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

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

3

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12

13 Running head: Inconsistent detection of changes in cerebral blood volume

14

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21

22

23 Abstract

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

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

38 While THLe and tHbe generally provided concordant indications, tHbi and THli exhibited
39 opposite-sign changes in a high percentage of cases (VM: 46%; HV: 31%; HUT: 40%).

40 Moreover, tHbi was correlated with THli only during HV ($p < 0.05$), not during VM and HUT,
41 while it correlated with PPG in all 3 maneuvers ($p < 0.01$). These results evidence that
42 extracranial circulation may markedly affect BL parameters in a high percentage of cases,
43 even during standard clinical tests. Surface plethysmography at the forehead is suggested
44 as complementary monitoring helpful in the interpretation of cerebral NIRS parameters.

45

46 **Keywords:** near infrared spectroscopy, cerebrovascular reactivity,

47 hyperventilation, Valsalva maneuver, head-up tilt

48 Introduction

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

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

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

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

73 frequently used in clinical investigations and the risk of interference from extracerebral
74 compartments is still underestimated.

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

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

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

97

98

99 **Methods**

100 *Subjects*

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

105

106 *Protocols*

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

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

113 Valsalva maneuver

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

118 Hyperventilation

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

122 Head-up tilt

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

125

126 *Near Infrared Spectroscopy*

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

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

135 BL parameters measure concentration changes in oxyhemoglobin (O₂Hb),
136 deoxyhemoglobin (HHb) and total hemoglobin (tHb= O₂Hb+HHb) and are all expressed in
137 μM/L; they do not provide a measure of the absolute concentration of the chromophores,
138 but of concentration changes with respect to an arbitrary value (31).

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

143

144 *Intracranial measurements*

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

147 sufficiently lateral from the midline to exclude the superior sagittal sinus. NIRS optodes
148 were set at a distance of 5 cm by a rubber holder secured to the skin by bi-adhesive foam
149 and further stabilized by a crepe bandage around the head (2).

150 In addition blood velocity in the left middle cerebral artery (V_{MCA}) was monitored using a 2-
151 MHz transcranial Doppler ultrasonography (Multidop X, DWL, Germany), the Doppler
152 probe being held by a headset for bilateral monitoring.

153

154 *Extracranial measurements*

155 The second NIRO channel was used for extracranial monitoring, the probe being
156 positioned on the left cheek.

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

165

166 *Systemic measurements*

167 Continuous non-invasive measurement of arterial blood pressure (ABP) was performed by
168 photo-plethysmography (Finapres, Ohmeda 2300, USA) applied to the right third finger.

169 $P_{ET}CO_2$ was continuously recorded using a small nasal cannula connected to a
170 capnograph (Ohmeda 4700 OxiCap, USA).

171

172 *Signal Acquisition and Processing*

173 All NIRS signals from both channels, O₂Hbi, HHbi, tHbi, THli, TOli, O₂Hbe, HHbe, tHbe,
174 THle and TOle (the subscripts *i* and *e* indicate the intracranial and extracranial monitoring,
175 respectively) were continuously acquired and digitally transferred to PC by a proprietary
176 software (Hamamatsu Photonics) (sampling frequency: 2Hz) throughout the whole
177 session. These data were subsequently exported in text files for off-line analysis under
178 Microsoft Excel.

179 In addition, V_{MCA}, ABP, P_{ET-CO₂}, PPG and LDF, along with some of the NIRS signals (tHbi,
180 THli, tHbe and THle) were continuously acquired on PC (PowerLab ML 785
181 ADInstruments) (sampling freq = 200 Hz) throughout the whole session.

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

185

186 *Data analysis and statistics*

187 The response to the different maneuvers was assessed by computing absolute or relative
188 changes exhibited by the different variables with respect to the pre-test (control) value.

189 The control value was computed as the mean value over a 30-s interval immediately
190 before the beginning of the test, whereas mean values elicited by each maneuver were
191 computed over the phase II of the response to VM (46), over a 20-s interval starting 40 s
192 after the beginning of HV, and over a 30-s interval starting 3 min after the beginning of
193 HUT.

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

197 Data are presented as mean \pm SD.

198 Changes produced on the different variables (V_{MCA} , ABP, HR, TOli, THli, tHbi, TOle, THle,
199 tHbe and PPG) with respect to the pre-test (control) values were tested separately for the
200 three maneuvers by means of a multivariate ANOVA and the Tukey HSD post-hoc test.

201 Significance of changes in LDF, that was collected from a smaller number of subjects, was
202 separately assessed by the Student's t-test.

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

205

206 Results

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

211

212 *Valsalva Maneuver*

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

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

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

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

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

234 The scatter plots shown in Fig. 2 help to understand the correlation between the different
235 variables, while all R values are summarized in Tab. 2. In particular, Fig. 2a underlines the
236 agreement between THle and tHbe which never gave contradictory indications and
237 exhibited a correlation of $R = 0.54$ ($p < 0.05$) which rises to 0.74 after removing one outlier.
238 Conversely, a non significant correlation was observed between tHbi and THli (Fig. 2b). It
239 is interesting to observe that a significant correlation resulted between tHbi and tHbe ($R =$
240 0.48 , $p < 0.05$) (Fig. 2c) and between tHbi and PPG ($R = 0.50$, $p < 0.01$) (Fig. 2d).

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

244

245 *Hyperventilation*

246 The response to HV of a representative subject is shown in Fig. 3. $P_{ET}CO_2$ stabilizes at 20
247 mmHg during the maneuver and V_{MCA} exhibits a marked reduction with a latency of 10-15
248 s while ABP exhibits a transient increase. In this subject intracranial blood volume
249 indicators exhibit opposite changes, while THle and tHbe, as well as PPG, all indicate an
250 increase in extracranial blood volume. On average (Table 1), HV produced a significant
251 increase in HR (from 74.7 ± 10.4 to 104.2 ± 20.8 bpm), a small decrease in ABP (from
252 85.8 ± 13.2 to 80.5 ± 15.4 mmHg), along with a marked and sustained decrease in V_{MCA}
253 (from 62.4 ± 13.11 to 46.5 ± 7.3 cm/s).

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

260 tHbi resulted significantly correlated with the extracranial indicators tHbe ($R = 0.57$,
261 $p<0.01$) (Fig. 4c) and PPG ($R = 0.70$, $p<0.01$) (Fig. 4d), as well as with THli ($R=0.47$,
262 $p<0.05$) (Fig 4b).

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

265 50 % of the cases, while a 78% agreement and a significant correlation is observed
266 between LDF and PPG ($R = 0.86$, $P < 0.01$).

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

269

270 *Head-up tilt*

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

277 On average (see Table 1), HUT elicited a significant increase in HR (from 73.7 ± 9.8 to
278 89.6 ± 10.8 bpm), non-significant changes in ABP (at heart level), and a significant
279 decrease in V_{MCA} (from 64.2 ± 15.6 to 59.2 ± 11.2 cm/s).

280 Unlike previous maneuvers, oxygenation indices show concordant changes: a significant
281 decrease in both TOli and TOle.

282 At extracranial level blood volume does not appear to be univocally affected. At the cheek
283 level both THle and tHbe reveal a significant decrease (in 100% of the subjects) and a
284 good correlation ($R = 0.63$, $p < 0.01$) (Fig. 6a) while cutaneous blood volume index from
285 forehead (PPG) evidences a greater response variability (8 increases out of 29 subjects),
286 resulting in a non-significant change. In addition PPG variations were not correlated with
287 changes in tHbe ($R = -0.068$), the two variables exhibiting changes of opposite sign in 30
288 % of the cases.

289 With regard to the intracranial compartment, both THli and tHbi showed a large variability
290 and no significant changes on average; nevertheless, when looking at the individual trials,
291 the two parameters yielded contradictory indications in 40 % of the cases and resulted to
292 be non significantly correlated ($R = 0.27$) (Fig. 6b).

293 At variance with what observed for VM and HV, the scatter plots here evidenced a non
294 significant correlation between changes in tHbi and changes in tHbe ($R = 0.37$, Fig. 6c).
295 However, tHbi was still significantly correlated with PPG ($R = 0.55$, $p < 0.01$, Fig. 6d); in
296 particular, it can be observed that large changes in PPG are associated with large
297 changes in tHbi. This relationship also hold for responses to VM and HV (Fig. 2d, 4d and
298 Fig. 6d). PPG and LDF exhibited a 70 % agreement and a non significant correlation.

299

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

311

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

314 Discussion

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

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

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

329

330 *Valsalva maneuver*

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

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

339 BL-based NIRS, report increased cerebral blood volume and oxygenation during VM (34,
340 36, 49). We also consistently observed an increase in tHbi (100% of cases) however THli
341 decreased in 46% of subjects. This suggests that intracranial blood volume can possibly
342 be reduced during VM and that the increased volume of extracranial compartments heavily
343 affects the tHbi indicator. In addition, it cannot be excluded that also THli could have been
344 affected to a small extent, which would imply an overestimation of the intracranial blood
345 volume change by THli.

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

356

357 *Hyperventilation*

358 HV induces transient arterial hypocapnia and alkalosis provoking a rapid cerebral
359 vasoconstriction, cerebral blood flow reduction (21, 30) and increased cerebral oxygen
360 extraction (48). This results, as also observed in the present study, in both a marked
361 reduction of V_{MCA} (7, 39) and a decrease in cerebral oxygenation (TOli) (5, 44, 48, 55).
362 A reduction of cerebral blood volume as detected by THli (80% of subjects) is consistent
363 with the occurrence of a marked cerebral vasoconstriction and is supported by previous

364 studies (29, 35). On the other hand tHbi is not significantly affected by HV and gives in
365 28% of the subjects opposite indication to THli.

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

374

375 *Head-up tilt*

376 Response to HUT appears to be more complex.

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

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

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

386 Decreased transmural pressure at the venous side may decrease blood volume (venous
387 collapse) but local and neural mechanisms may also intervene. In particular the

388 sympathetic activation driven by the orthostatic stimulus is known to increase
389 vasoconstrictor tone in skeletal muscles but not in cutaneous tissues (52).
390 This may explain the consistent decrease in blood volume and oxygenation indicated by
391 the NIRS channel positioned on the cheek, which likely reflects perfusion of underlying
392 skeletal muscle layers. Conversely, forehead plethysmography, which mostly reflects
393 cutaneous perfusion, results in a variable response which poorly correlates with tHbe.
394 Notably, also in the HUT response a large percentage (38%) of inconsistent indications by
395 THli and tHbi was observed. At variance with what observed in the other maneuvers, tHbi
396 was poorly correlated with tHbe but still rather correlated with PPG (Figs. 6c; 6d).
397 Thus, also in this case the occurrence of inconsistencies between cerebral blood volume
398 indicators appears to be related to the influence of extracranial circulation on the BL
399 parameter tHbi.

400

401 *General considerations*

402 For the sake of simplicity, in the above discussion the assumption was made that
403 measurements were collected at “steady state”, and we did not consider the possibility of
404 different time course of the responses in the different tissues. However this assumption
405 does not undermine the main outcomes of the work.
406 The three maneuvers investigated in the present study elicit very different hemodynamic
407 responses at intra and extracranial levels. In particular: i) VM provokes a marked increase
408 in central venous pressure which results in a large increase in blood volume of extracranial
409 compartments with minor changes at intracranial level, ii) HV produces a marked
410 metabolic cerebrovascular regulation with minor changes at extracranial level, while iii)
411 HUT affects the hydrostatic gradients, and stimulates metabolic and neural regulation at
412 both intra and extracranial levels.

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

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

434 In general, awareness of perfusion changes occurring in the extracranial compartment
435 may be of good help in the interpretation of the NIRS recordings. Since NIRS is based on
436 changes in hemoglobin concentration, surface plethysmography is to be preferred to
437 flowmetry, also in consideration of the fact that blood flow and blood volume may not vary

438 in a concordant way. This was well evidenced in the response to VM: besides large
439 increases in forehead skin blood volume, a non significant blood flow decrease was
440 detected by LDF. In addition we observed that plethysmographic monitoring of the
441 extracranial compartment with a second NIRS channel placed on the cheek may not
442 always be appropriate, possibly due to the different neural regulation of skin and muscle
443 vascular beds, which makes the cheek (skin + muscle) not a good representation of the
444 forehead (mostly skin). Surface plethysmography at the forehead proved to be better
445 correlated to tHbi (particularly during HUT) and to better help in understanding the
446 inconsistency between tHbi and THli. On this basis, it is proposed as a valid and
447 inexpensive measure to be included in the experimental/clinical set-up.

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

453

454 Conclusions

455 In conclusion, the present study reveals that BL NIRS monitoring can be detrimentally
456 affected by changes in extracranial circulation also in routine
457 cardiovascular/neurovegetative tests and emphasizes its low reliability for the assessment
458 of cerebral perfusion. Postural, mechanical and neural changes, that may occur under
459 most investigative maneuvers, alter blood perfusion or/and distribution in the extracranial
460 compartment and affect BL NIRS variables to the extent that detected changes in cerebral
461 tissue blood volume and oxygenation can be frequently reversed. On the basis of the

462 present data, forehead cutaneous plethysmography is suggested as an additional
463 measure to complement the hemodynamic monitoring and help in the interpretation of
464 NIRS recordings.

465

466

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483 Figure legends

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

493

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

499

500

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

504

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

507

508

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

513

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

516

Fig.7 Graphic summary of the correlations among THli, 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.

517 TABLES

518 Table 1. Average changes produced by Valsalva maneuver (VM), Hyperventilation (HV)
 519 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 **
TOI _i (%)	-4.77 ± 2.96 **	-5.18 ± 3.04 **	-3.19 ± 4.22 **
THI _i (%)	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
TOI _e (%)	6.25 ± 4.98 **	3.41 ± 2.73 **	-6.78 ± 3.35
THI _e (%)	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 **

532

533 V_{MCA} = cerebral blood velocity, ABP=arterial blood pressure, HR=heart rate,

534 TOI=tissue oxygenation index, THI=total hemoglobin index, tHB= total hemoglobin

535 concentration, PPG= cutaneous plethysmography at the forehead; LDF= cutaneous Laser

536 Doppler flowmetry at the forehead. Subscripts *i* and *e* indicate intracranial (at the

537 forehead) and extracranial (at the cheek) NIRS monitoring. Relative changes are

538 expressed in %, absolute changes in the original units. * = $P < 0.05$; ** = $P < 0.01$

539

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

	VM	HV	HUT	
THIi vs. tHbi	0.27	0.47 *	0.27	542
tHbi vs. tHbe	0.48 *	0.57 **	0.37	543
tHbi vs. PPG	0.50 **	0.70 **	0.55 **	544
THIi vs. tHbe	0.10	0.10	0.41	545
THIi vs. PPG	0.37 *	0.20	-0.11	546
THIe vs. tHbe	0.54 *	0.73 **	0.63 **	547
PPG vs. tHbe	0.15	0.40	-0.068	548
LDF vs. PPG	-0.031	0.86 **	0.079	549
THIi vs. V _{MCA}	0.27	0.0087	0.092	550
tHbi vs. V _{MCA}	-0.18	-0.32	-0.14	551

552

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

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Fig. 1

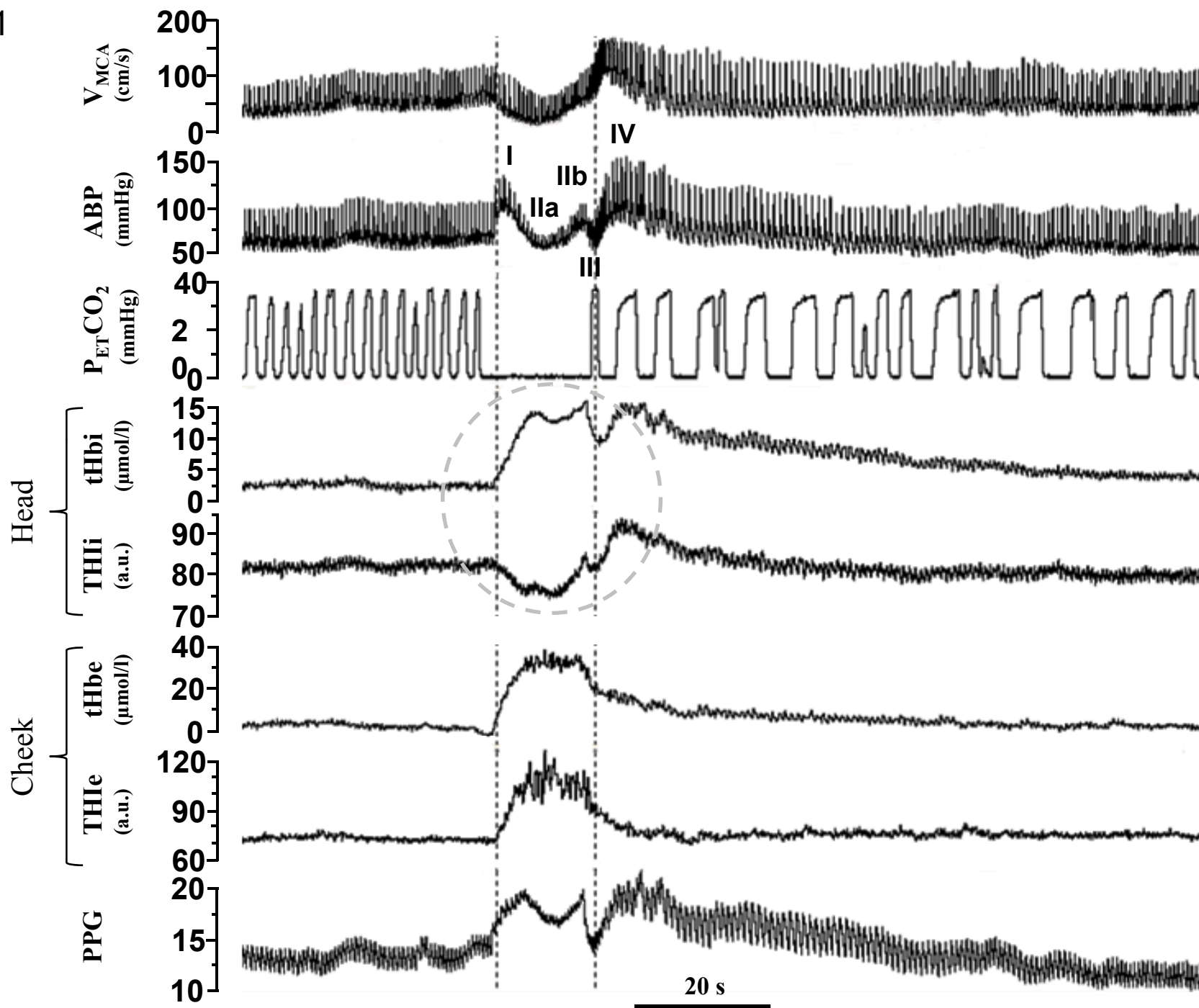


Fig. 2

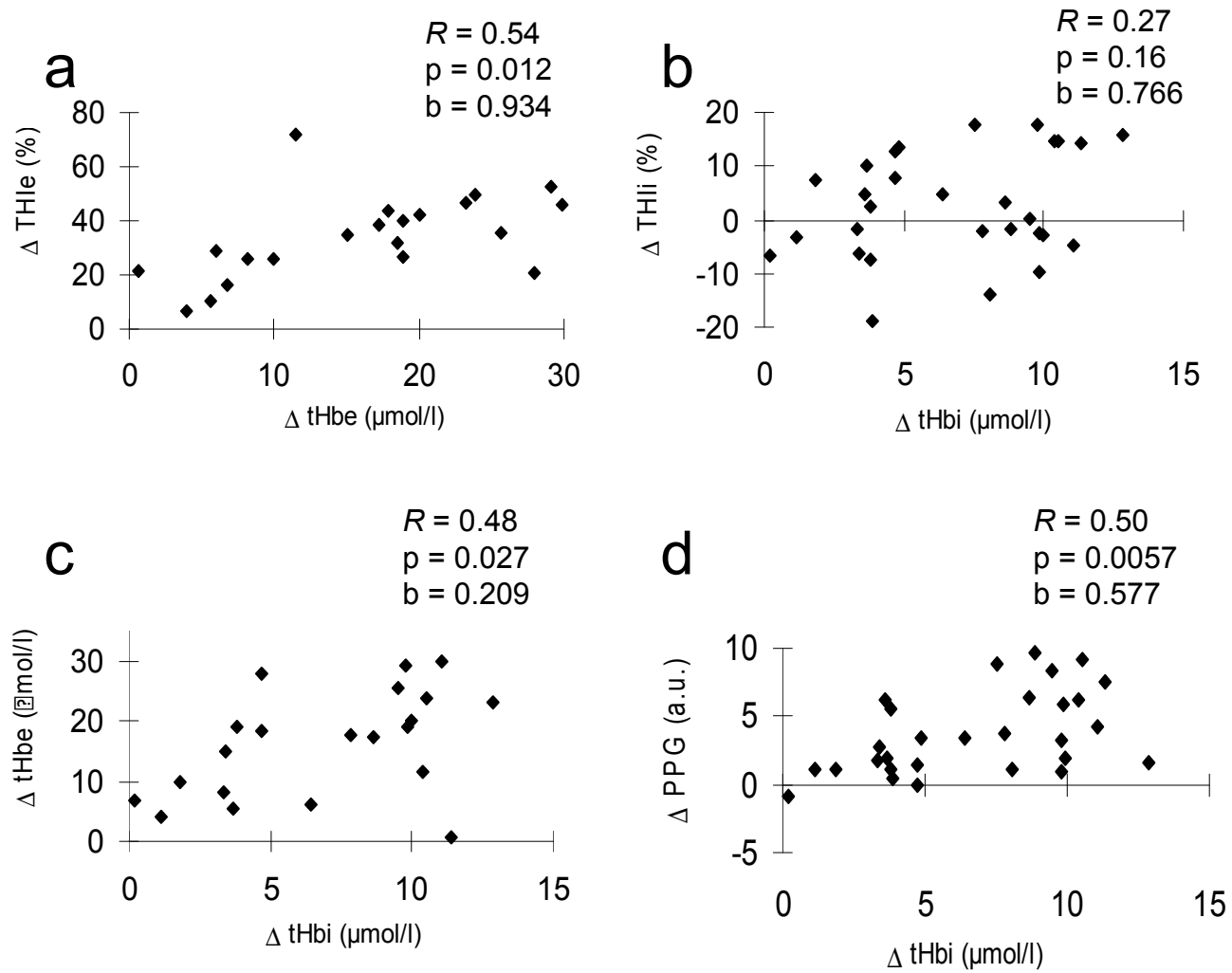


Fig. 3

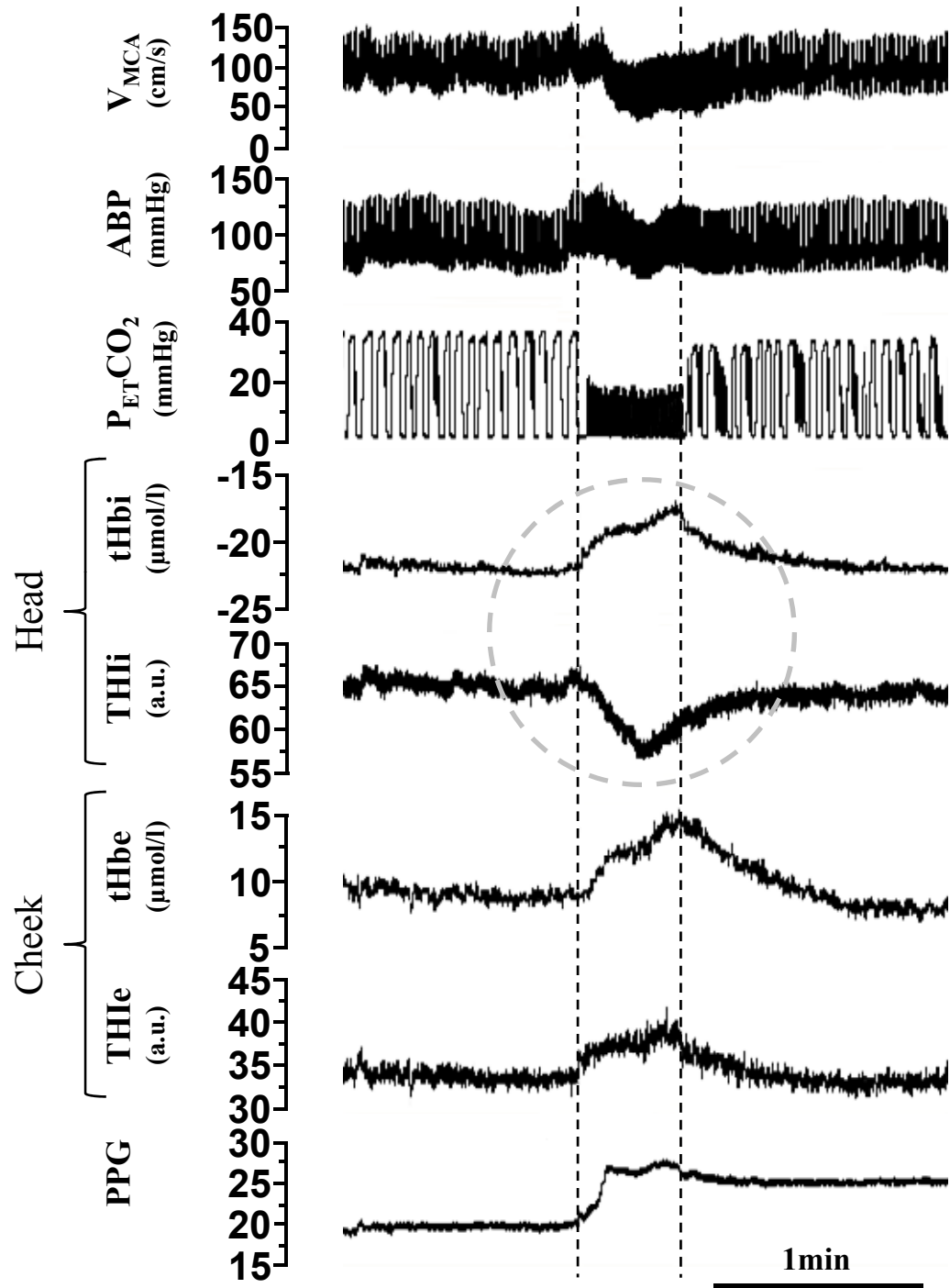


Fig. 4

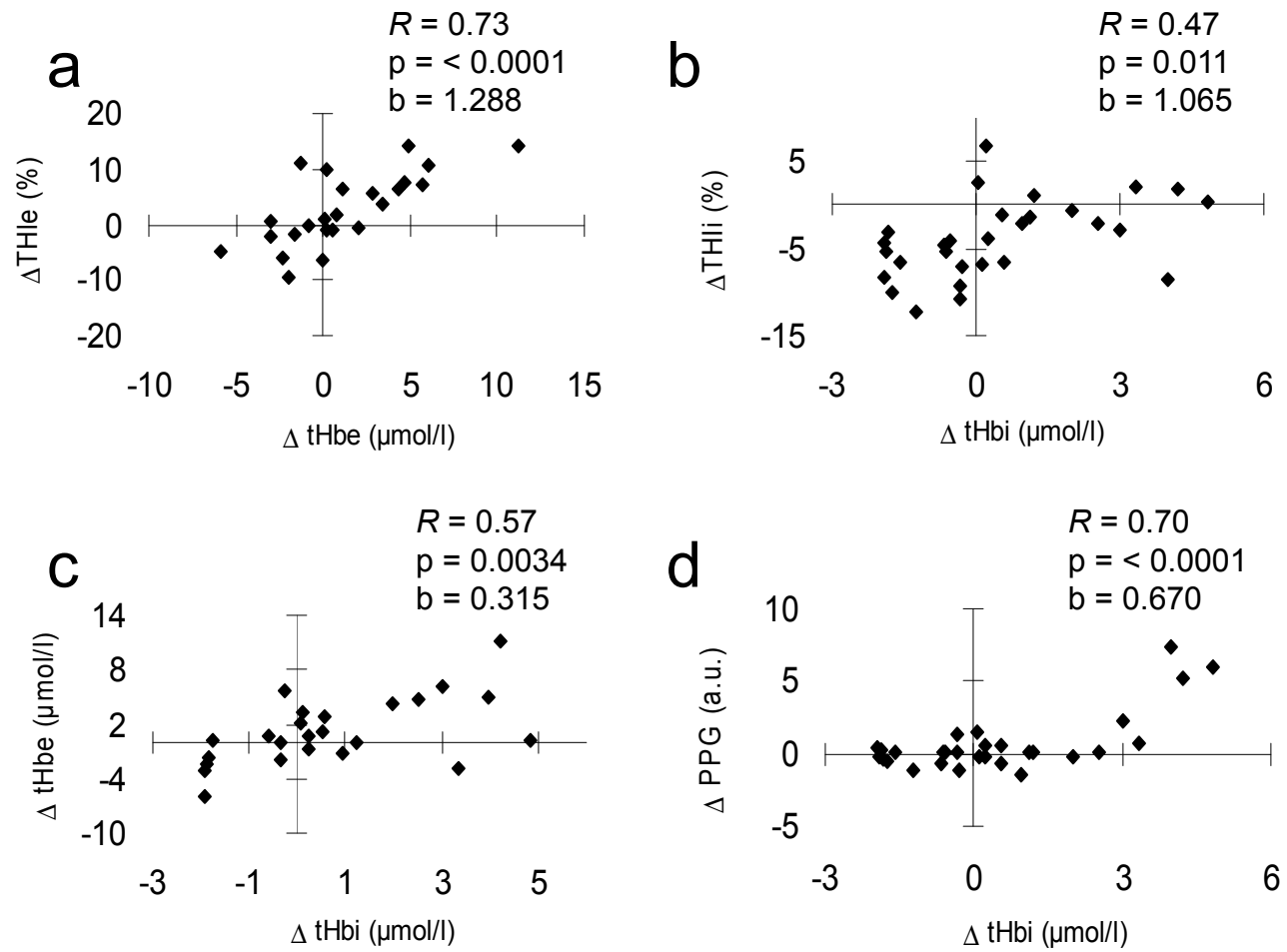


Fig. 5

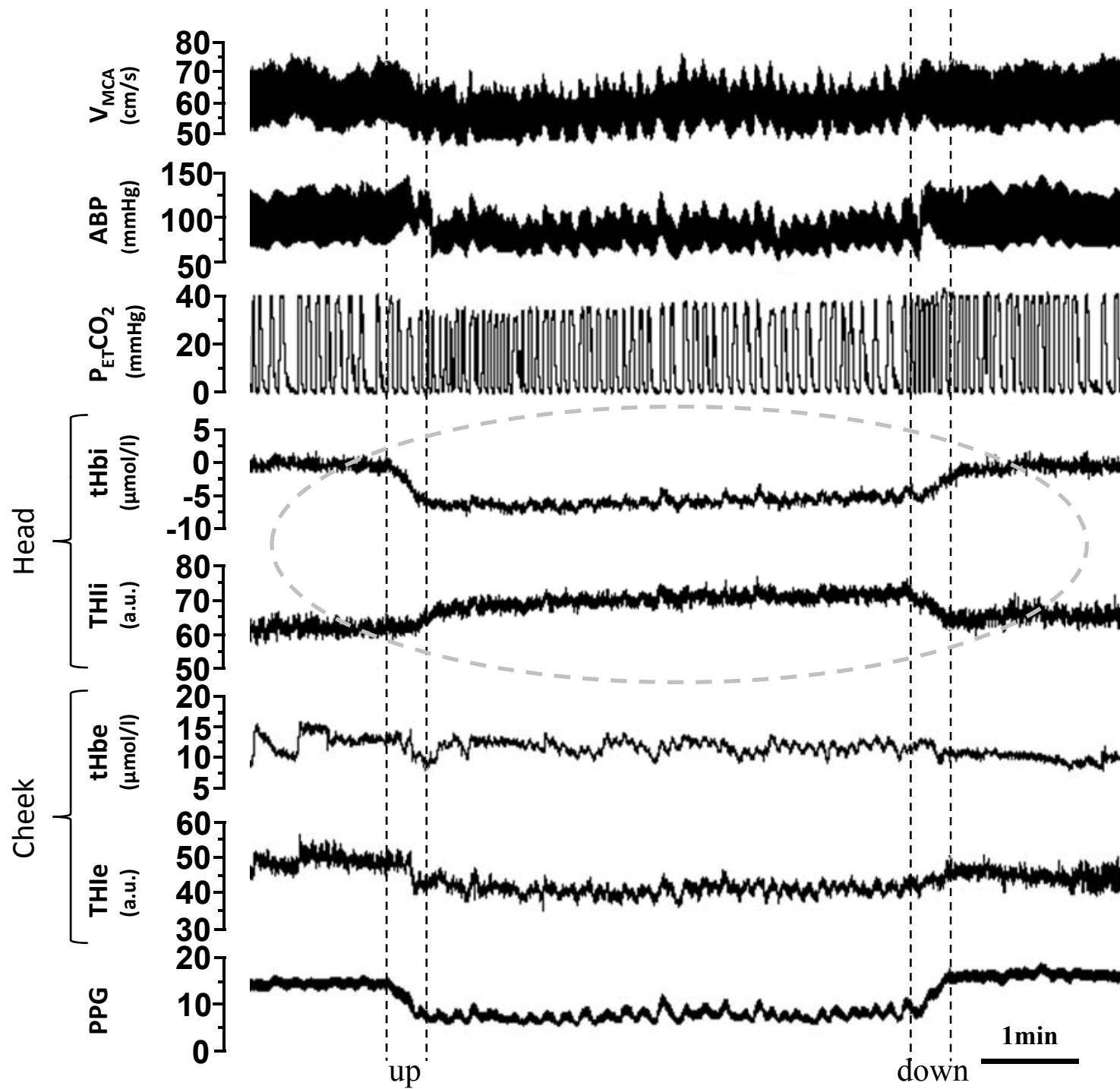


Fig. 6

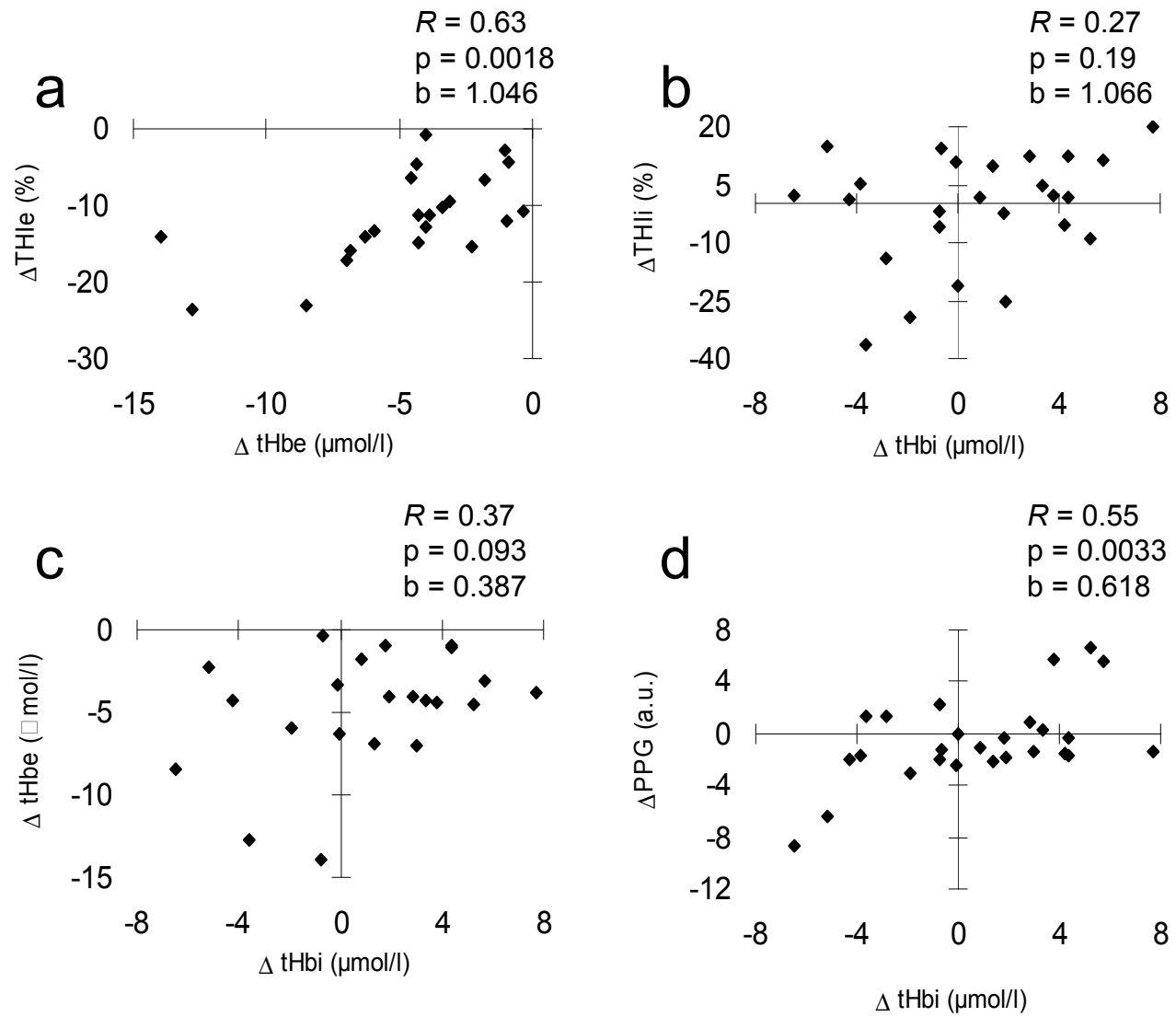


Fig. 7

