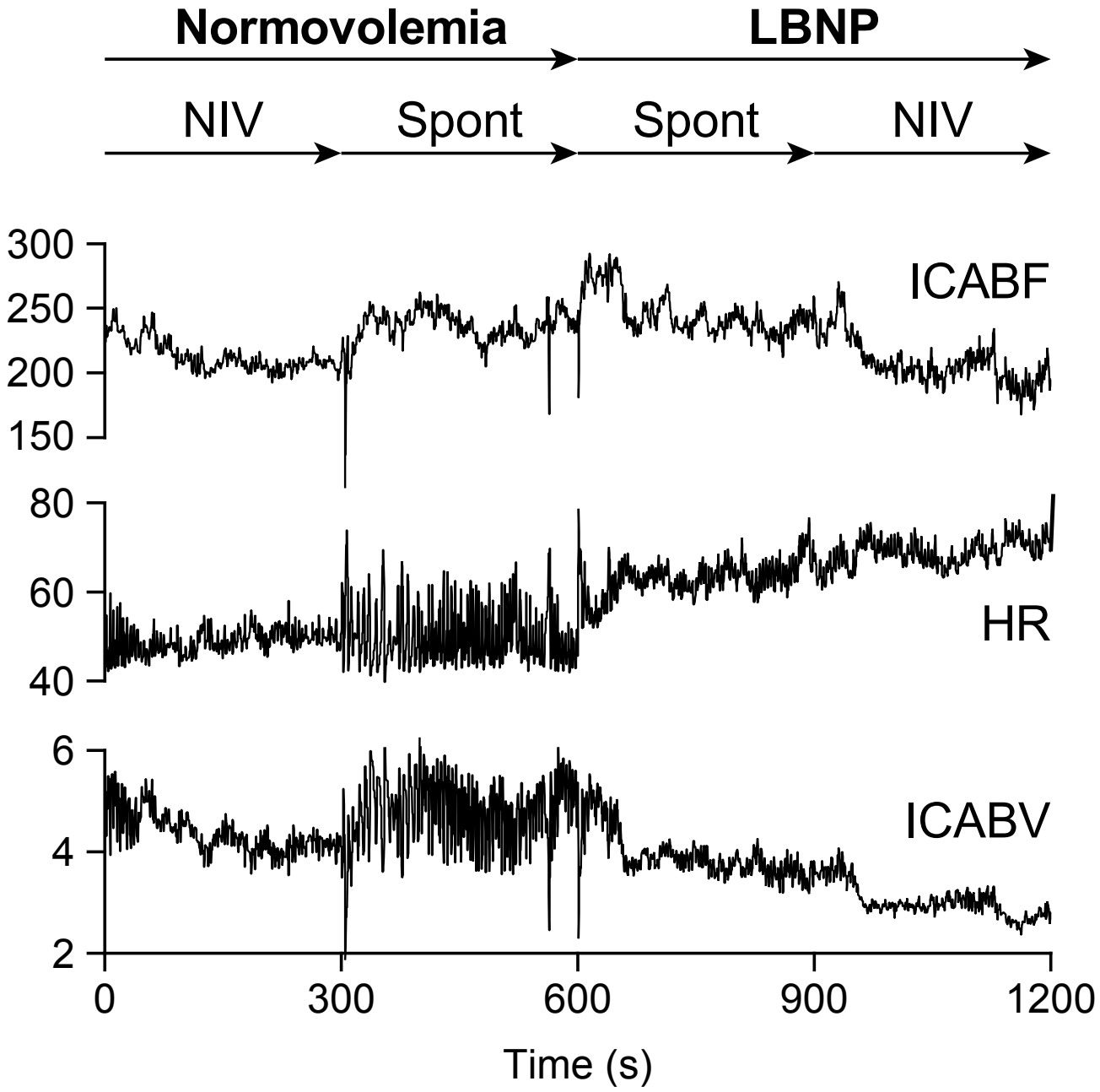
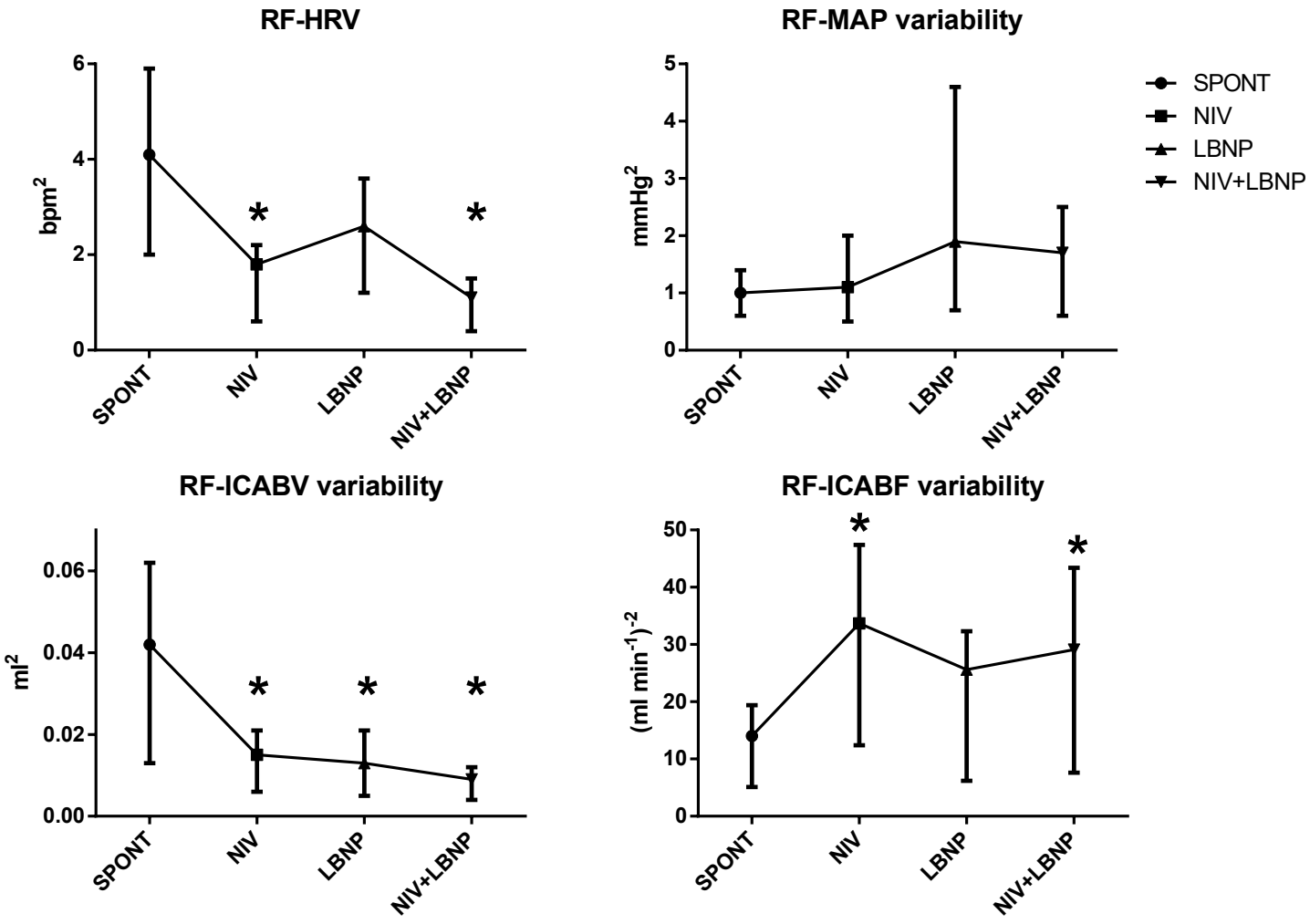
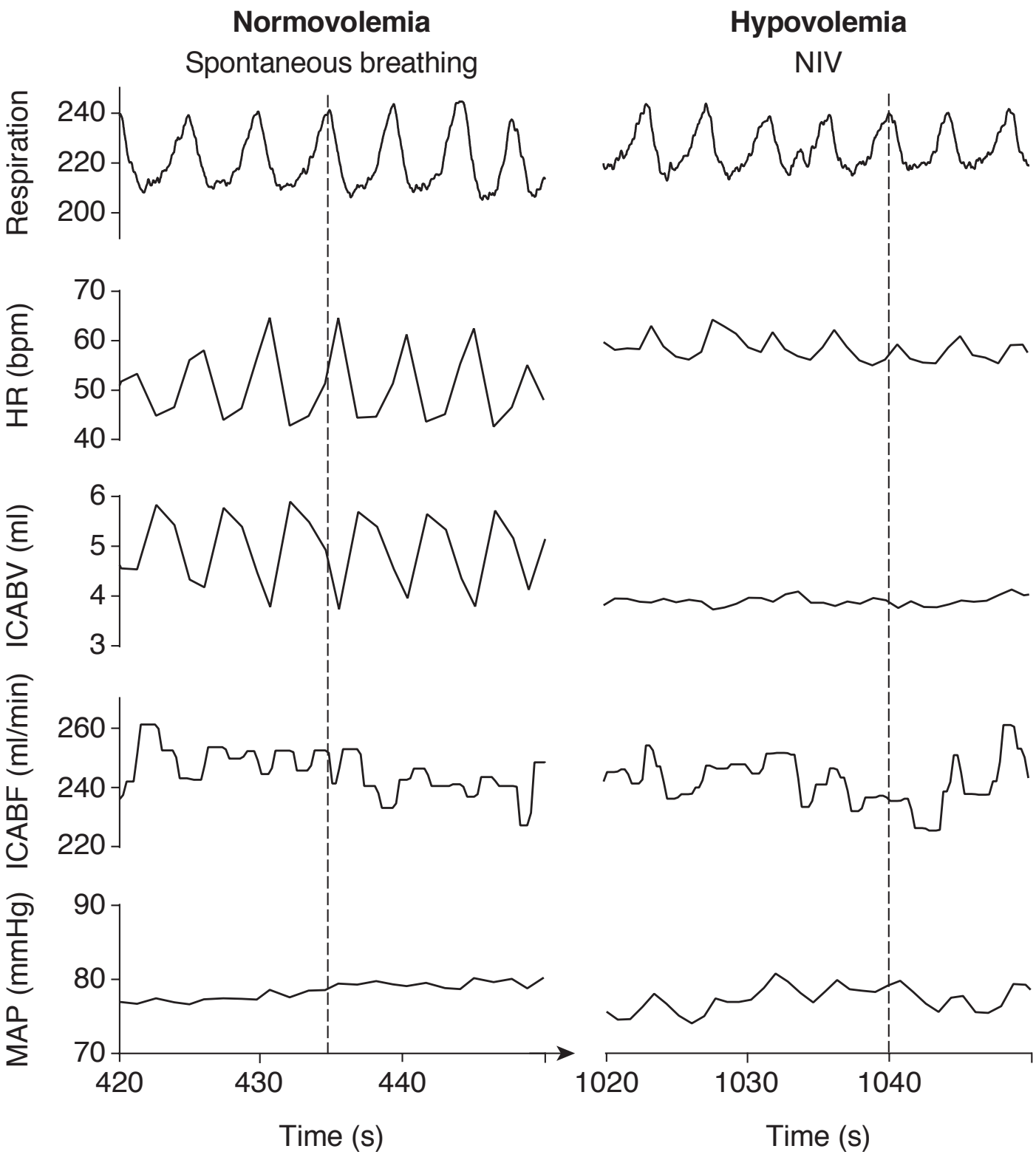
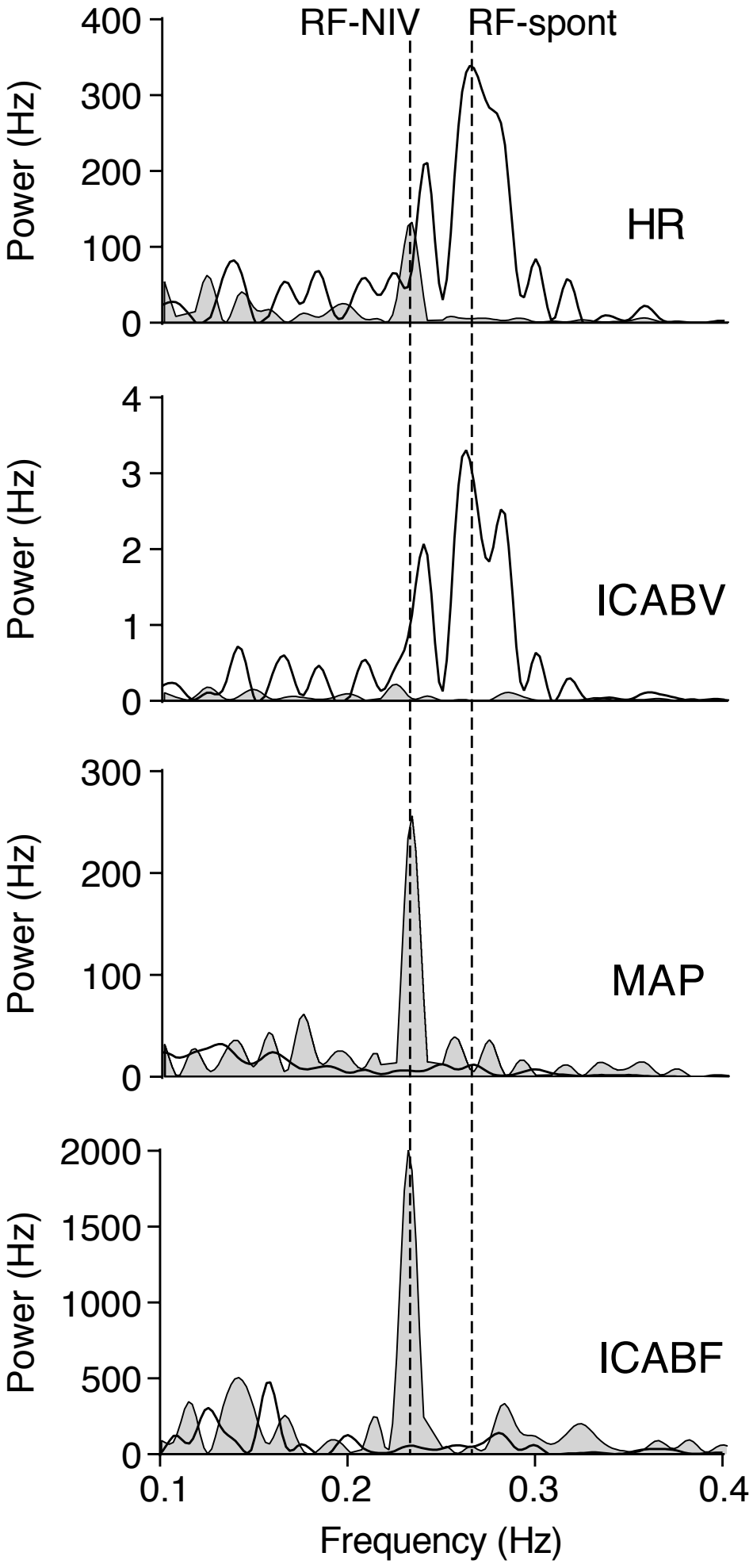
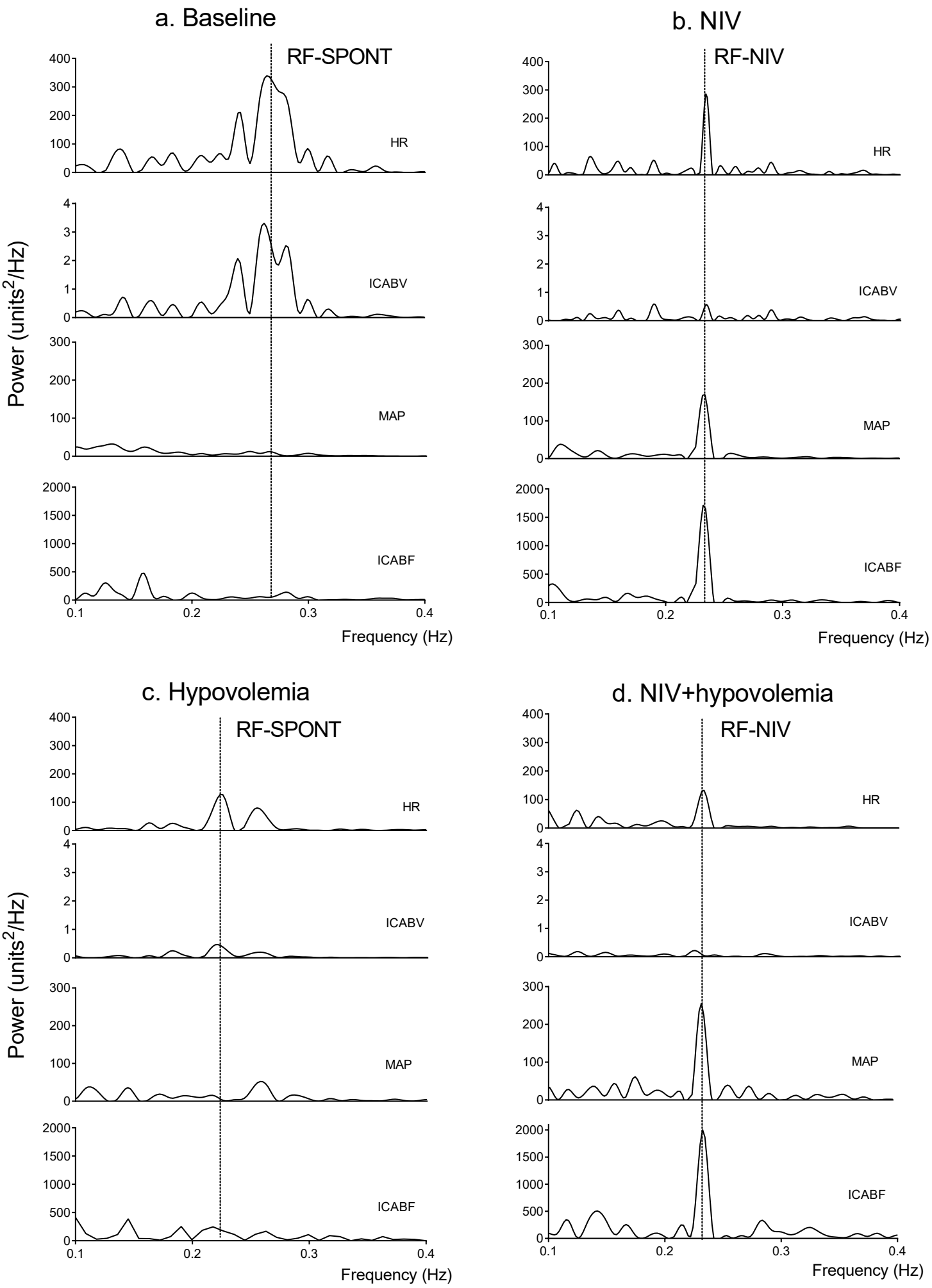

Normovolemia
LBNP
NIV
Spont
Spont
NIV
300
250
200
150
ICABF
80
60
40
HR
6
4
2
ICABV
0
300
600
900
1200
Time (s)


RF-HRV
bpm2
6
4
2
0
*
*
SPONT
NIV
LBNP
NIV+LBNP
RF-MAP variability
mmHg2
5
4
3
2
1
0
SPONT
NIV
LBNP
NIV+LBNP
SPONT
NIV
LBNP
NIV+LBNP
RF-ICABV variability
ml2
0.06
0.04
0.02
0.00
*
*
*
SPONT
NIV
LBNP
NIV+LBNP
RF-ICABF variability
(ml min-1)-2
50
40
30
20
10
0
*
*
SPONT
NIV
LBNP
NIV+LBNP


Normovolemia
Spontaneous breathing
Hypovolemia
NIV
Respiration
240
220
200
HR (bpm)
70
60
50
40
ICABV (ml)
6
5
4
3
ICABF (ml/min)
260
240
220
MAP (mmHg)
90
80
70
420
430
440
1020
1030
1040
Time (s)
Time (s)


RF-NIV
RF-spont
400
300
200
100
0
Power (Hz)
HR
4
3
2
1
0
Power (Hz)
ICABV
300
200
100
0
Power (Hz)
MAP
2000
1500
1000
500
0
Power (Hz)
ICABF
0.1
0.2
0.3
0.4
Frequency (Hz)


a. Baseline
RF-SPONT
b. NIV
RF-NIV
c. Hypovolemia
RF-SPONT
d. NIV+hypovolemia
RF-NIV
HR
ICABV
MAP
ICABF
Power (units²/Hz)
Frequency (Hz)
0.1
0.2
0.3
0.4