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Inconsistent detection of changes in cerebral blood volume by near infrared spectroscopy, in standard clinical tests

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Abstract

The attractive possibility of near infrared spectroscopy (NIRS) to non invasively assess cerebral blood volume and oxygenation is challenged by the possible interference from extracranial tissues. However to what extent this may affect cerebral NIRS monitoring during standard clinical tests is ignored.

To address this issue, 29 healthy subjects underwent a randomized sequence of 3 maneuvers that differently affect intra- and extracranial circulation: Valsalva Maneuver (VM), Hyperventilation (HV) and Head-up tilt (HUT). Putative intracranial ("i") and extracranial ("e") NIRS signals were collected from the forehead and from the cheek, respectively, and acquired together with cutaneous plethysmography at the forehead (PPG), cerebral blood velocity from the middle cerebral artery and arterial blood pressure Extracranial contribution to cerebral NIRS monitoring was investigated by comparing Beer-Lambert (BL) and spatially resolved spectroscopy (SRS) blood volume indicators (the total haemoglobin concentration, tHb, and the total haemoglobin index, THI, respectively) and by correlating their changes with changes in extracranial circulation.

While THIe and tHbe generally provided concordant indications, tHbi and THIi exhibited opposite-sign changes in a high percentage of cases (VM: 46%; HV: 31%; HUT: 40%). Moreover, tHbi was correlated with THIi only during HV ($p<0.05$), not during VM and HUT, while it correlated with PPG in all 3 maneuvers ($p<0.01$). These results evidence that extracranial circulation may markedly affect BL parameters in a high percentage of cases, even during standard clinical tests. Surface plethysmography at the forehead is suggested as complementary monitoring helpful in the interpretation of cerebral NIRS parameters.

Keywords: near infrared spectroscopy, cerebrovascular reactivity, hyperventilation, Valsalva maneuver, head-up tilt

Introduction

Near Infrared Spectroscopy is an attractive technology for non invasive monitoring tissue oxygenation and blood volume changes, also at cerebral level.

However, a number of factors limits its reliability in the clinical practice, at least in the adult subjects where NIRS must be applied in reflectance mode because transillumination is not possible (1). In fact this type of application has generated concerns about the actual sampling volume and, most significantly, the issue of signal contamination by the extracranial tissue layers (11, 15-17, 23, 32). The issue is particularly relevant if we consider that extracranial circulation may be heavily influenced by several factors including emotional stimuli, postural changes and thermoregulation. Thus, the possibility exists that changes in extracranial circulation are detected by the NIRS optodes, usually positioned on the forehead, and misinterpreted in terms of changes in cerebral parameters.

Aiming to minimize the influence of extracerebral circulation on NIRS measurements different algorithms have been applied (37, 47) and a number of techniques have been developed, such as time-resolved spectroscopy (8, 19), phase-resolved spectroscopy (14) and spatially resolved spectroscopy (SRS), the latter being based on collecting the backscattered light at multiple sites (3, 31, 43).

The interference from extracranial circulation on cerebral NIRS monitoring, particularly on classical Beer-Lambert (BL) parameters, was clearly evidenced during rather extreme maneuvers such as selective transient clamping of the external and internal carotid arteries, in surgical patients (2, 4). However, the contribution of extracerebral vascular beds to changes in putative intracerebral NIRS variables remains difficult to detect and quantify in physiological conditions and in response to standard hemodynamic tests. For this reason the higher cerebral specificity of SRS parameters is not considered to be particularly important in non-extreme conditions, standard BL parameters are still frequently used in clinical investigations and the risk of interference from extracerebral compartments is still underestimated.

We have reported preliminary observations about incongruous indications by BL and SRS parameters, which occasionally detected changes in blood volume or in tissue oxygenation of opposite sign, during neurovegetative tests (6). We hypothesized that the BL-SRS disagreement was due to a differential influence of extracranial circulation on the two sets of parameters or, in other words, that extracranial circulation was potentially capable of reverting a putatively cerebral NIRS indicator during standard clinical examinations.

With the present study we aimed to assess and document the role of extracranial circulation in disturbing NIRS monitoring of cerebral perfusion during three standard clinical tests, i.e., the Valsalva maneuver (VM), hyperventilation (HV) and head-up-tilt (HUT).These maneuvers, that are frequently adopted to investigate cerebrovascular reactivity and autoregulation also, but differently, affect extracranial circulation. For this reason they constitute a good model to reveal the possible extracranial influence on NIRS parameters. The study is based on the NIRO 300 (Hamamatsu Photonics) which implements both the original BL methodology with the more recently developed SRS and allows for direct comparison of the two sets of data.

The attention is focused in particular on the detection of changes in blood volume as detected by the change in total hemoglobin concentration (tHb), provided by BL algorithm, and the total hemoglobin index (THI), provided by SRS. Moreover, changes in blood volume occurring in the extracranial circulation were simultaneously monitored in different ways: by a second NIRS channel, the probe being applied on the cheek, and by a cutaneous photoplethysmographic device applied on the forehead. In a smaller number of subjects a cutaneous laser Doppler flowmeter applied to the forehead was also employed.

Methods

Subjects

Twenty-nine healthy volunteers, aged between 23 and 40 yr (8 males and 21 females), were enrolled in the study after providing written informed consent. The study was conducted at the “C Mondino” Neurological Hospital after approval by the local Ethical Committee.

Protocols

The study was performed in a quiet room at a constant ambient temperature (~ 23°C). The subjects were kept supine on an electrical auto-tilt table, were not allowed to speak during the experiment and were asked to keep their eyes closed and relax.

The three different maneuvers were performed sequentially in randomized order, separated by a resting period of 10-15 minutes during which the subjects remained in the supine position.

Valsalva maneuver

The subjects performed VM by expiring through a closed mouthpiece connected to a manometer that they could read (33). They were requested to generate and hold a positive alveolar pressure of 40 mmHg for 15 s, after a normal-size inspiration. A small leak in the tubing prevented the subjects from maintaining the pressure by closing the glottis (33).

Hyperventilation

The subjects were asked to hyperventilate to achieve and maintain for 1 min an end-tidal carbon dioxide pressure ($P_{ET}CO_2$) of 20 mmHg. Visual feedback was obtained directly from the display of the capnograph (20).

Head-up tilt

Passive head-up tilt to 70° for 5 min was performed. The bed was electrically operated by the experimenter; up and down rotation being performed in about 20s.

Near Infrared Spectroscopy

NIRS monitoring was performed by a two-channel NIRO 300 monitor (Hamamatsu Photonics K.K.).

The NIRO 300 is a noninvasive bedside monitor that employs 4 pulsed laser diodes (emitter optode) emitting light at different wavelengths (775, 810, 850 and 910 nm) and collects scattered light by 3 closely placed photodiodes (receiver optode). The device simultaneously provides 3 parameters by conventional differential spectroscopy, based on a modified BL law (12) and on the information collected by a single photodiode, and 2 parameters based on SRS (3, 31) that takes advantage of all three photodiodes (4).

BL parameters measure concentration changes in oxyhemoglobin (O_2Hb), deoxyhemoglobin (HHb) and total hemoglobin (tHb= O_2Hb+HHb) and are all expressed in µM/L; they do not provide a measure of the absolute concentration of the chromophores, but of concentration changes with respect to an arbitrary value (31).

SRS measures tissue oxygenation by the tissue oxygenation index (TOI), expressed in %, and total tissue hemoglobin concentration by the tissue hemoglobin index (THI) expressed in arbitrary units. These two parameters allow to assess relative changes in tissue oxygenation and tissue blood volume.

Intracranial measurements

One NIRS channel was used for intracranial monitoring, the probe being placed high on left side of the forehead to exclude the temporalis muscle from the sampling volume and

sufficiently lateral from the midline to exclude the superior sagittal sinus. NIRS optodes were set at a distance of 5 cm by a rubber holder secured to the skin by bi-adhesive foam and further stabilized by a crepe bandage around the head (2).
In addition blood velocity in the left middle cerebral artery (V_{MCA}) was monitored using a 2-MHz transcranial Doppler ultrasonography (Multidop X, DWL, Germany), the Doppler probe being held by a headset for bilateral monitoring.

Extracranial measurements

The second NIRO channel was used for extracranial monitoring, the probe being positioned on the left cheek.
In addition, extracranial circulation was monitored through an infrared plethysmograph transducer (MLT1020 IR Plethysmograph, PowerLab ADInstruments), detecting changes in blood volume from the cutaneous microcirculation (PPG), the probe being placed on the right side of the forehead. In a smaller group of subjects (n=9) laser Doppler flowmetry (LDF) (MBF3D, Moor Instruments Ltd, England) was also employed to monitor cutaneous blood flow at the forehead (right side). Application of LDF and PPG on the right side of the forehead, opposite to the intracranial NIRS monitoring, prevented possible interference with NIRS signals.

Systemic measurements

Continuous non-invasive measurement of arterial blood pressure (ABP) was performed by photo-plethysmography (Finapres, Ohmeda 2300, USA) applied to the right third finger.
$P_{ET}CO_2$ was continuously recorded using a small nasal cannula connected to a capnograph (Ohmeda 4700 OxiCap, USA).

Signal Acquisition and Processing

All NIRS signals from both channels, O_2Hbi, HHbi, tHbi, THIi, TOIi, O_2Hbe, HHbe, tHbe, THIe and TOIe (the subscripts *i* and *e* indicate the intracranial and extracranial monitoring, respectively) were continuously acquired and digitally transferred to PC by a proprietary software (Hamamatsu Photonics) (sampling frequency: 2Hz) throughout the whole session. These data were subsequently exported in text files for off-line analysis under Microsoft Excel.

In addition, V_{MCA}, ABP, $P_{ET}CO_2$, PPG and LDF, along with some of the NIRS signals (tHbi, THIi, tHbe and THIe) were continuously acquired on PC (PowerLab ML 785 ADInstruments) (sampling freq = 200 Hz) throughout the whole session.

The same software enabled off-line calculation of heart rate (HR) and was used to extract mean values and relative changes of the different signals throughout the different maneuvers.

Data analysis and statistics

The response to the different maneuvers was assessed by computing absolute or relative changes exhibited by the different variables with respect to the pre-test (control) value. The control value was computed as the mean value over a 30-s interval immediately before the beginning of the test, whereas mean values elicited by each maneuver were computed over the phase II of the response to VM (46), over a 20-s interval starting 40 s after the beginning of HV, and over a 30-s interval starting 3 min after the beginning of HUT.

Inconsistency between BL and SRS parameters was assessed by detecting opposite changes in the blood volume indicators tHbi and THIi in response to the different maneuvers.

Data are presented as mean±SD.

Changes produced on the different variables (V_{MCA}, ABP, HR, TOIi, THIi, tHbi, TOIe, THIe, tHbe and PPG) with respect to the pre-test (control) values were tested separately for the three maneuvers by means of a multivariate ANOVA and the Tukey HSD post-hoc test. Significance of changes in LDF, that was collected from a smaller number of subjects, was separately assessed by the Student's t-test.

Pearson correlation coefficient was calculated to assess the correlation between different parameters.

Results

Out of the 29 recruited subjects, two failed to complete the HUT, in 5 subjects the extracranial NIRS recording was not performed because of one probe being under scheduled maintenance and in 2 subjects changes in extracranial NIRS variables during VM could not be assessed due to saturation of the signals.

Valsalva Maneuver

The response to VM from a representative subject is shown in Fig. 1. In the ABP trace the different phases of the response can be identified, labeled from I to IV (46). V_{MCA} exhibited the initial decrease, followed by the gradual recovery starting before the end of the maneuver.

It can be observed that, while the extracranial NIRS variables tHbe and THIe concordantly detect an increase in tissue blood volume at the cheek, the two cerebral parameters give a contradictory indication: a decrease in THIi and a clear-cut increase in tHbi. Notably, the same pattern of increase exhibited by tHbi is also exhibited by skin plethysmography at the forehead (PPG).

Average changes, evaluated in phase II, are shown in Table 1 for the different variables. VM systemic effects resulted in non significant changes in ABP and significant HR increase (from 75.8 ± 10.2 to 100.3 ± 18.3 bpm) . At cerebral level both V_{MCA} and TOI significantly decreased while inconsistent indication about cerebral blood volume is provided by THIi and tHbi, the former was not significantly affected while the latter was significantly increased. In particular, tHbi increased in 100% of cases while THIi decreased in 46% which means that THIi and tHbi provided a contradictory indication, as pointed out for the subject recorded in Fig. 2, in almost half of the population examined.

The lower part of Table 1 reports values obtained from extracerebral monitoring.

While LDF produced variable and non significant results, both NIRS (cheek) and surface photoplethysmography applied to the forehead reported very significant increases in the blood volume indicators THIe, tHbe, PPG and TOIe.

The scatter plots shown in Fig. 2 help to understand the correlation between the different variables, while all R values are summarized in Tab. 2. In particular, Fig. 2a underlines the agreement between THIe and tHbe which never gave contradictory indications and exhibited a correlation of R = 0.54 ($p<0.05$) which rises to 0.74 after removing one outlier. Conversely, a non significant correlation was observed between tHbi and THIi (Fig. 2b). It is interesting to observe that a significant correlation resulted between tHbi and tHbe (R = 0.48, $p<0.05$) (Fig. 2c) and between tHbi and PPG (R = 0.50, $p<0.01$) (Fig. 2d).

Notably, cutaneous plethysmography at the forehead was not correlated with tHbe (R=0.15), although they both increased in 100 % of subjects, nor with LDF (R = -0.031). LDF and PPG showed concordant changes in 30% of the cases.

Hyperventilation

The response to HV of a representative subject is shown in Fig. 3. $P_{ET}CO_2$ stabilizes at 20 mmHg during the maneuver and V_{MCA} exhibits a marked reduction with a latency of 10-15 s while ABP exhibits a transient increase. In this subject intracranial blood volume indicators exhibit opposite changes, while THIe and tHbe, as well as PPG, all indicate an increase in extracranial blood volume. On average (Table 1), HV produced a significant increase in HR (from 74.7 ± 10.4 to 104.2 ± 20.8 bpm), a small decrease in ABP (from 85.8 ± 13.2 to 80.5 ± 15.4 mmHg), along with a marked and sustained decrease in V_{MCA} (from 62.4 ± 13.11 to 46.5 ± 7.3 cm/s).

Blood volume in the extracranial compartment (cheek) exhibited changes of variable sign in the different subjects resulting in non significant average change. However a good correlation resulted between THIe and tHbe (R=0.73, p<0.01) (Fig. 4a). At cerebral level tHbi was not significantly affected, while THIi exhibited on average a significant decrease (it was reduced in 80% of the subjects). When looking at individual trials, the two parameters provided contradictory indications in 31 % of the cases (Fig. 4b).

tHbi resulted significantly correlated with the extracranial indicators tHbe (R = 0.57, p<0.01) (Fig. 4c) and PPG (R = 0.70, p<0.01) (Fig. 4d), as well as with THIi (R=0.47, p<0.05) (Fig 4b).

With respect to VM, a lower agreement is here observed between extracranial blood volume changes at the cheek (tHbe) and at the forehead (PPG), exhibiting opposite sign in

50 % of the cases, while a 78% agreement and a significant correlation is observed between LDF and PPG (R = 0.86, P<0.01).

Notably, oxygenation indices show again opposite average changes at intracranial (increase) and extracranial (decrease) level.

Head-up tilt

The response to HUT in a representative subject is shown in Fig. 5. A slight decrease in V_{MCA}, ABP and $P_{ET}CO_2$ can be observed, particularly in the first minutes after the tilt-up. NIRS blood volume indices exhibit discordant patterns at cerebral level with a decrease in tHbi and an increase in THIi. At the cheek level no appreciable change in tHbe and a clear decrease in THIe are exhibited while forehead skin blood volume (PPG) decreased with a time course remarkably similar to tHbi's.

On average (see Table 1), HUT elicited a significant increase in HR (from 73.7 $\pm$ 9.8 to 89.6 $\pm$ 10.8 bpm), non-significant changes in ABP (at heart level), and a significant decrease in V_{MCA} (from 64.2 $\pm$ 15.6 to 59.2 $\pm$ 11.2 cm/s).

Unlike previous maneuvers, oxygenation indices show concordant changes: a significant decrease in both TOIi and TOIe.

At extracranial level blood volume does not appear to be univocally affected. At the cheek level both THIe and tHbe reveal a significant decrease (in 100% of the subjects) and a good correlation (R = 0.63, p<0.01) (Fig. 6a) while cutaneous blood volume index from forehead (PPG) evidences a greater response variability (8 increases out of 29 subjects), resulting in a non-significant change. In addition PPG variations were not correlated with changes in tHbe (R = -0.068), the two variables exhibiting changes of opposite sign in 30 % of the cases.

With regard to the intracranial compartment, both THIi and tHbi showed a large variability and no significant changes on average; nevertheless, when looking at the individual trials, the two parameters yielded contradictory indications in 40 % of the cases and resulted to be non significantly correlated (*R* = 0.27) (Fig. 6b).
At variance with what observed for VM and HV, the scatter plots here evidenced a non significant correlation between changes in tHbi and changes in tHbe (*R* = 0.37, Fig. 6c). However, tHbi was still significantly correlated with PPG (*R* = 0.55, $p<0.01$, Fig. 6d); in particular, it can be observed that large changes in PPG are associated with large changes in tHbi. This relationship also hold for responses to VM and HV (Fig. 2d, 4d and Fig. 6d). PPG and LDF exhibited a 70 % agreement and a non significant correlation.

Figure 7 provides a summary of the correlations among the following variables: tHbi, THIi (intracranial variables), PPG and tHbe (extracranial variables). In order to graphically emphasize the degree of correlation between two given variables, these have been connected by lines whose thickness is proportional to the *R* value. In addition, dashed instead of continuous lines have been used whenever the correlation was not statistically significant. By considering the three maneuvers all together it can be observed that tHbi, the putative intracranial BL parameter, exhibits a stronger correlation with extracranial indicators, i.e., tHbe (2 out of 3 correlations are statistically significant) and PPG (all correlations are significant), than with THIi (only 1 correlation is significant). Conversely, THIi exhibits a weak correlation with the same extracranial indicators PPG (only 1 of the correlations is significant) and tHbe (none of the correlations is significant).

The correlations between V_{MCA} and the two intracranial NIRS parameters tHbi and THIi are always non-significant (Tab. 2).

Discussion

The present study shows that tHbi and THIi, respectively the BL and SRS NIRS indicators of cerebral blood volume, give contradictory information in a high percentage of cases, ranging between 31 and 46% (average 39%), during maneuvers routinely used in clinical investigations. Such inconsistency is observed to a much lesser extent in extracranial NIRS monitoring (occurrence of discordant indications between tHbe and THIe: 6.6 %, on average).

The strong correlation observed between the tHbi and the extracranial indicators of blood volume, as compared to the weak or absent correlation between THI and the same parameters (Fig. 7), suggests that extracranial circulation is responsible for the observed inconsistency between BL and SRS. This interpretation is supported by the notion that BL parameters are intrinsically more sensitive than SRS parameters to extracranial circulation.

The results will be separately discussed for the different maneuvers before final considerations are drawn.

Valsalva maneuver

VM produces a large increase in intrathoracic pressure, which hinders venous return and increases blood pressure in venous compartments (18, 33, 41, 46). The resulting marked increase of blood volume in extracranial compartments has been clearly detected by PPG, THIe and tHbe.

The effect on blood volume at intracranial level is not as straightforward. In fact, cerebral blood volume can increase only if cerebrospinal fluid volume decreases (total volume of the cranium cannot change) however both central venous pressure and central spinal fluid pressure are increased during the maneuver (22, 24, 33). A number of studies, employing

BL-based NIRS, report increased cerebral blood volume and oxygenation during VM (34, 36, 49). We also consistently observed an increase in tHbi (100% of cases) however THIi decreased in 46% of subjects. This suggests that intracranial blood volume can possibly be reduced during VM and that the increased volume of extracranial compartments heavily affects the tHbi indicator. In addition, it cannot be excluded that also THIi could have been affected to a small extent, which would imply an overestimation of the intracranial blood volume change by THIi.

SRS-derived information about tissue oxygenation also deserves consideration. In fact it is interesting to observe that, while TOIe was significantly increased (cheek level), TOIi consistently decreased at intracranial level. This is at variance with other studies in which a paradoxical increase in cerebral oxygenation was detected by BL-based NIRS (34, 36). The VM-induced increase in central venous and intracranial pressures produces a decrease in cerebral perfusion pressure that impairs cerebral blood flow (10, 46). Such a situation fits well with the observed decrease in V_{MCA} and the decrease in cerebral oxygenation, as detected by the SRS parameter TOIi. It is possible that, also in this case, the disagreement with BL-based data from the literature is due to the greater sensitivity of BL parameters to changes in extracranial circulation, as compared to SRS's.

Hyperventilation

HV induces transient arterial hypocapnia and alkalosis provoking a rapid cerebral vasoconstriction, cerebral blood flow reduction (21, 30) and increased cerebral oxygen extraction (48). This results, as also observed in the present study, in both a marked reduction of V_{MCA} (7, 39) and a decrease in cerebral oxygenation (TOIi) (5, 44, 48, 55). A reduction of cerebral blood volume as detected by THIi (80% of subjects) is consistent with the occurrence of a marked cerebral vasoconstriction and is supported by previous studies (29, 35). On the other hand tHbi is not significantly affected by HV and gives in 28% of the subjects opposite indication to THIi.

In extracranial compartments, information about perfusion changes in response to HV is scanty. In one study increases in cutaneous blood flow have been reported (40). We here observe that both the NIRS signals from the cheek and cutaneous plethysmography (forehead) detected increase in blood volume in a large percentage of cases, all these signals being moderately correlated with tHbi. In particular it can be observed from the scatter plots (Figs. 4c; 4d) that largest PPG and tHbe increases are associated to the largest increases in tHbi, supporting the notion of extracranial interference on BL parameters (20, 51).

Head-up tilt

Response to HUT appears to be more complex.

At intracranial level the postural change is considered to produce a decrease in cerebral perfusion pressure which, together with the hyperventilation-induced hypocapnia induced by the hypotensive stimulus (45), may results in cerebral hypoperfusion which in turn accounts for the reduction in V_{MCA} and TOI (9, 26, 28, 39, 42), also observed in the present study.

In agreement with other studies from the literature (26, 27), significant changes in cerebral blood volume are neither detected by THIi nor by tHbi, which is possibly due to the prompt activation of local myogenic and metabolic compensatory mechanisms (25, 38).

Also at the extracranial compartment the response to HUT is not clear cut.

Decreased transmural pressure at the venous side may decrease blood volume (venous collapse) but local and neural mechanisms may also intervene. In particular the sympathetic activation driven by the orthostatic stimulus is known to increase vasoconstrictor tone in skeletal muscles but not in cutaneous tissues (52). This may explain the consistent decrease in blood volume and oxygenation indicated by the NIRS channel positioned on the cheek, which likely reflects perfusion of underlying skeletal muscle layers. Conversely, forehead plethysmography, which mostly reflects cutaneous perfusion, results in a variable response which poorly correlates with tHbe. Notably, also in the HUT response a large percentage (38%) of inconsistent indications by THIi and tHbi was observed. At variance with what observed in the other maneuvers, tHbi was poorly correlated with tHbe but still rather correlated with PPG (Figs. 6c; 6d). Thus, also in this case the occurrence of inconsistencies between cerebral blood volume indicators appears to be related to the influence of extracranial circulation on the BL parameter tHbi.

General considerations

For the sake of simplicity, in the above discussion the assumption was made that measurements were collected at "steady state", and we did not consider the possibility of different time course of the responses in the different tissues. However this assumption does not undermine the main outcomes of the work.

The three maneuvers investigated in the present study elicit very different hemodynamic responses at intra and extracranial levels. In particular: i) VM provokes a marked increase in central venous pressure which results in a large increase in blood volume of extracranial compartments with minor changes at intracranial level, ii) HV produces a marked metabolic cerebrovascular regulation with minor changes at extracranial level, while iii) HUT affects the hydrostatic gradients, and stimulates metabolic and neural regulation at both intra and extracranial levels.

The present results emphasize the notion that BL parameters are particularly sensitive to extracranial circulation (4, 51, 54), to the extent that extracranial interference may considerably alter the measured variables. This may occur not just in response to invasive interventions, like occlusion of external and internal carotid arteries, but also during the standard maneuvers commonly employed in the clinical routine, as well as in experimental investigations.

The inconsistency between tHbi and THIi represents a marker of extracranial interference on the putative cerebral NIRS monitoring. Such marker is quite easy to spot-out on the NIRO 300 that displays both SRS and BL variables at the same time, however the following few issues deserve consideration: 1) the inconsistency may only occur if extra and intracranial circulations undergo opposite changes, which is not necessarily the rule. If, for example, both compartments exhibit a simultaneous increase in blood volume tHbi would overestimate intracranial changes but would probably not disagree with THIi; 2) the interference from the extracranial compartment was here evidenced on blood volume indicators but may equally affect BL assessment of tissue oxygenation; 3) although previous studies (4, 51, 54) and the present data quite clearly demonstrate that BL parameters can be affected by extracranial circulation, whether and to what extent SRS parameters can also be affected remains to be ascertained; 4) although the present results, obtained with a specific NIRS device (the Hamamatsu Photonics, NIRO 300), cannot be readily extended to other devices, they suggest that uncorrected BL parameters should be interpreted with caution to infer hemodynamic changes at cerebral level.

In general, awareness of perfusion changes occurring in the extracranial compartment may be of good help in the interpretation of the NIRS recordings. Since NIRS is based on changes in hemoglobin concentration, surface plethysmography is to be preferred to flowmetry, also in consideration of the fact that blood flow and blood volume may not vary in a concordant way. This was well evidenced in the response to VM: besides large increases in forehead skin blood volume, a non significant blood flow decrease was detected by LDF. In addition we observed that plethysmographic monitoring of the extracranial compartment with a second NIRS channel placed on the cheek may not always be appropriate, possibly due to the different neural regulation of skin and muscle vascular beds, which makes the cheek (skin + muscle) not a good representation of the forehead (mostly skin). Surface plethysmography at the forehead proved to be better correlated to tHbi (particularly during HUT) and to better help in understanding the inconsistency between tHbi and THIi. On this basis, it is proposed as a valid and inexpensive measure to be included in the experimental/clinical set-up.

The importance of specifically monitoring cutaneous circulation at the forehead is emphasized by the peculiar neural control of this area, which is profoundly affected by cognitive and emotional aspects (13, 50, 53). These factors are experimentally difficult to control and are likely to account for the observed variability of responses in the extracranial compartments.

Conclusions

In conclusion, the present study reveals that BL NIRS monitoring can be detrimentally affected by changes in extracranial circulation also in routine cardiovascular/neurovegetative tests and emphasizes its low reliability for the assessment of cerebral perfusion. Postural, mechanical and neural changes, that may occur under most investigative maneuvers, alter blood perfusion or/and distribution in the extracranial compartment and affect BL NIRS variables to the extent that detected changes in cerebral tissue blood volume and oxygenation can be frequently reversed. On the basis of the present data, forehead cutaneous plethysmography is suggested as an additional measure to complement the hemodynamic monitoring and help in the interpretation of NIRS recordings.

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References

1. **Al-Rawi PG**. Near infrared spectroscopy in brain injury: today's perspective. *Acta Neurochir Suppl* 95: 453-457, 2005.

2. **Al-Rawi PG, and Kirkpatrick PJ**. Tissue oxygen index: thresholds for cerebral ischemia using near-infrared spectroscopy. *Stroke* 37: 2720-2725, 2006.

3. **Al-Rawi PG, Smielewski P, Hobbiger H, Ghosh S, and Kirkpatrick PJ**. Assessment of spatially resolved spectroscopy during cardiopulmonary bypass. *Journal of Biomedical Optics* 4: 208-216, 1999.

4. **Al-Rawi PG, Smielewski P, and Kirkpatrick PJ**. Evaluation of a near-infrared spectrometer (NIRO 300) for the detection of intracranial oxygenation changes in the adult head. *Stroke* 32: 2492-2500, 2001.

5. **Bosone D, Canova, D., Roatta, S., Marcheselli, S., Passatore, M., Micieli, G.** Correlation between tissue oxygenation index (TOI) and CBV: a tool to explore CBF. *Cerebrovascular Disease* 13 (suppl 4): 36, 2002.

6. **Bosone D, Canova, D., Roatta, S., Tosi, P., Micieli, G.** Contribution of extracranial circulation in cerebral hemodynamics evaluated by Near Infrared Spectroscopy. *Cerebrovascular Disease* 16 (suppl 2): 8, 2003.

7. **Bradac GB, Simon RS, and Heidsieck CH**. Angiographically verified transient alteration of the intracranial arteries and veins in dependence of different CO2 tensions. *Neuroradiology* 10: 257-262, 1976.

8. **Calderon-Arnulphi M, Alaraj A, and Slavin KV**. Near infrared technology in neuroscience: past, present and future. *Neurol Res* 31: 605-614, 2009.

9. **Carey BJ, Manktelow BN, Panerai RB, and Potter JF**. Cerebral autoregulatory responses to head-up tilt in normal subjects and patients with recurrent vasovagal syncope. *Circulation* 104: 898-902, 2001.

10. **Dawson SL, Panerai RB, and Potter JF**. Critical closing pressure explains cerebral hemodynamics during the Valsalva maneuver. *J Appl Physiol* 86: 675-680, 1999.

11. **Dehghani H, and Delpy DT**. Near-infrared spectroscopy of the adult head: effect of scattering and absorbing obstructions in the cerebrospinal fluid layer on light distribution in the tissue. *Appl Opt* 39: 4721-4729, 2000.

12. **Delpy DT, Cope M, van der Zee P, Arridge S, Wray S, and Wyatt J**. Estimation of optical pathlength through tissue from direct time of flight measurement. *Phys Med Biol* 33: 1433-1442, 1988.

13. **Drummond PD**. Sweating and vascular responses in the face: normal regulation and dysfunction in migraine, cluster headache and harlequin syndrome. *Clin Auton Res* 4: 273-285, 1994.

14. **Duncan A, Whitlock TL, Cope M, and Delpy DT**. A multiwavelenght, wideband, intensity modulated optical spectrometer for near infrared spectroscopy and imaging. *Proc SPIE* 1888: 248-257, 1993.

15. **Germon TJ, Evans PD, Barnett NJ, Wall P, Manara AR, and Nelson RJ**. Cerebral near infrared spectroscopy: emitter-detector separation must be increased. *Br J Anaesth* 82: 831-837, 1999.

16. **Germon TJ, Kane NM, Manara AR, and Nelson RJ**. Near-infrared spectroscopy in adults: effects of extracranial ischaemia and intracranial hypoxia on estimation of cerebral oxygenation. *Br J Anaesth* 73: 503-506, 1994.

17. **Germon TJ, Young AE, Manara AR, and Nelson RJ**. Extracerebral absorption of near infrared light influences the detection of increased cerebral oxygenation monitored by near infrared spectroscopy. *J Neurol Neurosurg Psychiatry* 58: 477-479, 1995.

18. **Gisolf J, van Lieshout JJ, van Heusden K, Pott F, Stok WJ, and Karemaker JM**. Human cerebral venous outflow pathway depends on posture and central venous pressure. *J Physiol* 560: 317-327, 2004.

19. **Gratton E, Fantini S, Franceschini MA, Gratton G, and Fabiani M**. Measurements of scattering and absorption changes in muscle and brain. *Philos Trans R Soc Lond B Biol Sci* 352: 727-735, 1997.

20. **Grubhofer G, Tonninger W, Keznickl P, Skyllouriotis P, Ehrlich M, Hiesmayr M, and Lassnigg A**. A comparison of the monitors INVOS 3100 and NIRO 500 in detecting changes in cerebral oxygenation. *Acta Anaesthesiol Scand* 43: 470-475, 1999.

21. **Halpern P, Neufeld MY, Sade K, Silbiger A, Szold O, Bornstein NM, and Sorkine P**. Middle cerebral artery flow velocity decreases and electroencephalogram (EEG) changes occur as acute hypercapnia reverses. *Intensive Care Med* 29: 1650-1655, 2003.

22. **Hamilton WF, Woodbury, R.A., Harper, H.T.** Arterial, cerebrospinal and venous pressures in man during cough and strain. *Am J Physiol* 141: 42-50, 1944.

23. **Harris DN, Cowans FM, and Wertheim DA**. NIRS in the temporal region--strong influence of external carotid artery. *Adv Exp Med Biol* 345: 825-828, 1994.

24. **Haykowsky MJ, Eves ND, DE RW, and Findlay MJ**. Resistance exercise, the Valsalva maneuver, and cerebrovascular transmural pressure. *Med Sci Sports Exerc* 35: 65-68, 2003.

25. **Hughson RL, Edwards MR, O'Leary DD, and Shoemaker JK**. Critical analysis of cerebrovascular autoregulation during repeated head-up tilt. *Stroke* 32: 2403-2408, 2001.

26. **Hunt K, Tachtsidis I, Bleasdale-Barr K, Elwell C, Mathias C, and Smith M**. Changes in cerebral oxygenation and haemodynamics during postural blood pressure changes in patients with autonomic failure. *Physiol Meas* 27: 777-785, 2006.

27. **Krakow K, Ries S, Daffertshofer M, and Hennerici M**. Simultaneous assessment of brain tissue oxygenation and cerebral perfusion during orthostatic stress. *Eur Neurol* 43: 39-46, 2000.

28. **Kurihara K, Kikukawa A, and Kobayashi A**. Cerebral oxygenation monitor during head-up and -down tilt using near-infrared spatially resolved spectroscopy. *Clin Physiol Funct Imaging* 23: 177-181, 2003.

29. **Leung TS, Tachtsidis I, Tisdall MM, Pritchard C, Smith M, and Elwell CE**. Estimating a modified Grubb's exponent in healthy human brains with near infrared spectroscopy and transcranial Doppler. *Physiol Meas* 30: 1-12, 2009.

30. **Markwalder TM, Grolimund P, Seiler RW, Roth F, and Aaslid R**. Dependency of blood flow velocity in the middle cerebral artery on end-tidal carbon dioxide partial pressure--a transcranial ultrasound Doppler study. *J Cereb Blood Flow Metab* 4: 368-372, 1984.

31. **Matcher SJ, Kirkpatrick, P.J., Nahid, K., Cope, M., Delpy, D.T.** Absolute quantification methods in tissue near infrared spectroscopy. *Proc SPIE* 2389: 486-495, 1995.

32. **Okada E, Firbank M, and Delpy DT**. The effect of overlying tissue on the spatial sensitivity profile of near-infrared spectroscopy. *Phys Med Biol* 40: 2093-2108, 1995.

33. **Pott F, van Lieshout JJ, Ide K, Madsen P, and Secher NH**. Middle cerebral artery blood velocity during a valsalva maneuver in the standing position. *J Appl Physiol* 88: 1545-1550, 2000.

34. **Pott F, Van Lieshout JJ, Ide K, Madsen P, and Secher NH**. Middle cerebral artery blood velocity during intense static exercise is dominated by a Valsalva maneuver. *J Appl Physiol* 94: 1335-1344, 2003.

35. **Rasmussen P, Dawson EA, Nybo L, van Lieshout JJ, Secher NH, and Gjedde A**. Capillary-oxygenation-level-dependent near-infrared spectrometry in frontal lobe of humans. *J Cereb Blood Flow Metab* 27: 1082-1093, 2007.

36. **Saager R, and Berger A**. Measurement of layer-like hemodynamic trends in scalp and cortex: implications for physiological baseline suppression in functional near-infrared spectroscopy. *J Biomed Opt* 13: 034017, 2008.

37. **Schelkanova I, and Toronov V**. Optimal quantitation of the cerebral hemodynamic response in functional near-infrared spectroscopy. *Opt Express* 18: 19386-19395, 2010.

38. **Schondorf R, Benoit J, and Stein R**. Cerebral autoregulation is preserved in postural tachycardia syndrome. *J Appl Physiol* 99: 828-835, 2005.

39. **Serrador JM, Picot PA, Rutt BK, Shoemaker JK, and Bondar RL**. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* 31: 1672-1678, 2000.

40. **Smielewski P, Kirkpatrick P, Minhas P, Pickard JD, and Czosnyka M**. Can cerebrovascular reactivity be measured with near-infrared spectroscopy? *Stroke* 26: 2285-2292, 1995.

41. **Stewart JM, Medow MA, Bassett B, and Montgomery LD**. Effects of thoracic blood volume on Valsalva maneuver. *Am J Physiol Heart Circ Physiol* 287: H798-804, 2004.

42. **Suzuki K, Asahina M, Suzuki A, and Hattori T**. Cerebral oxygenation monitoring for detecting critical cerebral hypoperfusion in patients with multiple system atrophy during the head-up tilt test. *Intern Med* 47: 1681-1687, 2008.

43. **Suzuki S, Takasaki S, Ozaki T, and Kobayashi Y**. A tissue oxygenation monitor using NIR spatially resolved spectroscopy. *Proc SPIE* 3597: 582-592, 1999.

44. **Thavasothy M, Broadhead M, Elwell C, Peters M, and Smith M**. A comparison of cerebral oxygenation as measured by the NIRO 300 and the INVOS 5100 Near-Infrared Spectrophotometers. *Anaesthesia* 57: 999-1006, 2002.

45. **Thomas KN, Cotter JD, Galvin SD, Williams MJ, Willie CK, and Ainslie PN**. Initial orthostatic hypotension is unrelated to orthostatic tolerance in healthy young subjects. *J Appl Physiol* 107: 506-517, 2009.

46. **Tiecks FP, Lam AM, Matta BF, Strebel S, Douville C, and Newell DW**. Effects of the valsalva maneuver on cerebral circulation in healthy adults. A transcranial Doppler Study. *Stroke* 26: 1386-1392, 1995.

47. **Umeyama S, and Yamada T**. Monte Carlo study of global interference cancellation by multidistance measurement of near-infrared spectroscopy. *J Biomed Opt* 14: 064025, 2009.

48. **Vanderhaegen J, Naulaers G, Vanhole C, De Smet D, Van Huffel S, Vanhaesebrouck S, and Devlieger H**. The effect of changes in tPCO2 on the fractional tissue oxygen extraction--as measured by near-infrared spectroscopy--in neonates during the first days of life. *Eur J Paediatr Neurol* 13: 128-134, 2009.

49. **Vanhatalo S, Tallgren P, Becker C, Holmes MD, Miller JW, Kaila K, and Voipio J**. Scalp-recorded slow EEG responses generated in response to hemodynamic changes in the human brain. *Clin Neurophysiol* 114: 1744-1754, 2003.

50. **Vassend O, and Knardahl S**. Personality, affective response, and facial blood flow during brief cognitive tasks. *Int J Psychophysiol* 55: 265-278, 2005.

51. **Virtanen J, Noponen T, and Merilainen P**. Comparison of principal and independent component analysis in removing extracerebral interference from near-infrared spectroscopy signals. *J Biomed Opt* 14: 054032, 2009.

52. **Vissing SF**. Differential activation of sympathetic discharge to skin and skeletal muscle in humans. *Acta Physiol Scand Suppl* 639: 1-32, 1997.

53. **Voncken MJ, and Bogels SM**. Physiological blushing in social anxiety disorder patients with and without blushing complaints: two subtypes? *Biol Psychol* 81: 86-94, 2009.

54. **Wabnitz H, Moeller M, Liebert A, Obrig H, Steinbrink J, and Macdonald R**. Time-resolved near-infrared spectroscopy and imaging of the adult human brain. *Adv Exp Med Biol* 662: 143-148, 2010.

55. **Yoshitani K, Kawaguchi M, Tatsumi K, Kitaguchi K, and Furuya H**. A comparison of the INVOS 4100 and the NIRO 300 near-infrared spectrophotometers. *Anesth Analg* 94: 586-590; table of contents, 2002.

Figure legends

Fig.1 The response to VM in a representative subject. From top to bottom: cerebral blood velocity (V_{MCA}), arterial blood pressure (ABP), end tidal CO_2 ($P_{ET}CO_2$), BL and SRS blood volume indices (tHb and THI, respectively) from the intracranial and extracranial compartments (i and e subscripts, respectively), as detected by the probes positioned on the forehead and on the cheek, respectively, and surface forehead plethysmography (PPG). Maneuver start-end points are marked by dashed lines. Labels indicating the different phases of the response are placed on the ABP trace (I = phase I, IIa-IIb = phase II, III = phase III, IV = phase IV). Disagreement between intracranial blood volume indices is evidenced by a dashed circle.

Fig.2 Scatter plots illustrating the correlation between VM-induced changes in different variables: a) SRS vs. BL extracranial blood volume indicators; b) SRS vs. BL intracranial blood volume indicators c) Extra- vs. intra-cranial BL blood volume indicators d) BL intracranial blood volume vs. forehead skin blood volume. Abbreviations as in Fig. 1; For correlation coefficients see text.

Fig.3 The response to HV from a representative subject. Abbreviations as in Fig.1. HV start-end points are marked by dashed lines. Disagreement between intracranial blood volume indices is evidenced by a dashed circle.

Fig.4 Scatter plots illustrating the correlation between HV-induced changes in different variables. Explanation as in Fig. 2.

Fig.5 The response to HUT in a representative subject. Abbreviations as in Fig. 1. The two leftmost vertical dashed lines indicate the tilt-up phase while the rightmost indicate the tilt-down. The dashed circle evidences the contradictory information provided by the two intracranial indices.

Fig.6 Scatter plots illustrating the correlation between HUT-induced changes in different variables. Explanation as in Fig. 2.

Fig.7 Graphic summary of the correlations among THIi, tHbi, PPG and tHbe for the different maneuvers: VM (black), HV (dark grey) and HUT (light grey). Solid lines indicate significant correlations ($p<0.05$), broken lines indicate non significant correlations; line thickness is proportional to R value. Abbreviations as in Fig. 1.

TABLES

Table 1. Average changes produced by Valsalva maneuver (VM), Hyperventilation (HV) and head-up tilt (HUT) on the different variables.

	VM	HV	HUT
V_{MCA} (%)	-6.75 ± 7.78 **	-23.63 ± 14.46 **	-6.23 ± 6.10 *
ABP (%)	4.84 ± 11.44	-4.10 ± 7.53 *	3.71 ± 19.52
HR (%)	32.42 ± 17.55 **	40.25 ± 24.43 **	21.41 ± 12.20 **
TOIi (%)	-4.77 ± 2.96 **	-5.18 ± 3.04 **	-3.19 ± 4.22 **
THIi (%)	2.30 ± 9.80	-3.90 ± 4.48 *	-1.02 ± 14.61
tHbi (μM)	6.73 ± 3.49 **	0.48 ± 1.97	0.77 ± 3.68
TOIe (%)	6.25 ± 4.98 **	3.41 ± 2.73 **	-6.78 ± 3.33 **
THIe (%)	33.85 ± 14.97 **	2.80 ± 6.56	-11.61 ± 5.87 **
tHbe (μM)	16.13 ± 8.81 **	1.17 ± 3.73	-4.75 ± 3.52 **
PPG (a.u.)	3.74 ± 3.02 **	0.71 ± 2.07	-0.58 ± 3.30
LDF (%)	-16.71 ± 37.00	32.89 ± 43.30	-27.47 ± 13.24 **

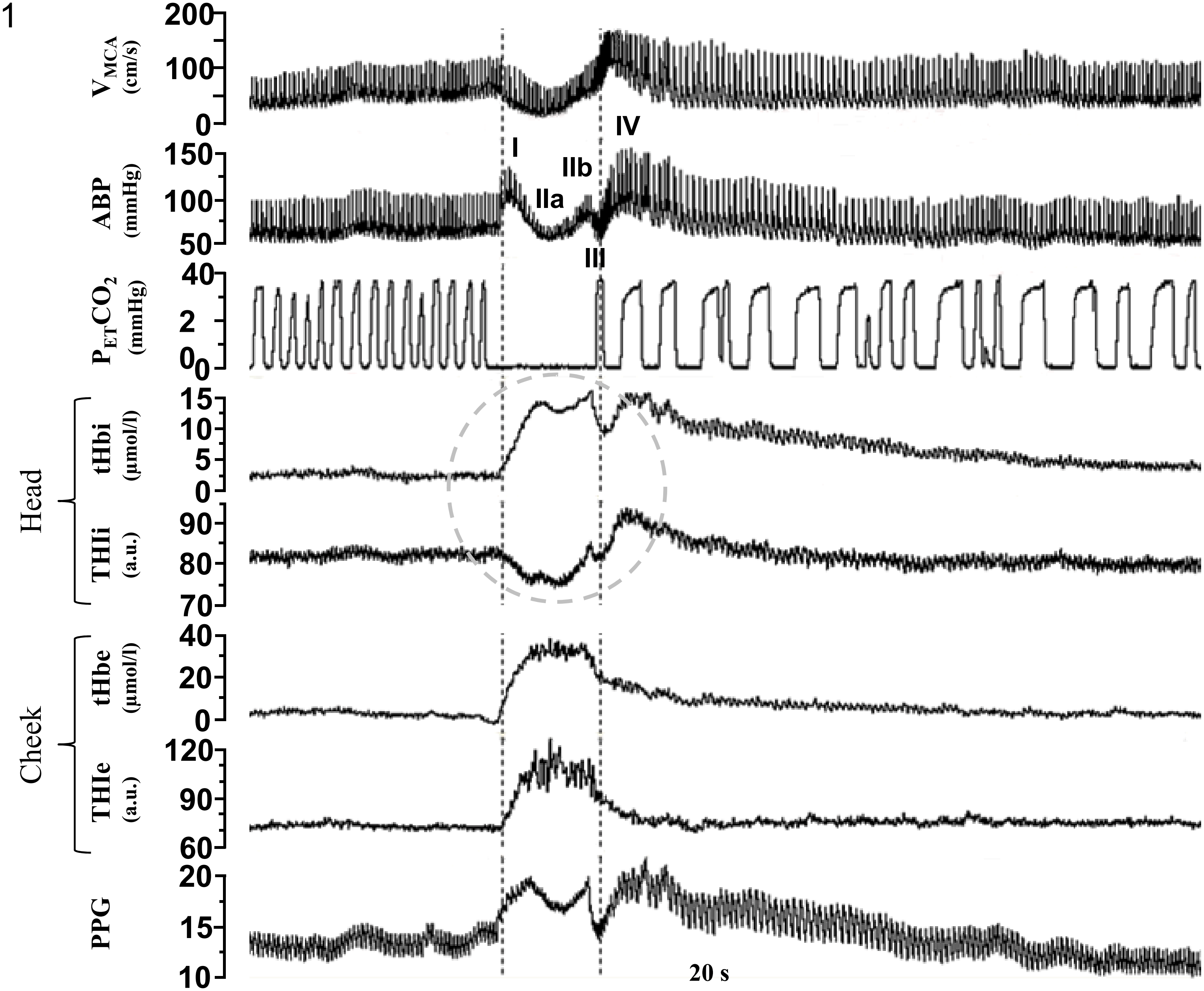
V_{MCA}= cerebral blood velocity, ABP=arterial blood pressure, HR=heart rate, TOI=tissue oxygenation index, THI=total hemoglobin index, tHB= total hemoglobin concentration, PPG= cutaneous plethysmography at the forehead; LDF= cutaneous Laser Doppler flowmetry at the forehead. Subscripts *i* and *e* indicate intracranial (at the forehead) and extracranial (at the cheek) NIRS monitoring. Relative changes are expressed in %, absolute changes in the original units. * = $P < 0.05$; ** = $P < 0.01$

Table 2. Strength of the linear correlation (R) between changes exhibited by different pairs of variables in response to the 3 maneuvers.

	VM	HV	HUT
THIi vs. tHbi	0.27	0.47 *	0.27
tHbi vs. tHbe	0.48 *	0.57 **	0.37
tHbi vs. PPG	0.50 **	0.70 **	0.55 **
THIi vs. tHbe	0.10	0.10	0.41
THIi vs. PPG	0.37 *	0.20	-0.11
THIe vs. tHbe	0.54 *	0.73 **	0.63 **
PPG vs. tHbe	0.15	0.40	-0.068
LDF vs. PPG	-0.031	0.86 **	0.079
THIi vs. V_{MCA}	0.27	0.0087	0.092
tHbi vs. V_{MCA}	-0.18	-0.32	-0.14

Abbreviations as in Tab. 1. * = $P < 0.05$; ** = $P < 0.01$

Fig. 1



V_{MCA} (cm/s)
200
100
0
I
IIa
IIb
III
IV
ABP (mmHg)
150
100
50
$P_{ET}CO_2$ (mmHg)
40
2
0
0
Head
tHbi (µmol/l)
15
10
5
0
THIi (a.u.)
90
80
70
Cheek
tHbe (µmol/l)
40
20
0
THIe (a.u.)
120
90
60
PPG
20
15
10
20 s

Fig. 2

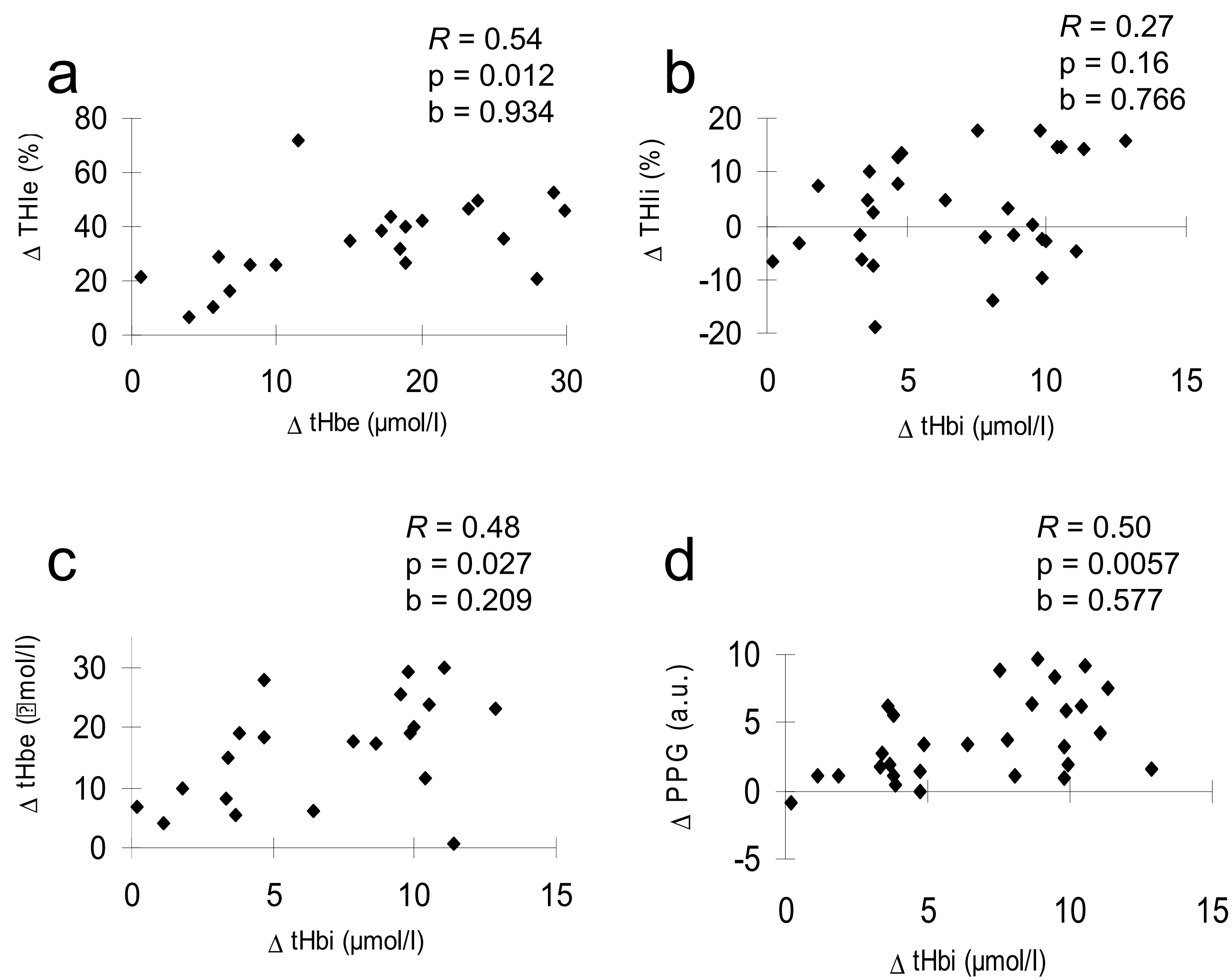
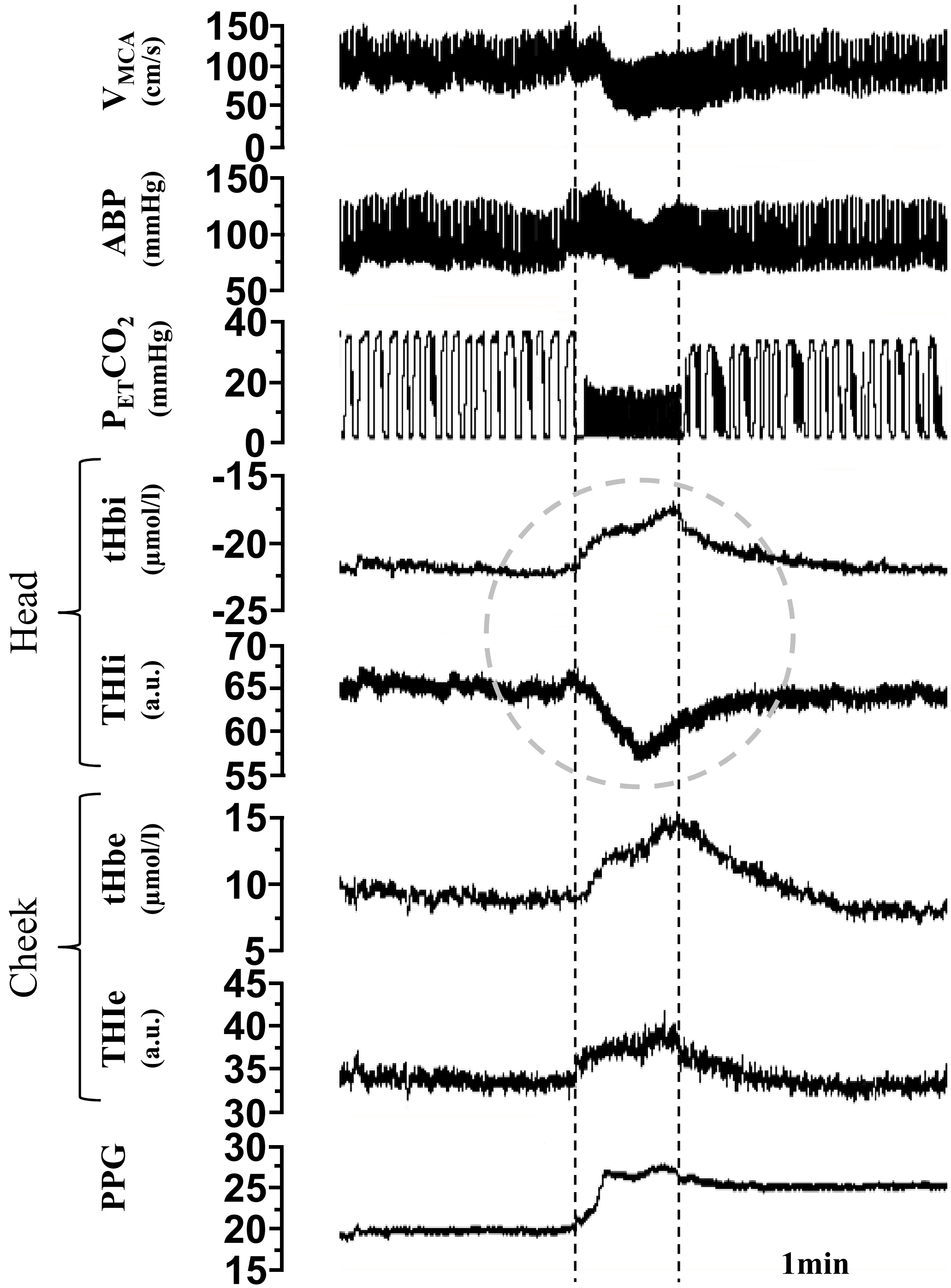
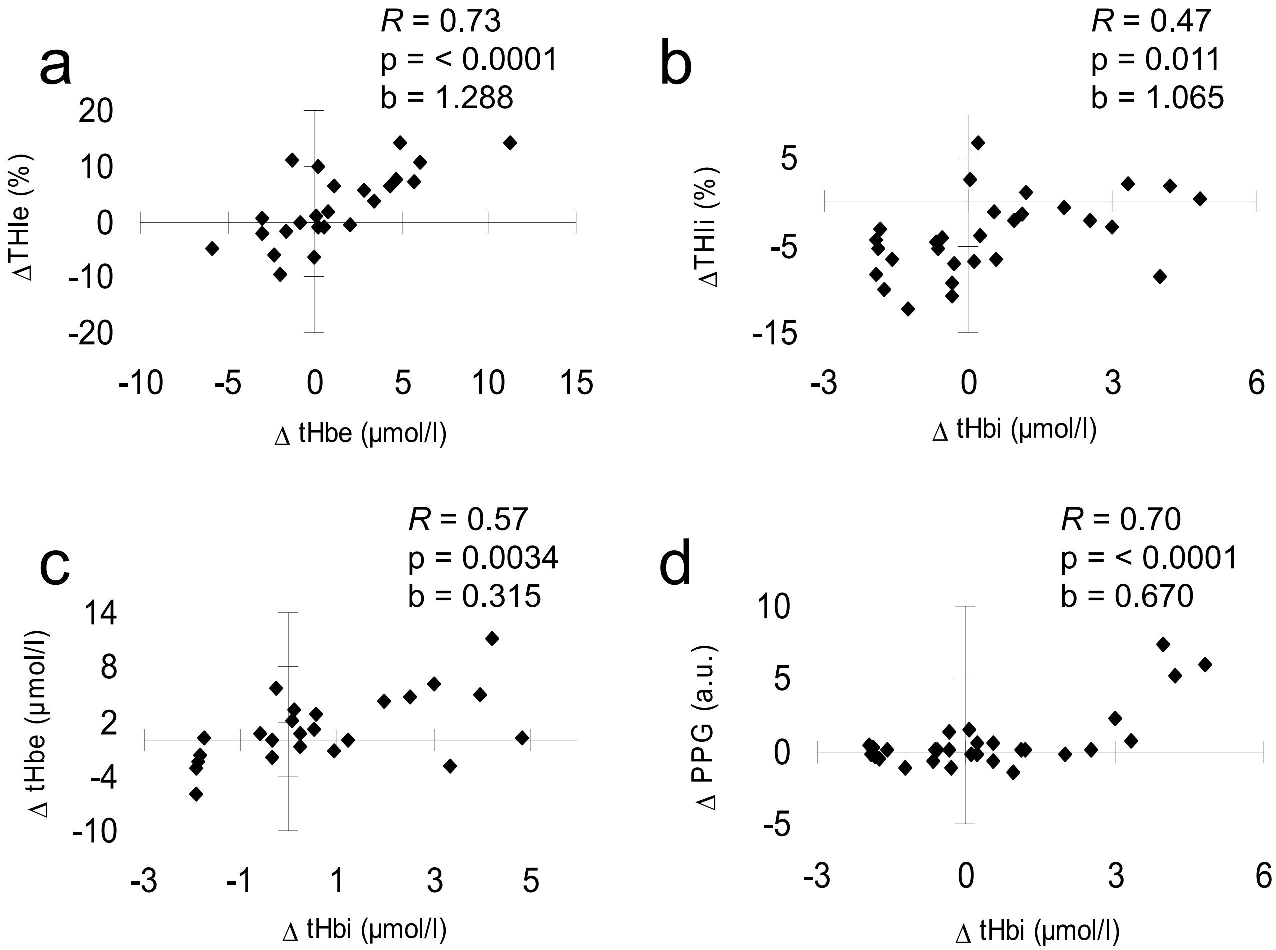

a
R = 0.54
p = 0.012
b = 0.934
Δ THIe (%)
Δ tHbe (µmol/l)
b
R = 0.27
p = 0.16
b = 0.766
Δ THIi (%)
Δ tHbi (µmol/l)
c
R = 0.48
p = 0.027
b = 0.209
Δ tHbe (µmol/l)
Δ tHbi (µmol/l)
d
R = 0.50
p = 0.0057
b = 0.577
Δ PPG (a.u.)
Δ tHbi (µmol/l)

Fig. 3



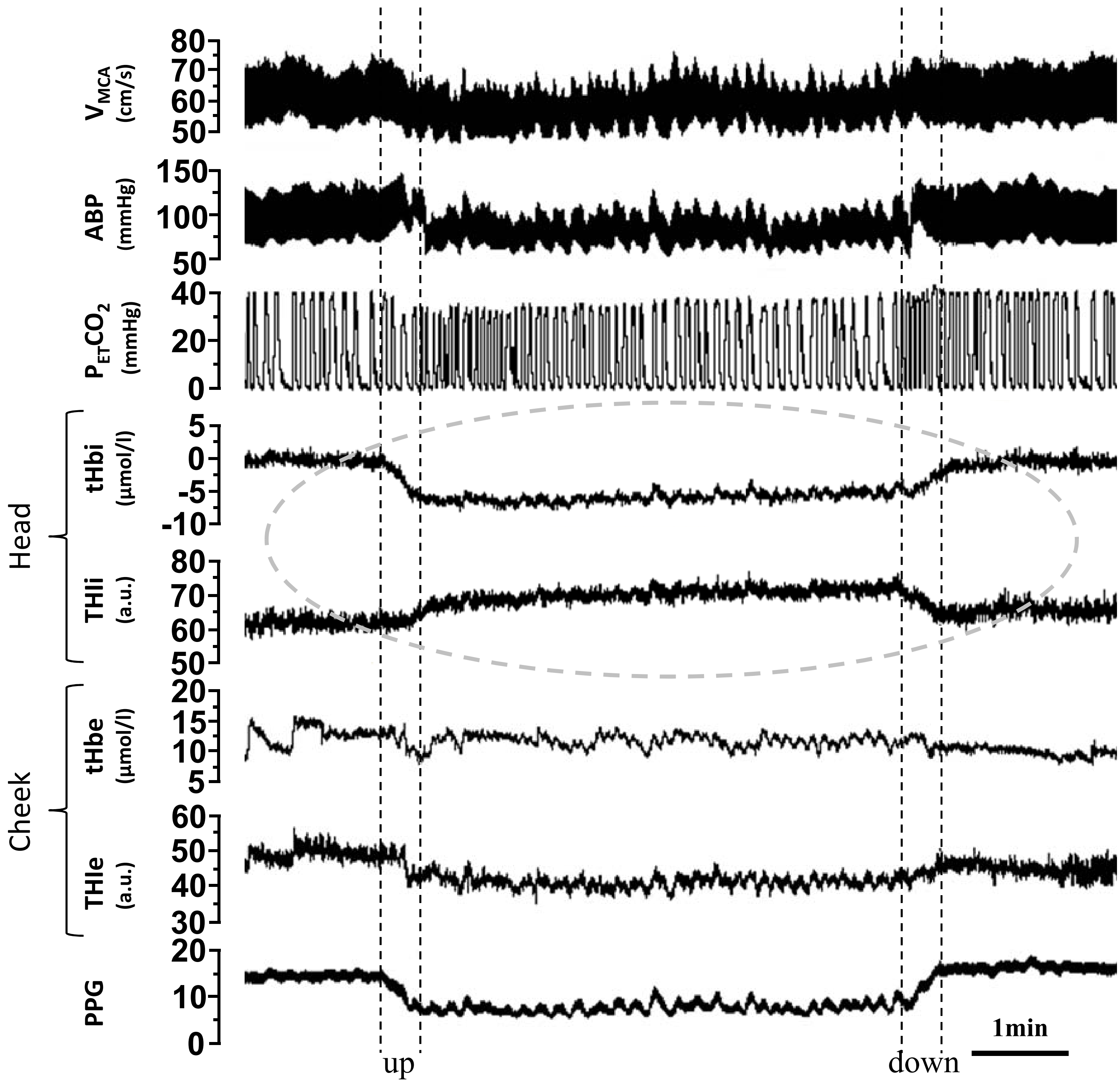
V_{MCA} (cm/s)
150
100
50
0
ABP (mmHg)
150
100
50
$P_{ET}CO_2$ (mmHg)
40
20
0
Head
tHbi (μmol/l)
-15
-20
-25
THIi (a.u.)
70
65
60
55
Cheek
tHbe (μmol/l)
15
10
5
THIe (a.u.)
45
40
35
30
PPG
30
25
20
15
1min

Fig. 4



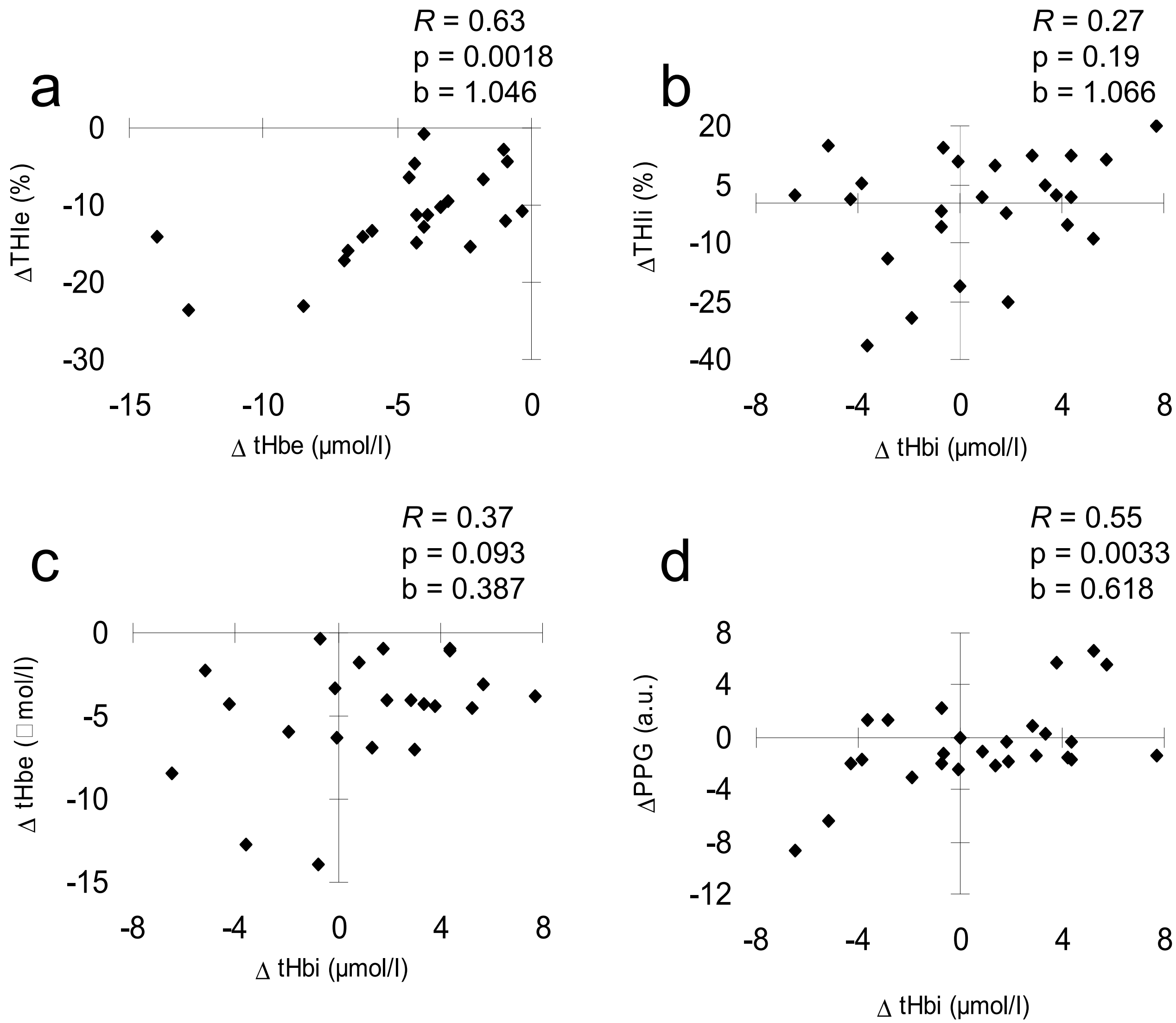
a
R = 0.73
p = < 0.0001
b = 1.288
ΔTHIe (%)
Δ tHbe (μmol/l)
b
R = 0.47
p = 0.011
b = 1.065
ΔTHIi (%)
Δ tHbi (μmol/l)
c
R = 0.57
p = 0.0034
b = 0.315
Δ tHbe (μmol/l)
Δ tHbi (μmol/l)
d
R = 0.70
p = < 0.0001
b = 0.670
Δ PPG (a.u.)
Δ tHbi (μmol/l)

Fig. 5



V_MCA (cm/s)
80
70
60
50
ABP (mmHg)
150
100
50
P_ETCO_2 (mmHg)
40
20
0
Head
tHbi (µmol/l)
5
0
-5
-10
THIi (a.u.)
80
70
60
50
Cheek
tHbe (µmol/l)
20
15
10
5
THIe (a.u.)
60
50
40
30
PPG
20
10
0
up
down
1min

Fig. 6



a
R = 0.63
p = 0.0018
b = 1.046
ΔTHIe (%)
Δ tHbe (μmol/l)
0
-10
-20
-30
-15
-10
-5
0
b
R = 0.27
p = 0.19
b = 1.066
ΔTHIi (%)
Δ tHbi (μmol/l)
20
5
-10
-25
-40
-8
-4
0
4
8
c
R = 0.37
p = 0.093
b = 0.387
Δ tHbe (□mol/l)
Δ tHbi (μmol/l)
0
-5
-10
-15
-8
-4
0
4
8
d
R = 0.55
p = 0.0033
b = 0.618
ΔPPG (a.u.)
Δ tHbi (μmol/l)
8
4
0
-4
-8
-12
-8
-4
0
4
8

Fig. 7