

Cerebral **blood** volume and
ventilation in severe COPD



M.J.T. VAN DE VEN

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Maria Josephina Tonia van de Ven
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Promotores:

Prof. Dr. H.Th.M. Folgering

Prof. Dr. B. Oeseburg†

Co-promotor:

Dr. W.N.J.M. Colier

Manuscriptcommissie:

Prof. Dr. T. van der Werf, voorzitter

Prof. Dr. C.L.A. van Herwaarden

Dr. L.J. Teppema (LUMC)

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CHAPTER 1

General introduction

CLINICAL BACKGROUND

CEREBROVASCULAR REACTIVITY AND VENTILATION

Effects of respiratory acid-base changes

In the group of patients with chronic obstructive pulmonary diseases (COPD), two extreme forms of the control of breathing exist: normocapnic and hypercapnic patients^[1]. Normocapnic patients are sometimes called pink and puffing patients ("pink puffers", fighters) ; they maintain relatively normal blood gas tensions at the expense of breathlessness. The other extreme form is called blue and bloated ("blue bloaters", quitters); they "choose" to be free of breathlessness at the expense of abnormal blood gas tensions and right heart failure^[2]. Within the hypercapnic COPD group, some patients have a blunted ventilatory response during inhalation of gas mixtures enriched with carbon dioxide (CO₂). This diminished response has been ascribed to either mechanical limitations imposed by the disease process itself (called "can't breathe") or to reduced sensitivity of the respiratory centers to the CO₂ stimulus ("won't breathe")^[3,4].

Scano et al.^[5] performed a study to ascertain whether, and to what extent, the reduced ventilatory response to a hypercapnic stimulus in COPD patients depends on a blunted chemoresponsiveness of central origin or to mechanical impairment. They studied two groups of COPD patients without and with chronic hypercapnia, but with similar degrees of airway obstruction and hyperinflation. During a CO₂ rebreathing test, ventilation, mouth occlusion pressure (P_{0.1}), and the electromyographic activity of diaphragm (E_d) were recorded and then plotted against end-tidal carbon dioxide tension (Pco₂). At a Paco₂ level of 8.65 kPa, a decreased ventilatory response was seen in the hypercapnic COPD group. Furthermore, the hypercapnic group showed a reduced P_{0.1} response and E_d responses to carbon dioxide stimulation. They concluded that, apart from mechanical impairment, an inadequate chemoresponsiveness is likely to contribute to the low ventilatory response to CO₂ stimulation in chronic hypercapnic COPD patients.

The importance of the control of cerebral blood flow (CBF) as a crucial link in stimulus-response studies of ventilatory control, was first pointed out by the classical study of Kety and Schmidt^[6]. It is well known that cerebral blood flow (CBF) and cerebral blood volume (CBV) increases by hypercapnia^[6,7]. In healthy subjects, an increase in CBF and CBV would increase CO₂ washout and lead to central hypocapnia^[8]. The effect of CBF and CBV alterations on central chemosensitivity however, remains unclear. While Berkenbosch, Olivier and DeGoede^[9,10] predicted a substantial role for CBF on central chemosensitivity in anaesthetized cats, Poulin and Robbins^[11] suggested a "quantitatively insufficient" role in conscious humans. However, in contrast to their

conclusions, the latter investigators noted a correlation between central ventilatory sensitivity to hypercapnia and the magnitude of hypoxic ventilatory decline, suggesting at least some influence of alterations in CBF on ventilation. These results emphasized the relevance of simultaneous measurements of cerebrovascular and of ventilatory reactivity.

Effects of metabolic acid-base changes

One of the major mechanisms for changes in blood flow and vascular resistance during changes in Paco_2 appears to be the local action of H^+ ions on cerebral blood vessels. This response is mediated by changes in extracellular fluid pH in the immediate vicinity of vascular muscle of cerebral vessels. CBF has been reported to increase during perfusion with acid cerebrospinal fluid (CSF) in cats^[12]. Betz et al.^[13] reported direct measurements of pH of the brain surface during CO_2 inhalation and during changes in acid-base status of blood produced by intravenous injection of HCl or NaHCO_3 in cats. Cortical vascular resistance correlated positively with brain surface pH. These changes in cortical vascular resistance could not be explained by changes in arterial blood pH or Paco_2 or by alterations in arterial blood pressure. Gotoh et al.^[14] determined the role of acetazolamide and PCO_2 on CBF, using the intra-arterial $^{133}\text{Xenon}$ injection method. An increase in CBF with inhalation of carbon dioxide was markedly suppressed by intravenous administration of 2000 mg acetazolamide of the carbonic anhydrase inhibitor, suggesting that CO_2 itself is not the final factor in chemical control of cerebral vessels.

If the major mechanism of action of CO_2 on the cerebral circulation is mediated via changes in CSF pH, one would expect *metabolic changes* in blood pH, during a constant Paco_2 , to have negligible effects on CBF and on cerebral vascular resistance. This expectation is based on the fact that molecular CO_2 diffuses freely across the blood-brain barrier and therefore can readily alter CSF pH, whereas diffusion of H^+ and HCO_3^- across the barriers is much slower. McDowall and Harper^[15] saw no change in CBF during intravenous infusion of lactic acid during constant normal Paco_2 or during constant arterial hypercapnia. Prolonged hypercapnia induces a down-regulation on CBF: lower CBF than could be expected from the acute changes of blood gases^[16].

Acetazolamide

Acetazolamide is a powerful carbonic anhydrase (CA) inhibitor. This enzyme is present in the central nervous system, in red cells, peripheral chemoreceptors, muscle cells, in endothelium of capillaries supplying brain, kidneys, muscles and lungs^[17,18].

Acetazolamide increases ventilation, partly by CO₂ retention (respiratory acidosis) arising from inhibition of red cell carbonic anhydrase (CA) and tissue CA, and by the metabolic acidosis that develops several hours after administration as a consequence of renal CA inhibition^[19].

Due to its physical-chemical properties, acetazolamide does not easily cross the blood brain barrier^[17]. However, penetration of acetazolamide into erythrocytes and other peripheral tissues may result in effective inhibition of local CA, even if the drug is administered in low (clinical) doses (<5 mg/kg)^[17]. In animal studies, a low intravenous dose of acetazolamide (4mg/kg, a dose not causing effective inhibition of red cell CA) caused a reduction in the slopes of the CO₂ sensitivities of both the peripheral and central chemoreflex loops by 30%^[10]. When given at high doses (50 mg/kg, a dose causing total inhibition of red cell CA), it led to a decrease in activity of the peripheral chemoreceptors, and also a decrease of their sensitivity to Pao₂ changes. The increase in ventilation by acetazolamide was suggested to result from an action of the drug on the central nervous system, possibly on the central chemoreceptors^[20].

The effects of acetazolamide on the ventilatory CO₂ response slope are confusing and vary from no change^[21,22] or an increase^[23 26] after chronic application, to a decrease of the CO₂ sensitivity during hypoxia after acute administration^[25]. In COPD patients, acetazolamide treatment can improve blood gas values^[26] especially in cases with a metabolic alkalosis related to the use of steroids and loop-diuretics^[24]. Acetazolamide also decreased desaturation time and improved nocturnal Po₂^[26]. Some laboratories found more non-responders to acetazolamide, when severely obstructed^[27]. However, Vos et al.^[24] were unable to find any correlation between FEV₁ and magnitude of response to acetazolamide. Swenson and Hughes^[28] warned against CA inhibitors in patients with severe obstruction and CO₂ retention (FEV₁ < 25% predicted and Paco₂ > 60 mm Hg). The obligatory hyperventilation, necessary to compensate for metabolic acidosis, may cause respiratory muscle fatigue, and further deteriorate ventilation/perfusion matching of the lung.

However, the beneficial effects of acetazolamide in (severe) COPD patients in earlier studies performed at our institute^[24,26,29] encouraged us to continue using acetazolamide in the present clinical study.

Furosemide

Loop- and thiazide-diuretics are commonly associated with metabolic alkalosis, the severity of which varies directly with the degree of diuresis. Both volume contraction and increased urinary H⁺ loss contribute to this problem^[30].

In chronic respiratory failure, the blood gas value shows often a compensatory metabolic alkalosis. When loop diuretics are given, progression in metabolic alkalosis could appear, resulting in further respiratory failure and altered CBF and CBV responses to CO₂. In our study we want to examine this last assumption.

EVALUATION OF CEREBROVASCULAR REACTIVITY

Several semi-invasive methods to study CBF have been developed: positron emission tomography, xenon enhanced computed tomography, radio-active xenon clearance techniques and magnetic resonance imaging. Although these methods give adequate results, they have several disadvantages. The techniques are expensive and cannot be used on a routine basis.

A relatively new technique for cerebral tissue oxygen monitoring is *Near Infrared Spectroscopy* (NIRS), first described by Jöbsis in 1977^[31]. In contrast to other methods, repeated measurements are possible at the bedside and the method is non-invasive: no ionizing radiation or injection of an intravascular tracer is needed. Therefore, the method seems very attractive for practical clinical use.

The principles of NIRS are based on absorption of near infrared light, mainly by the chromophores of oxyhemoglobin [O₂Hb] and deoxyhemoglobin [HHb]. Changes of optical density of a perfused tissue are converted into concentration changes of [O₂Hb] and [HHb]^[32-34].

Near-infrared light was carried to and from a highly sensitive, pulsed continuous-wave NIRS instrument (OXYMON, Departments of Physiology and Instrumentation, University of Nijmegen, the Netherlands)^[35] through two fiber optic bundles (optodes) on the left side of the forehead. One optode emits near infrared light at three different wavelengths, which penetrates through the skull/brain. The receiving optode is positioned at a distance of 5.5 cm apart from the emitting optode. This distance ensures that most of the extracranial circulation is excluded from the detected signal^[36]. To correct for light scattering in the tissue, a fixed differential path length factor of 6.0 was used^[33].

CBF measurements

We used the "oxygen swing method" or "O₂-method" for measuring of CBF. The method is based on the Fick's principle in which a rapidly induced change in arterial oxyhemoglobin concentration [O₂Hb] is used as an intravascular tracer. The rate of accumulation of the tracer is monitored. Edwards^[37] described this CBF technique by NIRS in infants. Basically, the mathematical background for CBF measurement can be described by^[38,39]:

$$\text{CBF (mL.100g}^{-1}\text{.min}^{-1}\text{)} = \frac{K_1 * \Delta \text{OI}}{2 * [\text{Hb}] \int_0^t \Delta \text{Sao}_2 \text{ dt}}$$

where K_1 is a constant, which accounts for molecular weight of hemoglobin, cerebral tissue density and a metric conversion factor. $[\text{Hb}]$ represents the total hemoglobin concentration in blood (mmol.L^{-1}), acquired by a blood sample. The signal to noise ratio of the NIRS measurement can be improved by measuring the change in the difference between $[\text{O}_2\text{Hb}]$ and $[\text{HHb}]$, defined as the oxygenation index (OI). $[\text{tHb}]$ is the sum of $[\text{O}_2\text{Hb}]$ and $[\text{HHb}]$. Since the $[\text{tHb}]$ is constant, the change in $[\text{O}_2\text{Hb}]$ and $[\text{HHb}]$ will be equal and opposite and the difference between these changes will be twice the amplitude of the changes of $[\text{O}_2\text{Hb}]$. The Sao_2 is measured by pulse-oximetry in the beat-to-beat mode, the OI with NIRS. When the rate of accumulation is measured within the mean transit time of 6 to 10 s of the tracer^[40], the venous outflow will be zero at that moment. First, a gradual decrease in saturation is performed by lowering the FiO_2 to 11-12%. This results in a decrease of Sao_2 of about 10%. Next, after a stable baseline of at least 20 s at this level is reached, a bolus of pure oxygen is given in the inspiratory air during one breath to generate a quick change in ΔSao_2 .

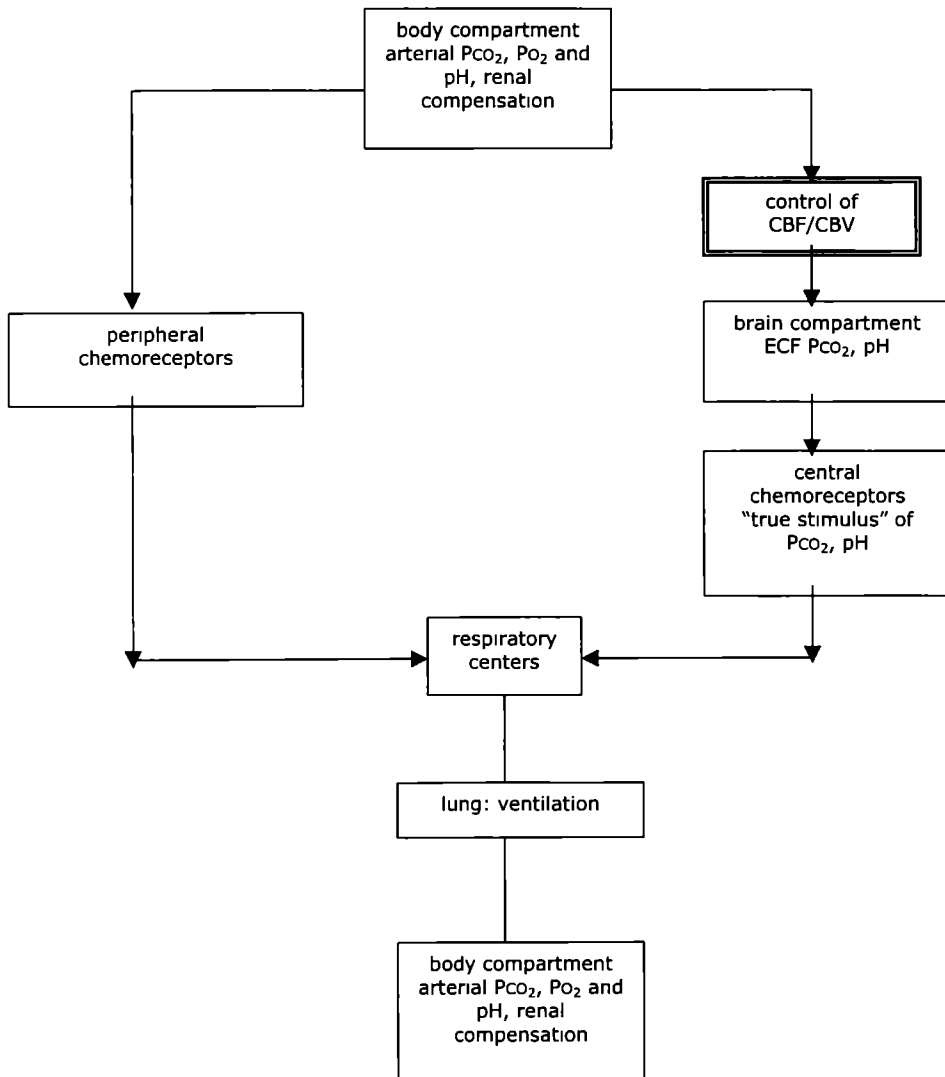
CBV measurements

It is important to consider the advantages of measurements of CBV for CBF measurements. Firstly, there is a close relationship ($r=0.9$) between CBV and CBF that has been extensively investigated by Grubb et al.^[7] and by van Zijl et al.^[41]. Secondly, the use of CBV instead of CBF eliminates the problems related to the mean cerebral transit time^[42]. Finally, near infrared absorption changes reflect changes in the oxygenation of the microvasculature, and thus the CBV of the brain tissue^[43]. Changes of CBV reflect capillary recruitment, which by some are considered a better reflection of cerebrovascular responses than CBF responses to acid-base stimuli^[42].

A slight change of saturation ($\sim 5\%$) is necessary to quantify CBV. Assuming a constant CBF, CBV and oxygen consumption during the short maneuver, absolute values of CBV can be calculated^[38,44], according to the following equation:

$$\text{CBV (mL.100g}^{-1}\text{)} = \frac{K_2 * \Delta \text{OI}}{(\text{Hb}) * \Delta \text{Sao}_2}$$

whereas K_2 is a constant representing the molecular weight of hemoglobin, cerebral tissue density, a large-to-small vessel hematocrit ration with a value of 0.69^[40] and a metric conversion factor.

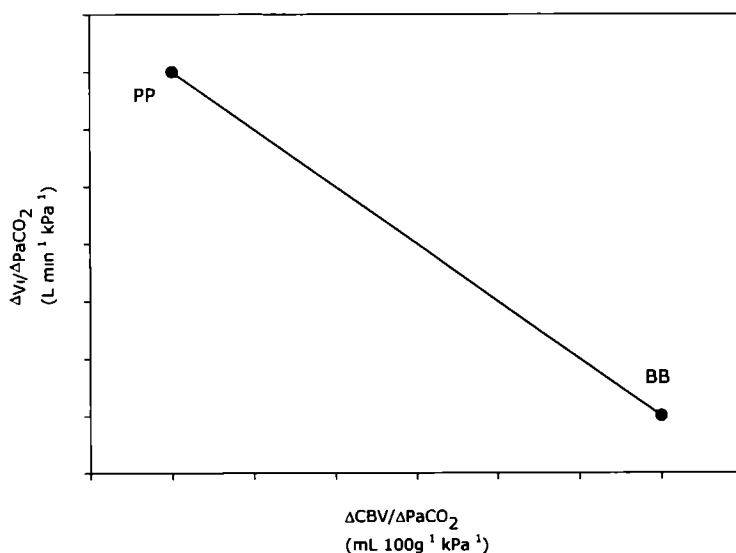
Figure 1. Hypothesis: Inverse relationship between CBV and ventilation

AIM OF THE THESIS

The basic research plan was to investigate whether the difference in the control of ventilation in normocapnic and hypercapnic patients with COPD can be explained by differences in the control of CBF (Figure1). We hypothesised that, in hypercapnic

patients with COPD, there is a high cerebral vasodilatory response to P_{CO_2}/pH , keeping the brain extracellular fluid (ECF) less hypercapnic, thus keeping the central chemoreceptor mediated ventilatory drive relatively low, and eventually resulting in systemic hypercapnia. In normocapnic COPD patients this autoregulatory system of CBF was hypothesised to be less reactive (Figure 2). The hypothesis predicts an inverse relationship between the ventilatory and cerebrovascular responses to changes in P_{aCO_2} . As a chronic respiratory acidosis is usually compensated via metabolic pathways, we have investigated the effects of superimposed chronic metabolic acid-base changes on the control of cerebrovascular and ventilatory responses. Therefore, a chronic metabolic acidosis and alkalosis was induced by orally administered drugs and ventilatory and cerebrovascular responses to changes in P_{aCO_2} were investigated.

Figure 2. Hypothesis. inverse relationship between CBV and ventilation in chronic hypercapnic and normocapnic COPD patients



Chronic hypercapnic COPD patient (BB: "Blue Bloater"):

$$\Delta CBV / \Delta P_{aCO_2} \uparrow \rightarrow \Delta V_I' / \Delta P_{aCO_2} \downarrow$$

Normocapnic COPD patient (PP: "Pink Puffer"):

$$\Delta CBV / \Delta P_{aCO_2} \downarrow \rightarrow \Delta V_I' / \Delta P_{aCO_2} \uparrow$$

OUTLINE OF THIS THESIS

Reproducibility measurements of CBF using NIRS were performed in healthy subjects and presented in chapter 2. Using $[O_2Hb]$ as a tracer to measure CBF, NIRS could *not* provide reproducible values of CBF. Therefore we abandoned CBF measurements with NIRS. As CBV is closely related to CBF[7,41], it is allowed to measure CBV instead of CBF under controlled circumstances. CBV could be measured reproducibly (within-subject coefficient of variation 10-12.6%), as described in chapter 3, and we adapted the initial research question from CBF into CBV measurements. CBV could be measured reproducibly in healthy subjects during hypercapnia and hypocapnia under baseline metabolic conditions (Chapter 4) and during acute metabolic acidosis induced by ammoniumchloride (NH_4Cl) (Chapter 6). Prior to the latter study, we investigated the induction of acute metabolic acid/base changes of ≥ 2 mEq/L change in base excess (BE) by using ammoniumchloride (NH_4Cl) for acidification, and furosemide for alkalization. Results are presented in chapter 5.

Cerebrovascular responses, expressed as a change of CBV (ΔCBV) to hypercapnia were studied for their relationship to ventilatory responses in normal healthy subjects, under baseline metabolic conditions and during acute metabolic acidosis (Chapter 7).

When these experiments were completed, normocapnic and chronic hypercapnic COPD patients could be measured and their results were compared to those obtained in healthy subjects (Chapter 8). Finally, effects of chronic metabolic alkalosis and chronic metabolic acidosis and their relation to ventilation were evaluated in normocapnic and chronic hypercapnic COPD patients. They were measured under baseline metabolic conditions and after one-week treatment with oral furosemide (40 mg, daily) or acetazolamide (500 mg, daily). Results of these experiments are presented in chapter 9.

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Cerebral blood flow in humans measured with near infrared spectroscopy is not reproducible

MJT van de Ven

WNJM Colier

D Walraven

B Oeseburg

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KEYWORDS

Brain - Near infrared spectroscopy - Cerebral blood flow - Human studies

INTRODUCTION

Theoretically, Near Infrared Spectroscopy (NIRS) can be used as a non-invasive and fast method to quantify cerebral blood flow (CBF) in neonates^[1] and adults^[2]. In contrast to current methods such as ^{133}Xe washout, positron emission tomography (PET) or magnetic resonance imaging (MRI), repeated measurements are possible at the bedside and the method is non-invasive: no ionizing radiation or injection of an intravascular tracer is needed. Therefore, the method seems very attractive for practical clinical use.

The principles of NIRS were first described in 1977^[3] and are based on absorption of near infrared light by chromophores, mainly oxyhemoglobin [O_2Hb] and deoxyhemoglobin [HHb]. Changes of optical density of a perfused tissue are converted into concentration changes of [O_2Hb] and [HHb]. When combining the changes of concentrations with an absolute value of arterial saturation obtained by pulse-oximetry, absolute values of CBF and cerebral blood volume (CBV) can be calculated.

However, the reproducibility is subject of discussion^[2,4,5].

Recently, Newton *et al.*^[5] validated NIRS measurements with other accepted techniques in dogs. They concluded that NIRS measurements in the reflectance mode using [O_2Hb] as an intravascular tracer, did not correlate with the microsphere technique nor with the venous outflow technique. They suggested that a faster sampling rate and better signal-to-noise (s/n) ratio might improve the accuracy of the measurements.

Another drawback of the method was the high rate of rejection of measurements after presetting criteria for reproducibility. In Fallon's study^[4], Elwell's study^[2], and in Newton's study^[5], 66% of all measurements could not be used as they did not fulfill preset criteria. The aim of our study was to evaluate the effect of improvements of NIRS instrumentation on the reproducibility of CBF in adults, using [O_2Hb] as an intravascular tracer. In contrast to other investigators, we used a highly sensitive continuous wave near infrared spectroscope (OXYMON, Physiology Department and Instrumentation Department, KU Nijmegen, The Netherlands) with faster sampling rates and a lower signal to noise (s/n) ratio^[6]. We focused on CBF measurements and the intra-individual variance on predefined well-controlled stable baseline values of arterial saturation (SaO_2), mean arterial pressure (MAP) and end-tidal CO_2 (PETCO_2), heart rate (HR) and respiratory rate (RR).

METHODS

NIRS

The OXYMON measures changes in optical density (OD) in the near infrared region at three different nominal wavelengths (λ): 905, 845 and 770 nm. We used a distance between transmitting and receiving fibers of 5.5 cm. This ensures deep enough penetration of light into the brain. A sampling rate of 10 Hz was used. A pathlength factor of 6.0 was used. The NIRS data were collected and displayed in real time. Data were stored on disk for off-line analysis and calculation of CBF.

CBF measurements

We used the "oxygen swing method" for measuring the CBF. The method is based on the Fick's principle in which a rapidly induced change in arterial oxyhemoglobin concentration [O_2Hb] is used as an intravascular tracer. The rate of accumulation of the tracer is monitored with NIRS. Edwards^[7] described this CBF technique using NIRS in infants. A detailed description of the analysis technique and quantification of CBF in adults using NIRS was described by Elwell^[2,8].

CBF is given by the formula:

$$\text{"Eq."} \quad K * \Delta OI$$

$$CBF \text{ (ml.100g}^{-1}\text{.min}^{-1}\text{)} = \frac{\text{-----}}{2 * [Hb]_0 \int^t \Delta SaO_2 dt}$$

where K is a constant, which accounts for molecular weight of hemoglobin, cerebral tissue density and the cerebral-to-large vessel hematocrit ratio. [Hb] represents the total hemoglobin concentration in blood (mmol/l), acquired by a blood sample. The s/n ratio of the NIRS data can be improved by measuring the change in the difference between [O_2Hb] and [HHb], defined as the oxygenation index (OI). [tHb] is the sum of [O_2Hb] and [HHb]. If [tHb] is constant, the absolute changes in [O_2Hb] and [HHb] will be equal. The difference between these changes will be approximately twice the amplitude of the changes of [O_2Hb]. The SaO_2 is measured by pulse-oximetry in the beat to beat mode, the OI with NIRS. When the rate of accumulation is measured within the mean transit time (MTT) of 6 to 10 s^[9] of the tracer, the venous outflow of the tracer will be zero. First, a slow gradual decrease in saturation is performed by lowering the FiO_2 to 11-12%. This results in a decrease of SaO_2 of about 10-15%. Next, after a stable baseline of at least 20 s at this level is reached, a bolus of pure oxygen is given in the inspiratory air during one breath.

Experimental procedure

The subjects were in supine position during the whole experiment. Peripheral SaO_2 and HR were monitored with a pulse oximeter (N200 Nellcor Puritan Bennett, St. Louis, USA), with the sensor attached to the right-frontal forehead. The optodes of the NIRS were positioned on the left side of the forehead with the receiving probe 2 cm from the midline. The optodes were held in position by an elastic band around the head.

Arterial blood pressure was measured from the middle finger of the right hand non-invasively with a finger plethysmographic device (Finapres, Ohmeda, USA). Blood pressure values were monitored as mean arterial blood pressure (MAP) during each step change in the protocol.

The subjects breathed through a facemask, covering mouth and nose. Separated valves for in- and expiratory gasmixture were integrated in the mask. Changes in inspiratory gasmixture of oxygen, nitrogen and carbon dioxide were induced by means of a computer controlled massflow system (Bronckhorst Hitec, Veenendaal, The Netherlands). This system generates maximum flow rates for O_2 and nitrogen (N_2) of 50 l/min and for CO_2 of 2.5 l/min. The FiO_2 was monitored continuously using an oxygen analyzer (OM-11 Beckman, Fullerton, CA., USA). Fast changes in inspiratory gasmixture could be generated; the aimed changes were reached within one breath. This is of prime importance to prevent slurring of the input function. The expiratory part of the system monitored PETCO_2 and the RR (N200 Nellcor Puritan Bennett, St. Louis, USA). The analogue data were linked directly to the NIRS computer for real time display and storage simultaneously with the NIRS data.

Protocols

Series 1

We started to measure seventeen healthy subjects, 9 male and 8 female, aged 21 to 54 years (mean 31years). Four measurements of CBF in baseline, normocapnic conditions were performed. After the first two measurements, the entire equipment was disconnected from the subjects, to determine the effect of repositioning of the optodes on the reproducibility of the measurements. This was done to get informed on the variation in measurements, due to optode localisation and attachment. Baseline, steady state conditions were defined as stable values of SaO_2 , MAP, PETCO_2 , RR, HR, [tHb], $[\text{O}_2\text{Hb}]$ and [HHb] for at least 20 s.

Series 2

After finishing the first experiments, alterations were made to the protocol. We emphasized more prolonged baseline steady states (30-60 sec). Ten healthy subjects, 5 males and 5 females, aged 21 to 54 years (mean 30) were investigated. After one try-out CBF procedure, we performed 4 repeated measurements in each subject during baseline normocapnic conditions without disconnection of the optodes. [Hb] was determined at the start of each series of experiments in each subject. Values of PETCO₂, HR, RR, MAP and CBF were calculated as means (SD). A paired *t*-test was used to compare the MAP data between baseline, normocapnic condition and just before and after the [O₂Hb] bolus was given. The level of statistical significance was set at $p < 0.05$. To compare reproducibility within subjects, the coefficient of variation (CV) was calculated.

All volunteers gave informed consent. This study was approved by the ethical committee of the Department of Pulmonology Dekkerswald, University of Nijmegen.

RESULTS

Figure 1 shows the upstroke of arterial saturation (upper panel) and OI (lower panel) versus time. For CBF calculations, we took the maximal value of the slope of the OI curve, plotted against the change in SaO₂. In our algorithm, we could observe an unstable ΔtHb or ΔSaO_2 , based on two measurements of 6 seconds, which were started at $t = -20$ s and at $t = -6$ s, before the takeoff upstroke point. To ensure that all of the CBF measurements took place within the mean cerebral transit time, an integration time of 3 sec was used.

Figure 1. Signal of SaO₂ and Oxygenation Index (OI); a bolus of 100% oxygen was given after a stable baseline of desaturation was reached.

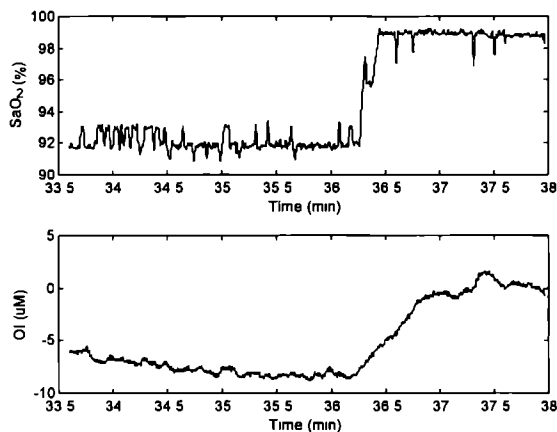


Table 1. Age and mean values (with SD) for cerebral blood flow (CBF), change in saturation (ΔSaO_2), heart rate, PetCO_2 , and respiratory rate in 17 healthy subjects; each subject challenged 4 measurements: two duplicated measurements, disconnection of the optodes and repeated measurements.

Subject	Age	CBF	delta SaO_2	MAP	PetCO_2	HR	RR
		($\text{ml} \cdot 100\text{g}^{-1} \cdot \text{min}^{-1}$)	(%)	(mm Hg)	(kPa)	(min^{-1})	(min^{-1})
		Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)
1	38	28(26)	11(3)	105(4)	4.47(0.25)	76(11)	14(2)
2	21	30(15)	10(1)	78(8)	4.12(0.26)	80(8)	10(2)
3	22	20(8)	10(2)	97(8)	4.55(0.26)	79(10)	12(1)
4	29	11(3)	11(5)	88(4)	5.43(0.07)	63(8)	11(1)
5	54	36(15)	11(7)	108(8)	4.95(0.28)	68(9)	8(5)
6	22	20(4)	12(4)	77(7)	4.27(0.18)	62(8)	19(2)
7	26	19(4)	13(5)	92(8)	4.26(0.33)	56(8)	14(2)
8	22	19(6)	11(2)	98(8)	4.22(0.42)	78(10)	15(2)
9	28	15(6)	14(7)	101(7)	5.14(0.41)	65(3)	9(2)
10	41	27(12)	11(1)	98(5)	4.40(0.56)	66(6)	12(3)
11	21	21(6)	15(1)	103(5)	4.46(0.68)	60(6)	16(2)
12	22	21(6)	14(2)	84(2)	4.24(0.27)	68(10)	11(3)
13	41	27(19)	11(2)	109(6)	4.26(0.61)	61(13)	16(1)
14	30	13(8)	10(1)	92(4)	4.30(0.80)	60(14)	17(2)
15	33	36(15)	11(1)	77(3)	4.65(0.11)	73(13)	13(2)
16	45	36(9)	11(3)	89(4)	4.72(0.33)	60(17)	8(3)
17	32	35(15)	10(2)	107(5)	4.83(0.29)	55(14)	9(2)
Mean	31(10)	26(9)	12(2)	94(6)	4.55(0.37)	66(8)	13(3)

Series 1

CBF was measured 4 times in each individual (table 1). The ΔSaO_2 , at which level a stable baseline was obtained, varied between 10 and 15% (mean 12%). Values of PetCO_2 varied little (mean SD of 0.37 kPa, range 0.07 to 0.80); the HR and RR varied by values of 8 min^{-1} and 3 min^{-1} respectively. The MAP within subjects, showed significant changes ($p < 0.01$) between the normocapnic baseline condition and just after the oxygen bolus was given. When a distinction was made between systolic and diastolic blood pressure, both systolic and diastolic blood pressure values were significant ($p < 0.05$ and $p < 0.01$, respectively). Individual values of CBF between 11 to 36 (mean 26) $\text{mL} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ brain tissue were found.

The within subject CV ranged from 22.3 to 92.4% (mean 42.4%). Figure 2 shows the considerable variability of CBF values within the subjects in the first setup.

Figure 2 The within subject variability of CBF in the first series measurements with connection of the optodes

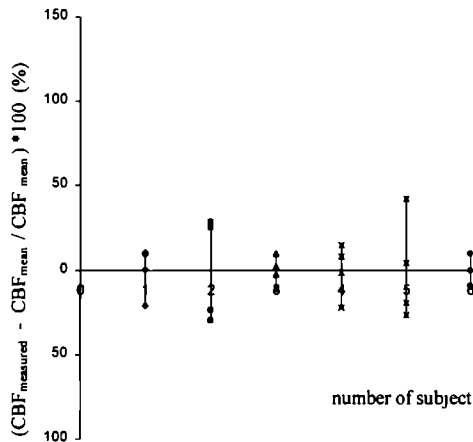


Table 2 Age and mean values (with SD) for cerebral blood flow (CBF), change in saturation (ΔSaO_2), heart rate (HR), endtidal CO_2 (P_{ETCO_2}), and respiratory rate (RR) in 10 healthy subjects, each subject 4 measurements without disconnection of the optodes

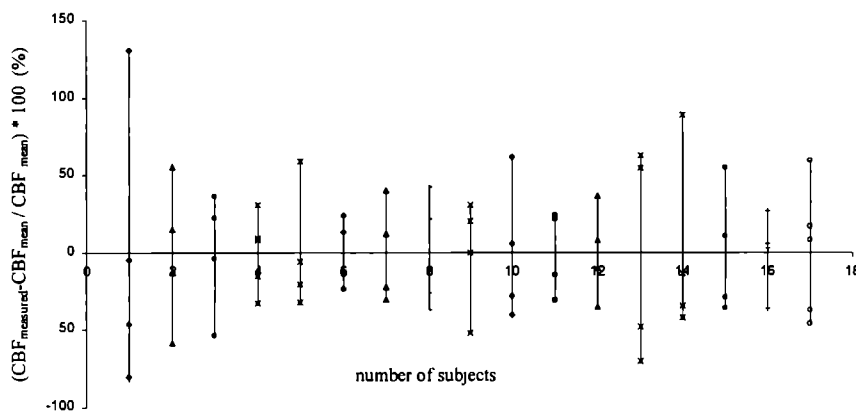
Subjects	Age	CBF ml 100g ⁻¹ min ⁻¹ Mean(SD)	delta SaO ₂ (%) mean(SD)	MAP (mm Hg) Mean(SD)	PetCO ₂ (kPa) Mean(SD)	HR (min ⁻¹) Mean(SD)	RR (min ⁻¹) Mean(SD)
1	21	27(4)	10(1)	75(5)	4.68(0.14)	80(5)	12(1)
2	22	15(5)	5(1)	91(3)	4.86(0.28)	55(3)	11(1)
3	29	16(1)	14(2)	83(8)	4.50(0.39)	76(7)	11(2)
4	54	25(4)	14(5)	86(6)	4.52(1.39)	72(6)	6(2)
5	44	37(12)	10(2)	100(9)	4.45(0.14)	73(5)	8(2)
6	21	20(2)	9(3)	71(5)	4.45(0.25)	65(3)	17(3)
7	30	12(6)	10(3)	85(3)	4.60(0.47)	50(5)	18(2)
8	22	16(2)	10(2)	94(4)	4.21(0.58)	66(5)	12(1)
9	21	20(3)	10(0)	92(4)	4.05(0.35)	72(5)	12(2)
10	41	14(5)	13(2)	95(7)	4.10(0.56)	65(4)	11(2)
mean	31(12)	20(8)	11(3)	87(5)	4.44(0.26)	67(9)	12(4)

Series 2

The results are shown in table 2. The ΔSaO_2 varied between 5 to 14% (mean 11%). Values of P_{ETCO_2} varied with a mean SD of 0.26 kPa (range 0.14 to 1.39); the HR and RR varied by 9 min⁻¹ and 4 min⁻¹ respectively. The MAP within subjects showed significant

changes ($p=0.03$) between the normocapnic baseline condition and just before the oxygen bolus was given. When a distinction was made between systolic and diastolic blood pressure, only systolic blood pressure was significant ($p<0.01$). Individual values of CBF between 12 to 37 (mean 20) $\text{mL} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ brain tissue were found. The within subject CV ranged from 31 to 52 (mean 22.6%). Figure 3 shows the variability of CBF values within the subjects during the second setup.

Figure 3 The within subject variability of CBF in the second series four consecutive measurements without disconnection of instrumentation.



DISCUSSION

In this study the reproducibility of CBF measurements in human adults was investigated. Using a new and improved continuous wave NIRS instrument, we fulfilled some of the recommendations by other authors to improve accuracy and reliability^[2,5] of CBF measurements in adults. However, the results showed a wide intra-subject variation of the CBF measurements, in standardized experimental conditions.

Physiological Considerations

CBF is controlled by metabolic, neural and chemical stimuli. The autoregulation of CBF, with a lower and an upper blood pressure limit^[10], can be influenced by a number of vascular mechanisms^[11,12]. A 7% increase in flow per 10-mmHg increase in MAP in normal humans was described^[13]. All subjects in our study were considered

normotensive, although we found mean MAP values up to 109 mm Hg. It is well known that within-individual alterations in blood pressure occur and differences between home and clinic measurements are common^[14]. Considering the results of Moyer^[13], alterations of (mean) 6-mmHg (*series 1*) and 5-mmHg (*series 2*) during the whole experiment could lead to an increase in (mean) flow of approximately 3.5 to 4 %. These small alterations are unlikely to be of relevant importance for the wide CBF variance.

In both series, the MAP showed significant changes within subjects, when mean blood pressure values were compared. In the first series, both systolic and diastolic blood pressure values were significantly different between normocapnic baseline condition and just after the oxygen bolus was given. However, these differences in MAP still fall well within the range of cerebral autoregulation. When a distinction was made between systolic and diastolic blood pressure in the second series, only systolic blood pressure was significant ($p < 0.01$). The latter can be influenced very easily by environmental factors^[14].

Alterations of heart rate values and respiratory rate values could have an influence on CBF variability. However, both variables showed no significant differences during the experiments and are therefore unlikely to be responsible for the CBF variability.

The main chemical stimuli of CBF are CO₂, pH and O₂. CO₂ is known as an important modulator of cerebral vascular resistance: hypocapnia induces cerebral vasoconstriction, whereas hypercapnia causes cerebral vasodilation. Concerning intrasubject alterations of PETCO₂ during series 1 and 2, changes ranged from 0.07 to 0.8 kPa in series 1 and from 0.14 to 1.39 kPa in series 2. Another chemical stimulus, hypoxia, could result in vasodilation and therefore increased CBF^[10]. We applied only a slight desaturation (mean Δ SaO₂ 12 and 11%, series 1 and 2, respectively). Previous physiological studies showed that CBF is virtually constant when arterial oxygen tension varies between 5.7 and 12.3 kPa^[15] and therefore no significant influence on CBF may be expected.

The calculated mean value of all CBF measurements from *series 1* was lower than the mean value of all CBF measurements of *series 2*. This could be partly due to the lower PETCO₂ seen in *series 2* (mean 4.44 kPa), compared to *series 1* (4.55 kPa). It is well known that hypocapnia leads to vasoconstriction with lowered CBF. As a broad rule of thumb, CBF increased by 46%/per kPa change in arterial PCO₂ during moderate hypercapnia when compared to the normocapnic value for blood flow^[16]. Hyperventilation (Paco₂ < 3.4 kPa) reduces flow by a maximum of 40 – 50%^[17]. CBF reactivity during moderate hyperventilation is not known in terms of percentage/kPa CO₂. Based on Olesen's values for CBF reactivity during moderate hypercapnia^[16], only a (mean) 4.5% lowered value of CBF could account for the lower PETCO₂ in our

experiments. This is not sufficient to explain the lowered (mean) CBF value of the second series.

Methodological considerations

We used an advanced NIRS instrument, the OXYMON, with improved noise level, making rapid sampling (10 Hz) possible. The maximal sampling rate of the peripheral arterial Sao_2 signal, using a pulse oximeter, however is limited to a beat-to-beat modus. This is a weakness, which cannot be solved.

We measured with 3 s integration time; due to the high sampling rate of the NIRS instrument we collected a semi-continuous signal, in contrast to the Sao_2 signal of which only 2 or 3 values can be collected due to the heart beat. This will mainly explain the high CV in our group and among others^[2,5] and may explain why neonates with higher heart rates have better intrasubject CV's^[18].

The influence of the disconnection of the optodes could cause a lower reproducibility; the CV of the second series however, showed little improvement compared to the first series. Therefore small positioning errors are not of relevant importance for the measurements.

The mean CBF in both protocols were in the same range as found by Elwell *et al.*^[2] and Owen-Reece *et al.*^[19].

As shown by others^[20,21], the interoptode distance should have a minimum distance of 5 cm for significant intracranial signal sampling. The suggestion that extracranial blood contents could disturb intracranial CBF measurements was investigated by Owen-Reece *et al.*^[19]. He investigated cerebral blood volume in healthy subjects, before and after inflating a pneumatic tourniquet proximal to the measurement site. He saw no effect on NIRS measurement of cerebral hemodynamics when the scalp blood flow was clamped. This finding was in contrast with the results of Newton *et al.* (1997)^[5] in dogs and Lam *et al.*^[21] in patients during carotid endarterectomy, in which the external carotid artery was clamped for a full 2 minutes. In 50% of the patients, they saw an extracranial contribution to the NIRS signal. In our experiments, disturbances due to extracranial contribution are not of great importance in terms of reproducibility, since the applied changes in the experiments were equal on internal and external carotid circulation.

In conclusion, measurements of CBF with the NIRS are not reproducible despite improved faster sampling and lower noise level. Generally, the measured values of CBF were in the same range as measured by other investigators, using the NIRS method. Considering methodological and physiological problems, the limited frequency of the pulse oximetry remains an important limitation to the use of O_2Hb as an indicator.

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**Can cerebral blood volume be measured reproducibly
with an improved near infrared spectroscopy system?**

MJT van de Ven

WNJM Colier

MC van der Sluijs

D Walraven

B Oeseburg

H Folgering

Journal of Cerebral Blood Flow and Metabolism 2001; 21, 110-113.

ABSTRACT

In some circumstances, cerebral blood volume (CBV) can be used as a measure for cerebral blood flow. A new near-infrared spectroscope was used for determining the reproducibility of CBV measurements assessed by the O₂-method. 27 healthy subjects were investigated. An intrasubject coefficient of variation (CV) was calculated, based on four identical episodes of desaturation-resaturation ("O₂-method") procedures for CBV measurements. Two trials were performed, with (trial 1) and without (trial 2) disconnecting the equipment. A mean CV of 12.6% and 10.0% was found in trial 1 and 2, respectively. CBV values yield $3.60 \pm 0.82 \text{ mL} \cdot 100 \cdot \text{g}^{-1}$. CBV could be measured reproducible in adults using near infrared spectroscopy, if the arterial desaturation is limited to approximately 5% from baseline level.

KEYWORDS

Brain - Near infrared spectroscopy - Cerebral blood volume - Human studies

INTRODUCTION

Near-infrared spectroscopy (NIRS) is a non-invasive continuous method to monitor brain oxygenation and hemodynamics. It has been used as a monitoring device during carotid, cerebrovascular and cardiopulmonary bypass surgery and on head-injured and other intensive care patients^[1]. By using oxyhemoglobin (O_2Hb) as an intravascular tracer it is possible to calculate cerebral blood volume (CBV) using the desaturation-resaturation method, also named the " O_2 -method"^[2]. A gradual change of arterial oxygen saturation (SaO_2) of approximately 10%, measured by pulse oximetry, induced by changing the inspired oxygen fraction (FiO_2), is related to a small change of $[O_2Hb]$ and allows calculation of an absolute value of CBV^[2,3]. However, the reproducibility of the measured values of CBV in adults is being questioned^[3]. Faster sampling rates and a good signal-to-noise (s/n) ratio has been suggested to improve reproducibility. Furthermore, a constant wide distance between emitter and detector (interoptode distance) is necessary to distinguish intra-cerebral from extra-cerebral tissue oxygenation^[4,5]. A new continuous wave (CW) NIRS instrument integrating these properties was used in this study^[6]. We set out to test the reproducibility of CBV assessment utilizing the O_2 -method using NIRS. Absolute CBV values were compared to those found in other studies using NIRS.

MATERIALS AND METHODS

After obtaining local research Ethics Committee approval and informed consent, we studied twenty-seven healthy subjects (14 men), mean age 31 (range 21-54) yr. The principles of CW NIRS and the O_2 -method are described in detail elsewhere^[2,3,7]. The CW NIRS instrument (OXYMON, Physiology and Instrumentation Departments, University Medical Centre Nijmegen, The Netherlands) measures changes in optical density (OD) in the near infrared region at three different wavelengths. The instrument has been compared with some others using a standardized phantom^[8]. It showed to be more sensitive (more than 9 OD). With this instrument it is, e.g., possible to show the vascular response to a single finger opposition task in the motor cortex area without any averaging^[9]. Furthermore it allows sampling average times down to 20 ms without deteriorating the sensitivity^[9]. Changes in OD are converted into concentration changes of O_2Hb ($[O_2Hb]$) and deoxyhemoglobin ($[HHb]$), using a modified Lambert-Beer law algorithm^[10]. Total hemoglobin concentration ($[tHb]$), which is a measure for blood volume, is defined as the sum of $[O_2Hb]$ and $[HHb]$. The sensitivity of the NIRS data is improved by measuring the change in the difference between $[O_2Hb]$ and $[HHb]$, defined as the oxygenation index (OI) and provided there is no change in $[tHb]$ ^[3]. Sampling was

performed at 10 Hz; transmitting and receiving fibres were placed on the left side of the forehead with the receiving probe 2 cm from the midline. A constant interoptode distance of 5.5 cm was used. The optodes were held in position by an elastic band around the head. To correct for light scattering in the tissue, a fixed differential path length factor of 6.0 was used^[11].

The subjects were in supine, comfortable position during the entire experiment. For each subject a try-out desaturation-resaturation episode preceded the actual measurements. CBV measurements were repeated 4 times. Mean arterial blood pressure (MABP), end tidal CO₂ (PETCO₂), cardiac frequency (f_H) and respiratory frequency (f_R) were continuously monitored. [tHb] was visualised continuously and used as an indicator for changes of CBV. The latter should be constant during the desaturation manoeuvre.

Reproducibility of CBV measurements might be worsened, due to optode displacement. To determine the effect of repositioning of the optodes on the reproducibility of the measurements, two different trials were performed. In the first trial, the optodes were disconnected after two episodes, to continue with the other two episodes after a short break. The second trial involved four consecutive episodes without disconnection of the equipment.

SaO₂ and f_H were monitored with a pulse oximeter (N200 Nellcor Puritan Bennett, St. Louis, USA), with the sensor attached to the right-frontal forehead. MABP was measured from the middle finger of the right hand non-invasively with a finger plethysmographic device (Finapres, Ohmeda, USA). The subjects breathed through a one-way facemask. Changes in inspiratory gas mixture were induced by means of a computer controlled mass flow system (Bronckhorst Hitec, The Netherlands). The F_{IO₂} was monitored using an oxygen analyser (OM-11 Beckman, Fullerton, CA., USA). At the expiratory port of the system, PETCO₂ and f_R were monitored with a capnograph (N1000, Nellcor Puritan Bennett, USA). All analogue data were stored simultaneously with the NIRS data. Arterialized capillary bloodgas samples were taken to determine the systemic [Hb] in each subject (Synthesis 25, Instrumentation Laboratory SpA, Italy).

Values of CBV were expressed as means \pm SD. Continuous measurements of PETCO₂, MABP, f_H, and f_R were averaged (\pm SD) to cancel out slight alterations during the experiments. Data were averaged for 1 minute prior to the start of the desaturation procedure and for 1 minute at the constant desaturated level. Mean values of CBV, PETCO₂, MABP, f_H, and f_R of both sexes were compared with a Student's unpaired test. The level of statistical significance was set at $p < 0.05$. Reproducibility of CBV was expressed as the intrasubject coefficient of variation (CV) among four measurements. Multiple regression analysis was used to relate CBV to PETCO₂, MABP, f_H, and f_R.

Table 1. Reproducibility measurements of 27 subjects

Subjects	Age	CBV (mL 100 g ⁻¹)	CV (%)	PETCO ₂ (mm Hg)	fh (min ⁻¹)	MABP (mm Hg)
1, f	21	6.25 ± 0.09	1.6	35 ± 1	80 ± 5	77 ± 5
2, m	22	3.88 ± 0.72	18.6	36 ± 2	55 ± 3	92 ± 3
3, f	29	4.36 ± 0.29	6.7	34 ± 3	76 ± 7	93 ± 8
4, m	54	3.26 ± 0.26	8.1	34 ± 5	72 ± 6	86 ± 6
5, m	44	4.28 ± 0.44	10.4	33 ± 1	73 ± 5	100 ± 9
6, m	21	4.06 ± 0.72	17.7	33 ± 2	65 ± 3	73 ± 5
7, m	30	4.14 ± 0.16	5.6	35 ± 4	50 ± 5	87 ± 3
8, f	22	4.01 ± 0.43	7.0	32 ± 4	66 ± 5	97 ± 4
9, f	21	4.25 ± 0.33	6.4	30 ± 3	72 ± 5	93 ± 4
10, f	41	3.1 ± 0.55	17.8	31 ± 4	65 ± 4	97 ± 7
mean	31 ± 12	4.16 ± 0.84	10.0 (5.7-14.2)	33 ± 3	67 ± 9	90 ± 9
11, f	38	3.22 ± 0.44	13.7	34 ± 2	76 ± 11	105 ± 4
12, f	21	4.5 ± 0.46	10.2	31 ± 2	80 ± 8	79 ± 5
13, f	22	4.2 ± 0.53	12.7	34 ± 2	79 ± 10	98 ± 8
14, m	29	3.19 ± 0.22	6.8	41 ± 1	63 ± 8	90 ± 4
15, m	54	2.98 ± 0.28	9.6	37 ± 2	68 ± 9	109 ± 8
16, f	22	4.0 ± 0.86	21.6	32 ± 1	62 ± 8	79 ± 7
17, f	26	2.75 ± 0.27	10.1	32 ± 2	56 ± 8	93 ± 8
18, f	22	4.65 ± 0.59	12.6	32 ± 3	78 ± 10	100 ± 8
19, f	28	3.22 ± 0.23	7.3	39 ± 3	65 ± 3	103 ± 7
20, m	41	4.05 ± 0.6	15.2	33 ± 4	66 ± 6	100 ± 5
21, m	21	2.16 ± 0.35	16.6	33 ± 5	60 ± 6	104 ± 5
22, f	22	3.4 ± 0.19	5.6	32 ± 2	68 ± 10	85 ± 2
23, m	41	2.99 ± 0.8	27.0	32 ± 5	61 ± 13	111 ± 6
24, m	30	2.67 ± 0.42	15.8	32 ± 6	60 ± 14	94 ± 4
25, m	33	3.01 ± 0.23	7.8	35 ± 1	73 ± 13	78 ± 3
26, m	45	2.91 ± 0.36	12.3	35 ± 2	60 ± 17	91 ± 4
27, m	32	3.24 ± 0.33	10.2	36 ± 2	55 ± 14	109 ± 5
mean	31 ± 10	3.36 ± 0.68	12.6 (9.8-15.4)	34 ± 3	66 ± 8	96 ± 11
total mean	31 ± 10	3.66 ± 0.82	11.7 (9.6-13.9)	34 ± 2	66 ± 8	93 ± 11

Values are the mean ± SD of 4 measurements. CBV, cerebral blood volume; PETCO₂, endtidal CO₂; fh, cardiac frequency; MABP, mean arterial blood pressure; CV, intrasubject coefficient of variation, mean (range, 95% C.I.); f, female; m, male. Measurements were performed without (subject 1-10) and with (subject 11-27) disconnection of equipment halfway the protocol.

RESULTS

The individual and mean (\pm SD) results of both trials are displayed in Table 1. The average decrease of SaO_2 was 12% (range 10-15%). The mean intrasubject CV of both trials was 11.7 %. The intersubject CV of CBV of both trials was 22% (20.2-24.4, 95% C.I.). Mean values of CBV were significantly higher ($p < 0.05$) in females relative to males, 3.99 ± 0.91 and 3.34 ± 0.64 mL.100g⁻¹ respectively. Significant differences between sexes ($p < 0.05$) were seen in age and PETCO_2 (both higher in males) and fh (lower in males). CBV was related to age and fh as follows:

$\text{CBV} = 1.17 - 0.03 \cdot \text{age} + 0.05 \cdot \text{fh}$ ($p < 0.01$, $r^2 = 0.40$; CBV: mL.100 g⁻¹).

DISCUSSION

In this study the reproducibility of CBV measurements in a large population of human adults was investigated with an improved continuous wave NIRS instrument. The present study showed a good reproducibility, even after reconnecting the equipment. Furthermore, this study showed sexual differences in CBV.

The experimental design to assess CBV followed Elwell's experimental setup (1994). They used an interoptode distance of 5 cm and a NIRO 500 (Hamamatsu Photonics KK, Japan) instrument with a sampling rate of 2 Hz and. On each of the 10 subjects 5 CBV measurements were performed using the O_2 -method. Only 22 measurements proved suitable for calculating CBV. A high intrasubject CV for the CBV measurements of 24% (range 6-38%) was found.

The present study also used the O_2 -method to calculate CBV. There is controversy whether the O_2 -method or the "[tHb]-method" is better for monitoring CBV. The latter method measures changes of [tHb] invoked by respiratory hypercapnia. Both methods can be used to measure cerebrovascular reactivity. However, baseline values during normocapnia can only be determined with the O_2 -method. Firbank et al. (1998) compared both methods, evaluating hypercapnic responsiveness. The O_2 -method was found to have a high CV (23%), whereas the other method had a low CV (3%). In contrast to the [tHb]-method to determine CBV, the O_2 -method was not able to distinguish CBV values on different levels of Paco_2 in their analysis. The explanation of the high CV was mainly based on the accuracy of the pulse oximeter, which is 3%^[12]. In contrast to Firbank, Totaro et al.^[13] found a good reproducibility for $\Delta[\text{OI}]$ and a bad reproducibility for $\Delta[\text{tHb}]$ while evaluating cerebrovascular reactivity. We favour the use of the O_2 -method to determine CBV at different Paco_2 levels; moreover, significant ($p < 0.001$) changes of CBV were found during hyper- and hypocapnia, as compared with normocapnia^[14].

The absolute CBV values ($3.66 \pm 0.82 \text{ mL} \cdot 100\text{g}^{-1}$, $n=27$) were comparable with values ($3.51 \pm 0.71 \text{ mL} \cdot 100\text{g}^{-1}$) found in earlier studies performed on 15 healthy subjects (5 men), mean age 56 yr^[14]. Values of CBV were slightly higher than CBV results of Elwell et al.^[3] ($2.85 \pm 0.97 \text{ mL} \cdot 100\text{g}^{-1}$, $n=10$, mean age 27.5 yr), but lower than those found by Gupta et al.^[15] ($5.38 \text{ mL} \cdot 100\text{g}^{-1}$, $n=13$, mean age 35 yr). As discussed by Gupta, an arterial desaturation of 10-15% may have been crossed the threshold for hypoxic vasodilatation. In the current study negligible values of $\Delta[\text{tHb}]/\Delta[\text{OI}]$ were seen, suggesting a constant CBV during the applied hypoxia even during mild hypocapnia (mean PETCO_2 4.50 kPa). As suggested by others^[15], a possible influence of hypoxia on cerebral vasoresponsiveness using the O_2 -method should be avoided and therefore desaturation should be limited to 5% from baseline saturation level.

The higher value of CBV in women in the present study is in line with the higher value of cerebral blood flow (CBF) in other studies^[16]. They suggest differences in functional organization of the cortex to be the main responsible. Although the younger age of women could contribute to a higher CBV, this effect is counteracted both by a lower PETCO_2 and fH . Previous studies showed a correlation between CBF and various physiological factors like age^[17], PETCO_2 and fH ^[18]. In the present study, we found fH and age to be significant determining factors for CBV.

In conclusion, CBV assessed by the O_2 -method, can be measured reproducibly in adults using near-infrared spectroscopy. The required desaturation to calculate CBV should preferably be limited to 5% from baseline saturation level to avoid confounding hypoxic cerebral vasodilatation.

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Induction of acute metabolic acid/base changes in humans

MJT van de Ven

WNJM Colier

B Oeseburg

H Folgering

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ABSTRACT

This study aimed at inducing acute metabolic acid/base changes of ≥ 2 mEq/L change in base excess (BE) in the perspective of future investigations of respiratory parameters in these conditions. Ammoniumchloride (NH_4Cl) was given for acidification, and furosemide for alkalization.

Nine healthy volunteers, (6 m, 3 f, 35 ± 18 yrs), ingested a calculated amount of NH_4Cl at $t=0$, and a repeated dose after 60 min. Eight healthy volunteers (3 m, 5 f, 37 ± 16 yrs) took 40 mg of furosemide. Arterialized capillary blood gases were measured at $t=0$, 30, 60, 90, 120 and 180 min.

Acute metabolic acidosis: the aimed acidification of 2 mEq/L was attained after 30 min. and reached the greatest change at 90 min: -4.9 (2.2) mEq/L. Acute metabolic alkalosis: the aimed alkalization of 2 mEq/L was reached between 120 and 180 min; the greatest change was seen at 180 min: $+2.2$ (1.4) mEq/L. Significant changes of acidification were found ($p<0.05$) from 60 to 180 min; significant changes of alkalization were found from 120 to 150 min ($p<0.05$), both compared with baseline BE values. PaCO_2 did not change significantly in either condition.

We conclude that NH_4Cl and furosemide induce a steady state of pure metabolic acid/base conditions in humans, that are buffered in an isocapnic way.

KEYWORDS

Ammoniumchloride, Furosemide, Base Excess, Control of Breathing, Extracellular Fluid.

INTRODUCTION

The chemical control of ventilation is dominated by three stimuli: changes in P_{CO_2} , P_{O_2} and pH. Changes in pH can result from a primary respiratory or metabolic origin. Acute primary respiratory acid-base changes involve both central and peripheral chemoreceptors, whereas acute primary metabolic changes have a larger effect on the peripheral and a lesser and slower effect on the central chemoreceptor^[1]. Oral administration of ammoniumchloride (NH_4Cl) has been shown to be effective at inducing acute metabolic acidosis^[2-6], which causes NH_3 to be trapped by the liver and metabolised to urea, leaving HCl in the systemic circulation.

Oral administration of furosemide causes alkalisation. Furosemide is a loop diuretic: in the thick ascending limb of the loop of Henle, 20 to 25% of the filtered Na^+ can be excreted with maximal diuresis^[7]. The drug induces a metabolic alkalosis due to increased urinary H^+ loss and contraction of the extracellular volume around a constant amount of extracellular HCO_3^- (called contraction alkalosis)^[7].

A change in base excess (BE) represents a nonrespiratory disturbance of the acid-base balance^[8]. Changes in BE cause shifting of the CO_2 dissociation curve, producing considerable changes in the total CO_2 content of the blood and consequently changes in ventilation^[8]. In the present study, we have sought to induce a change in base excess (BE) of at least 2.0 mEq.L^{-1} , which is approximately a ten-fold shift of the intra individual variability ($SD \text{ } 0.2 \text{ mEq.L}^{-1}$)^[9]. We analysed the changes in BE and P_{CO_2} following NH_4Cl and furosemide. Furthermore, we have described a model to calculate responses of minute ventilation and CO_2 output following an isocapnic, acutely induced change of BE of 2 mEq.L^{-1} .

METHODS

The administered dose of NH_4Cl was based on the assumption that extracellular fluid comprises one third of total body weight^[6]. This value (in litres) multiplied by 2 mmol provides the total amount of NH_4Cl necessary to reach the desired degree of acidification. Multiplication of this value by the molecular weight of NH_4Cl (53.5) gives the dosage in mg. Nine healthy volunteers (6 men and 3 women, aged 35 ± 18 years) took the calculated amount of NH_4Cl , ranging from 1.7 to 2.6 grams, at point $t=0$ and a repeated dose after 60 min, in order to keep plasma levels constant, since $t_{1/2}$ is approximately 60-75 min^[10]. Eight different volunteers (3 men and 5 women, aged 37 ± 16 years) took 40 mg of furosemide at $t=0$. It was not necessary to repeat the dose of furosemide, since this amount of furosemide provides a maximal diuretic effect for approximately 6 hours^[7].

At $t=0, 30, 60, 90, 120$ and 180 min, we took arterialized capillary blood from the fingertip and measured changes in pH, BE and PCO_2 . All subjects were at resting position, sitting on a chair. Before the sample was taken, the hand was warmed in hot water (40°), for at least 5 min. The arterialized samples were collected in heparinized glass capillaries (capillary tube kit, Instrumentation Laboratory SpA, Italy) and immediately introduced into the blood gas analyzer (Synthesis 25, Instrumentation Laboratory SpA, Italy). The statistical tests were all performed against baseline; we used a non-parametric repeated measures ANOVA (Friedman Test) with Dunn post test and $P<0.05$ was considered significant.

All volunteers gave informed consent and this study was approved by the ethical committee of the Department of Pulmonology Dekkerswald, Groesbeek.

RESULTS

Mean values of metabolic responses are presented in Table 1. Changes of base-excess (ΔBE) are shown in Figure 1. In response to oral administration of NH_4Cl , acute metabolic acidosis, measured by ΔBE , showed a highest value at $t=90$ min, 4.9 mEq.L^{-1} (SD 2.23). At $t=30$ min, the aimed acidification was reached on a group level; all (9) subjects reached the aimed acidification at $t=90$. ΔBE reached significance compared to baseline from $t=60$ - 180 min. In addition, at $t=90$ min ΔPCO_2 was decreased by an average value of -0.30 kPa (SD 0.5), which was not significant.

Acute metabolic alkalosis, induced by oral administration of furosemide, showed a highest value at $t=180$ min, 2.23 mEq.L^{-1} (SD 1.39). At $t=120$ - 180 min, the aimed alkalization was reached on a group level and ΔBE reached significance as compared to baseline. At $t=120$ - 180 min, 5 of 8 subjects reached a ΔBE of $\geq 2 \text{ mEq.L}^{-1}$ (range 2.3 to 4.9 mEq.L^{-1}); in the remaining 3 subjects, the ΔBE ranged from 1 to 1.6 mEq.L^{-1} . Simultaneous average measurements of ΔPCO_2 varied from -0.09 to -0.01 kPa (SD 0.25 to 0.43), which were not significant from baseline.

DISCUSSION

We wanted to find an appropriate method of inducing different metabolic acid-base conditions, defined as a change of base excess of at least two mEq.L^{-1} , within 1-2 hours and stabilisation of these conditions for at least 1-2 hours.

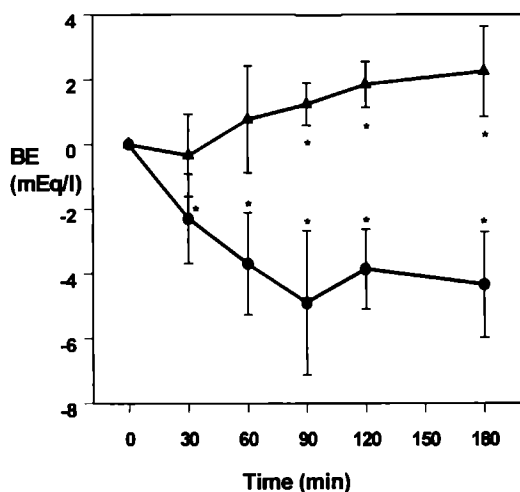
As Sauty *et al.*^[11] pointed out, the arterial-arterialized PCO_2 difference is negligible (0.067 kPa , $0.5 \pm 1.5 \text{ mmHg}$), because of the arteriovenous PCO_2 difference at rest is very small: 0.8 kPa (6.0 mmHg). Therefore, it can be justified to use arterialized capillary blood to measure the PCO_2 and BE changes.

Table 1. Mean values (and standard deviations) of pH, base excess (BE), changes of base excess (Δ BE), P_{CO_2} and ΔP_{CO_2} at different time intervals (min) after administration of ammoniumchloride (NH_4Cl) and furosemide.

	time	0	30	60	90	120	180
NH_4Cl	pH	7.44 (0.02)	7.43 (0.03)	7.40 (0.02)	7.39 (0.04)	7.39 (0.02)*	7.39 (0.03)*
	BE (mEq/L)	2.24 (1.32)	0.01 (1.68)	-1.45 (1.27)	-2.6 (1.35)	-1.68 (1.16)	-2.10 (0.57)
	Δ BE (mEq/L)		-2.30 (1.38)	-3.70 (1.58)*	-4.90 (2.23)***	-3.86 (1.23)**	-4.34 (1.64)**
	P_{CO_2} (kPa)	4.95 (0.37)	4.85 (0.33)	4.60 (0.42)	4.65 (0.38)	4.74 (0.24)	4.75 (0.47)
	ΔP_{CO_2} (kPa)		-0.2 (0.35)	-0.35 (0.36)	-0.30 (0.50)	-0.28 (0.33)	-0.20 (0.42)
Furo-Semide	pH	7.42 (0.02)	7.42 (0.02)	7.43 (0.02)	7.43 (0.01)	7.44 (0.01)	7.45 (0.01)
	BE (mEq/L)	1.60 (1.35)	1.44 (1.06)	2.36 (1.94)	2.83 (1.07)	3.53 (1.11)	3.84 (1.42)
	Δ BE (mEq/L)		-0.34 (1.27)	0.76 (1.64)	1.23 (0.65)	1.84 (0.70)*	2.23 (1.39)***
	P_{CO_2} (kPa)	5.23 (0.46)	5.15 (0.24)	5.11 (0.48)	5.21 (0.32)	5.14 (0.31)	5.21 (0.30)
	ΔP_{CO_2} (kPa)		0.01 (0.28)	-0.12 (0.59)	-0.09 (0.25)	-0.09 (0.36)	-0.01 (0.43)

Statistical tests were all performed against baseline (*= $P<0.05$; **= $P<0.01$; ***= $P<0.001$).

Figure 1. Changes of base excess (mEq/L) after administration of NH_4Cl (●) and furosemide (▲) at different time interval (min) Error bars: SD. *= $P<0.05$, **= $P<0.01$; ***= $P<0.001$.



Ammoniumchloride has been used previously to measure ventilatory responses. Tojima *et al.*^[4] used metabolic changes induced by acetazolamide and NH_4Cl to measure effects on ventilatory responses. They gave a dose of 8 grams NH_4Cl daily for 3 days and determined the effect of acidification, expressed as a reduction in standard plasma bicarbonate (mean of $5.6 \pm 1.8\text{mM}$) and pH (0.08 ± 0.03). These changes corresponded with significant decreases in Paco_2 : 0.48 ± 0.33 kPa ($p < 0.02$). Lerche *et al.*^[2] administered a total amount of 0.3 g/kg NH_4Cl partitioned over 3-4 doses. They found a decrease in whole blood buffer base of 6.0 mval l^{-1} and a leftward shift of the ventilatory response curve to CO_2 . Langbroek *et al.*^[5] measured ventilatory responses to a metabolic acidosis induced by oral administration of NH_4Cl in awake dogs. After administration of 10 mmol per kg body mass NH_4Cl , they saw a significant decrease of PCO_2 from 4.61(0.18) kPa in the control group, to 4.36 (0.10) kPa and 3.57 (0.10) kPa, after 1 hour and 4 hours respectively. In the present study, we used a lower total dose, which induced a pure metabolic change without significant concomitant lowering of CO_2 .

The extra amount of CO_2 and minute ventilation as a result from the induced metabolic acidification can be estimated. Assuming a total body weight (TBW) of 75 kg, extracellular fluid distribution as one third of TBW and a desired change in BE of 2 mmol, the desired dose is 50 ($75 \times 0.33 \times 2$) mmol NH_4Cl . To maintain the isocapnic acid-base homeostasis of body fluid, an excess of 50 mmol CO_2 will have to be blown-off. Multiplication of this amount with the CO_2 gas conversion factor of 22.26[2], yields 1.11 liter CO_2 at STPD or 1.35 liter CO_2 at BTPS as an index of the increased CO_2 production (ΔVCO_2) generated by this metabolic acidification.

Calculation of excess in minute ventilation (ΔVe) equals the quotient of ΔVCO_2 and the fractional concentration of CO_2 (Feco_2). Assuming a mixed expired Feco_2 of 4%, ΔVe is approximately 34 L/min. Within 30 minutes, a decrease of BE of 2 mEq/L is reached, resulting in a mean extra ventilation of 1.1 L/min. This is much lower than the calculated ventilation changes found by Lerche, who saw an increased ventilation of $2.7 \text{ L} \cdot \text{min}^{-1}$ per $-1 \text{ mEq} \cdot \text{L}^{-1}$ isocapnic change of whole blood buffer base. Two main reasons could explain the lower value, found in the present study. The first reason could be the measured time interval after 30 min; if the aimed acidification of $2 \text{ mEq} \cdot \text{L}^{-1}$ would have been reached after 15 min, the excess in ventilation would have to be two times higher. Secondly, an assumed lower mixed expired Feco_2 of 3.0% would have given rise to a ΔVe of approximately $45 \text{ L} \cdot \text{min}^{-1}$, this again would have resulted in a higher excess of ventilation of $1.5 \text{ L} \cdot \text{min}^{-1}$. Lerche *et al.*^[2] did not take the time to induce an acidification, nor the endtidal PCO_2 into account. They measured the quotient of change in ventilation and change in BE during a constant Fico_2 of zero (room air) and 2% in control and acidosis experiments.

In the present study, NH_4Cl was well tolerated, only minor complaints of nausea were mentioned in 3 of 9 subjects.

In our study, changes in BE obtained after NH_4Cl administration exceeded the aimed minimum of 2 mEq.L^{-1} . This can be explained by our calculation method of NH_4Cl quantity as this was partly based on the assumption, that extracellular fluid comprises a third of total body weight. However, the quantity of extracellular water in proportion to total body weight varies between $23 \pm 3\%^{[12]}$ and $30\%^{[6]}$. Therefore, if the extracellular water proportion was actually less than 30%, a lower dose should be sufficient to reach the 2 mEq.L^{-1} BE change.

The role of furosemide to induce acute metabolic alkalosis has not been reported previously. Furosemide resulted in a significant rise in ΔBE , what was still rising. Again the ventilatory compensating reaction was complete as we obtained isocapnic bloodgas values through the study period. The subjects reported no significant side effects of furosemide, apart from the expected diuresis.

We conclude that NH_4Cl and furosemide induce a clear pattern of pure metabolic acid/base conditions in normal volunteers, that are buffered in an isocapnic way. Both drugs were well tolerated and the ability to induce metabolic acidosis or alkalosis suggests that the agents may be useful in the future determination of respiratory changes induced by acute ΔBE .

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Chapter 5

Cerebral blood volume responses to acute PaCO₂ changes in humans, assessed with near infrared spectroscopy

MJT van de Ven

WNJM Colier

BTP Kersten

B Oeseburg

H Folgering

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KEYWORDS

Carbondioxide, NIRS, cerebral blood volume, human studies, brain.

INTRODUCTION

Near Infrared Spectroscopy (NIRS) can be used to measure cerebral blood volume (CBV), and its reactivity to changes in PaCO_2 . NIRS is a promising technique, which offers continuous measurements of CBV at bedside and is relatively cheap. Moreover, invasiveness is not necessary, which makes NIRS a very interesting tool for clinical use. The assessment of CBV reactivity in response to PaCO_2 changes is not as common as the use of cerebral blood flow (CBF). However, there is a close relationship between CBV and CBF that has been extensively investigated by Grubb *et al.*^[1] and by Van Zijl *et al.*^[2]. They showed a significant, nonlinear relationship between CBF and CBV ($r=0.9$). Moreover, the use of CBV in stead of CBF eliminates the problems related to the mean transit time^[3].

The changes in CBF hemodynamics due to changes in arterial PCO_2 are well known^[1,4]; hypocapnia results in vasoconstriction with a decrease in CBV, whereas hypercapnia results in vasodilation with increased CBV^[5].

The optical technique of NIRS is based on the principle, that light in the NIR region is absorbed by chromophores of oxyhemoglobin [O_2Hb] and deoxyhemoglobin [HHb]^[6]. Changes in light absorption are converted into concentration changes of these chromophores. By monitoring changes in [O_2Hb], it should be possible to measure and quantify CBV^[7,8].

In this study, we used NIRS to determine the cerebrovascular reactivity to acute hypo- and hypercapnia, expressed as CBV/PaCO_2 , in healthy conscious humans. Secondly, we set out to establish reference values of $\Delta\text{CBV}/\Delta\text{PaCO}_2$ during hypercapnia and hypocapnia. These reference values of $\Delta\text{CBV}/\Delta\text{PaCO}_2$, measured in healthy subjects, will help to evaluate the response to CO_2 in patients with chronic obstructive pulmonary disease (COPD).

METHODS

Subjects

Fifteen healthy volunteers –5 male and 10 female, mean age 56 years (range 46 to 68 years) –participated in the study. None of them were on medication. At least two hours prior to the experiments, they had to abstain from caffeinated drinks and smoking. A short physical examination was done with a supplementary brief lungfunction test. All dynamic lung volumes (FEV_1) were within the normal range. All volunteers gave informed consent. The study was approved by the ethical committee of the Department of Pulmonology Dekkerswald, Groesbeek.

NIRS

We used a new NIRS system (OXYMON, Departments of Physiology and Instrumentation, University of Nijmegen, the Netherlands). This instrument allowed us to use a high sampling rate of 10 Hz. Furthermore, this instrument shows an improvement of 1 OD in sensitivity in contrast to other NIRS instruments^[9].

Changes in optical density (OD) in the near infrared region are measured at three different wavelengths: 905, 845 and 770 nm. A distance of 5.5 cm between transmitting and receiving fibers was used. This ensures deep enough light penetration into the brain^[10]. A pathlength factor of 6.0 was used^[11]. Data were stored on disk for off-line analysis and calculation of CBV.

CBV measurements

Owing to light scattering in the tissue, only changes in concentration in the chromophores from an arbitrary zero can be determined. A modified Lambert-Beer law gives the relationship between the changes in concentration and absorption. The difference between $[O_2Hb]$ and $[HHb]$ is defined as the oxygenation-index $[OI]$.

To quantify CBV, a slight desaturation is necessary. This was performed by lowering the FiO_2 . The change in arterial saturation (SaO_2) was measured with a pulse oximeter (N200 Nellcor Puritan Bennett, St. Louis, USA), on the forehead, in the beat to beat mode. When the change in SaO_2 was related to the change of OI , absolute values of CBV could be calculated. A detailed description of the analysis technique and quantification of CBV in adults using NIRS was described by Elwell *et al.*^[8], under the assumption that blood flow, blood volume and oxygen consumption remain constant during the desaturation procedure.

In formula, CBV is given by:

"Eq."

$$CBV \text{ (ml.100g}^{-1}\text{)} = \frac{K * \Delta[OI]}{[Hb] * \Delta SaO_2}$$

where K is a constant, which accounts for molecular weight of hemoglobin, cerebral tissue density and the cerebral-to-large vessel hematocrit ratio. $[Hb]$ represents the total hemoglobin concentration in blood (mmol.L^{-1}), measured from a blood sample.

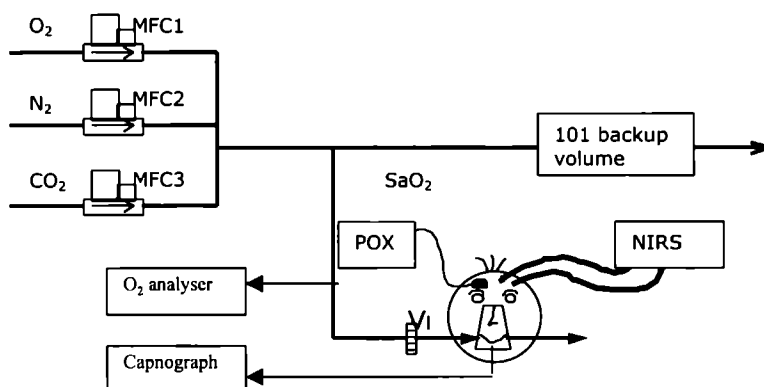
When stable readings of $[O_2Hb]$, $[HHb]$ and SaO_2 at a lowered saturation level of 6-8% were seen, resaturation followed. Subsequently, a FiO_2 of 21% was given to maintain normoxia. Values of $[OI]$ and SaO_2 at the applied desaturation level were obtained in a 20 s time prior to resaturation. Values of $[OI]$ and SaO_2 during normoxia were calculated

from a 20 s average. Steady state conditions are needed to obtain reproducible values of $[OI]$ and SaO_2 at two levels. A steady state condition was predefined as a condition with stable values of $[O_2Hb]$, $[HHb]$ and $[tHb]$, SaO_2 , mean arterial pressure (MAP), endtidal PCO_2 ($PETCO_2$), heart rate (HR) and respiratory rate (RR) for at least 20 s. $[tHb]$ is the sum of $[O_2Hb]$ and $[HHb]$.

General procedure

The subjects were in supine position during the whole experiment. Figure 1 shows the experimental setup. SaO_2 and HR were monitored with a pulse-oximeter, with the sensor attached to the right-frontal forehead. The optodes of the NIRS were positioned on the left side of the forehead with the receiving probe 2 cm from the midline. The optodes were held in position by an elastic band around the head. Both optodes of the NIRS and the sensor of the pulse-oximeter, were not disconnected until the experiment was finished.

Figure 1. Experimental setup MFC1-3. flow of oxygen (O_2), nitrogen (N_2), and carbon dioxide (CO_2), regulated with massflow controller. POX: pulse-oximeter; SaO_2 tensions of arterial saturation, measured in a beat-to-beat mode NIRS: Near Infrared Spectroscopy V_i inspiratory minute ventilation (l/min), measured by pneumotachography.



Arterial blood pressure was measured continuously from the middle finger of the right hand with a finger plethysmographic device (Finapres Ohmeda, USA). Blood pressure values were monitored as mean arterial blood pressure (MAP) from each step change in the protocol.

The subjects breathed through a facemask with separated valves for in- and expiratory gasmixture. Changes in inspiratory gasmixture of oxygen, nitrogen and carbon dioxide

were induced by means of a computer controlled massflow system (Bronckhorst, Hitec, The Netherlands). The FiO_2 was monitored continuously using an oxygen analyzer (OM-11 Beckman, Fullerton, CA., USA). Fast changes in inspiratory gasmixture could be performed; the aimed changes were reached within one breath. The expiratory part of the mask was connected to a combined capnograph/pulse-oximeter (N1000, Nellcor Puritan Bennett, St. Louis, USA) to monitor PETCO_2 (kPa), respiratory rate (min^{-1}) and inspiratory CO_2 (PiCO_2) (kPa). The analogue data were linked directly to the NIRS computer for real time display and storage simultaneously with the NIRS data.

Three different respiratory PaCO_2 conditions were measured. First, normocapnic $\text{PETCO}_{2,\text{norm}}$ baseline conditions were measured with a FiO_2 of 21%. Then, inspired CO_2 was given to induce hypercapnia. We aimed at a hypercapnic value of ($\text{PETCO}_{2,\text{norm}} + 1$) kPa. For that purpose, the FiCO_2 could vary between 3.5% and 6%. For hypocapnia, we predefined a PETCO_2 value of ($\text{PETCO}_{2,\text{norm}} - 1$) kPa. This condition could be performed by making the subject increase the respiratory rate and/or tidal volume. The subject had a visual feedback of his/her PETCO_2 on a meter. All three respiratory conditions were duplicated. When a stable respiratory PETCO_2 condition was reached, a slight desaturation was induced for calculation of CBV by lowering the FiO_2 to 11-13%. Arterialized capillary samples were taken during (each) stable CO_2 challenge, from a warmed fingertip, and analyzed within 2 minutes (Synthesis 25, Instrumentation Laboratory SpA, Italy). The Hb concentration, was determined on the same analyzer.

Statistical Analysis

Each subject was measured twice during three respiratory PaCO_2 conditions. Duplicate values of CBV and PaCO_2 were averaged afterwards.

During the whole experiment, values of PETCO_2 , HR, RR and MAP were recorded continuously; they were expressed as means with standard deviation during each CO_2 challenge. A paired *t*-test was used to compare mean CBV and MAP values in normocapnic condition to mean CBV and MAP values during hypercapnic and hypocapnic conditions. The level of statistical significance was set at $p < 0.01$. Linear regression analysis was performed on the individual slopes of CBV/PaCO_2 .

RESULTS

Table 1 shows the results of all measurements of CBV, PaCO_2 , MAP, HR, PETCO_2 and RR, expressed as means with standard deviation. Compared to normocapnic CBV values, higher and lower values of CBV were seen in hypercapnia and hypocapnia, respectively ($p < 0.001$). The PETCO_2 in hypercapnia showed a value of ($\text{PETCO}_{2,\text{norm}} + 1.2$) kPa, with concomitant

values of ($\text{PaCO}_{2\text{norm}} + 0.5$) kPa. In hypocapnia, a ($\text{PETCO}_{2\text{norm}} - 1.1$) kPa was achieved, with concomitant values of ($\text{PaCO}_{2\text{norm}} - 1.3$) kPa. During each experiment, the same amount of desaturation was established. HR and RR remained stable during the three measured conditions. MAP increased significantly ($p < 0.001$) during both hyper- and hypocapnia. When values of systolic and diastolic arterial blood pressure are evaluated separately, mean systolic blood pressure alters more than mean diastolic blood pressure in all CO_2 conditions; mean sd in changes of systolic blood pressure was 10 mm Hg, whereas the mean sd in changes of diastolic blood pressure was 5 mm Hg.

Figure 2 Outline of NIRS measurements ($[\text{O}_2\text{Hb}]$, $[\text{HHb}]$, $[\text{OI}]$, $[\text{tHb}]$) and measurements of the pulse oximeter (SaO_2) during slight desaturation, necessary for each CBV calculation. Events moments to collect the data (which does not automatically correspond to applied changes in gasmixture)

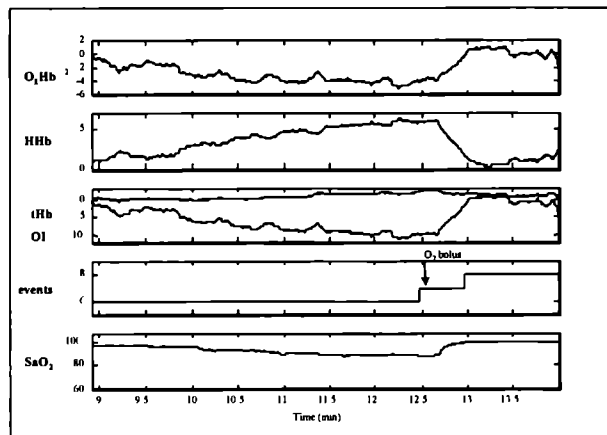
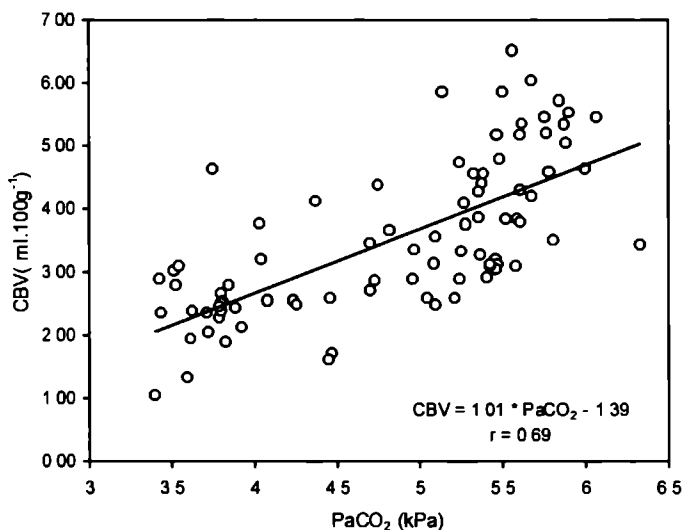


Table 1 Age and mean values (with SD) for cerebral blood volume (CBV), capillary PaCO_2 , change in saturation (dSaO_2), mean arterial pressure (MAP), heart rate (HR), endtidal CO_2 (PetCO_2) and respiratory rate (RR) during normocapnia, hypercapnia and hypocapnia

		age	CBV ml 100g-1 Mean(SD)	PaCO_2 (kPa) Mean(SD)	delta sat (%) Mean(SD)	MAP (mmHg) Mean(SD)	HR (beat/min) Mean(SD)	PetCO_2 (kPa) Mean(SD)	RR (beat/min) Mean(SD)
n=15	normocapnia	56(7)	3 51(0 71)	5 14(0 36)	7 83(2 29)	84(11)	72(10)	4 33(0 42)	14(3)
	hypercapnia		4 82(1 12)	5 63(0 29)	5 89(1 75)	93(12)	71(10)	5 57(0 46)	15(4)
	hypocapnia		2 55(0 72)	3 80(0 24)	6 12(0 85)	95(11)	70(8)	3 21(0 14)	19(4)

Figure 3 shows the raw data of all subjects in three conditions; from each individual, 6 values of PaCO_2 with concomitant CBV values are plotted. Linear regression analysis was performed, resulting in the equation $\text{CBV} = 1.01 * \text{PaCO}_2 - 1.39$ ($r = 0.69$). (CBV: $\text{mL} \cdot 100\text{g}^{-1}$; PaCO_2 : kPa).

Figure 3. CBV/ PaCO_2 reactivity from hypo- to hypercapnia: raw data of all subjects.



We determined the individual $\Delta\text{CBV}/\Delta\text{PaCO}_2$ reactivity for the hypercapnic and for the hypocapnic response, taking the normocapnic situation as a starting point.

Figure 4 shows the results. Mean slopes in the hypercapnic and hypocapnic ranges were 2.60 (1.32 - 3.88) $\text{mL} \cdot 100\text{g}^{-1} \cdot \text{kPa}^{-1}$ and 0.83 (0.64 - 1.02) $\text{mL} \cdot 100\text{g}^{-1} \cdot \text{kPa}^{-1}$ (95% confidence interval), respectively.

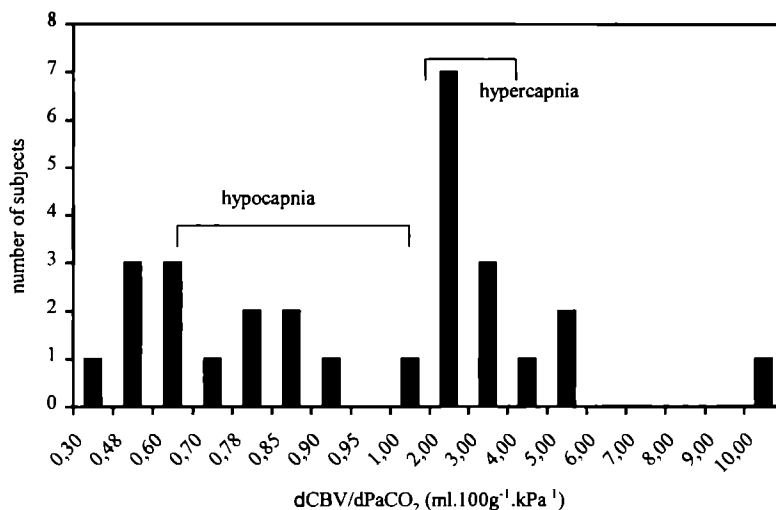
DISCUSSION

We investigated cerebrovascular reactivity to acute induced hypercapnia and hypocapnia (hyperventilation), expressed ΔCBV over ΔPaCO_2 ($\text{mL} \cdot 100\text{g}^{-1} \cdot \text{kPa}^{-1}$) in healthy conscious volunteers with NIRS.

There was a difference between the PetCO_2 and PaCO_2 . The PetCO_2 is always expected to be lower than the PaCO_2 . We aimed at a higher level of hypercapnia, but this is difficult to

induce in conscious humans due to buffering of CO_2 in the blood. However, despite the small increase in PaCO_2 , a considerable increase in CBV was found.

Figure 4. Individual $\Delta\text{CBV}/\Delta\text{PaCO}_2$ reactivity for the hypercapnic and hypocapnic response.



During hypocapnia as well as during hypercapnia, the MAP rose in both cases with about 10 mm Hg. This rise, probably due to sympathetic activity, falls well within the range of cerebral autoregulation^[12].

How does $\Delta\text{CBV}/\Delta\text{PaCO}_2$ from hypo- to hypercapnia compare to other studies? Brun *et al.*^[13] did a study on mechanically “ventilated preterm infants with arterial blood pressure and blood gases within the normal ranges in 24-48% oxygen supply”. Using NIRS and $[\text{O}_2\text{Hb}]$ as a tracer, the CBV/CO_2 reactivity was in the same range ($0.89 \text{ mL} \cdot 100\text{g}^{-1} \cdot \text{kPa}^{-1}$, 95% confidence interval = $0.63\text{-}1.26$) as in the present study. Grubb *et al.*^[1] investigated the CBV/PaCO_2 reactivity in anaesthetized, intubated rhesus monkeys using a free diffusible (^{15}O -labeled water) and vascular tracer (^{15}O -labeled carboxyhemoglobin) with detection by PET. They found a slope of $0.3 \text{ mL} \cdot 100\text{g}^{-1} \cdot \text{kPa}^{-1}$. However, the study was done in animals, they used a different method and they measured during different circumstances (anaesthetized, mechanically ventilated).

Figure 4 shows the slopes of ΔCBV over ΔPaCO_2 reactivity in the hypercapnic and hypocapnic region. Hypocapnia led to a much lower slope than hypercapnia. Hypocapnia results in lower responses of CBF and CBV than hypercapnia^[5]. Interpretation of the CBV response to PaCO_2 must therefore be divided into a reactivity from normo- to hypercapnia

and normo- to hypocapnia. A linear relationship was seen in both responses, but with a significantly different slope.

The use of CBV, instead of CBF, may be discussed. Using $[O_2Hb]$ as a tracer to measure CBF, NIRS cannot provide the standards to obtain reproducible values of CBF^[14,15]. Prior to this study, we determined the reproducibility for CBV measurements with NIRS, which was good (coefficient of variation (CV) $\pm 10\%$,^[16]), whereas the "reproducibility" for NIRS-CBF varied from (CV) 22 to 92%.

Table 2 Comparison of mean (normocapnic) CBV value to other CBV values *, $p < 0.01$

Investigators	Number of subjects	Method	CBV(mL 100g ⁻¹)
Van de Ven <i>et al</i> (1998)	15	NIRS	3.51 ± 0.71
Elwell <i>et al</i> (1994)	10	NIRS	2.85 ± 0.97
Leenders <i>et al</i> (1990)[21]	32	PET	4.3 ± 0.8 *
Muizelaar <i>et al</i> (1997)	10	CT	5.3 ± 0.4 *

The absolute CBV values found for normocapnia (3.51 ± 0.71 mL.100g⁻¹) were compared to other CBV measurements in humans. They are in good agreement with other studies using NIRS^[8]. However, other methods showed significant ($p < 0.01$) higher CBV values (table 2). An explanation for this discrepancy was given by Owen-Reece *et al.*^[17]. They quantified cerebral blood flow on the scalp and then on the dura after craniotomy in humans. Compared to the CBF values measured with fibres on the dura, they found an approximately threefold underestimate of CBF with fibres on the scalp. The role of extracerebral tissue on transmission of light through the cranial cavity seemed to be the main responsible. Due to this underestimation, lowered values of CBV could be expected, when the fibres are placed on the scalp. The slightly higher CBV value found by Muizelaar *et al.*^[18] using Computed Tomography are based on independent measurements of CBF and transit time; he supposed an overestimation for CBF (and therefore for CBV) using Xenon/CT.

MRI did not report normal values of CBV in humans yet. However, there is a report studying CBV values measured by PET and MRI in pigs^[19]. PET CBV values were 2.5 times larger than absolute MRI CBV values. They explained this difference as a result of the (hypothetized) sensitivity of MRI to small vessels. This hypothesis is in agreement with the NIRS theory. Near infrared absorption changes reflect changes in the oxygenation of the microvasculature, i.e., tissue oxygenation at the level of small blood vessels, capillaries, and intracellular sites of oxygen uptake^[20].

We conclude that CBV responses to acute $PaCO_2$ changes in humans can adequately be assessed by NIRS: hypercapnia results in an increase of CBV, whereas hypocapnia decreases CBV. Furthermore, the results of $\Delta CBV/\Delta PaCO_2$ reactivity are in agreement

with other studies in human neonates using NIRS. Separated evaluation of CBV reactivity to hypo- and hypercapnia resulted in a linear relationship with significantly different slopes. The latter approach to evaluate CBV reactivity might be important for further analysis of pathological respiratory CO₂ conditions as in chronic obstructive pulmonary disease.

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Cerebrovascular response to acute metabolic acidosis in humans

MJT van de Ven

WNJM Colier

BTP Kersten

B Oeseburg

H Folgering

Submitted

ABSTRACT

Objectives: Evaluation of the cerebrovascular response ($\Delta\text{CBV}/\Delta\text{Paco}_2$) during baseline metabolic conditions and acute metabolic acidosis. **Methods:** 15 healthy subjects, 5 m, 10 f, 56 ± 10 yrs were investigated. For acidification, NH_4Cl was given orally. CBV was measured using Near Infrared Spectroscopy (OXYMON) during normo-, hyper- and hypocapnia. **Results:** Acute metabolic acidosis was realised: mean ΔBE -2.7 mEq.L^{-1} ($p < 0.001$) with mean ΔPaco_2 -0.2 kPa ($p < 0.01$). During normo-, hyper- and hypocapnia, CBV values of 3.51, 4.82 and $2.55 \text{ mL.100g}^{-1}$ were calculated during baseline metabolic conditions and 3.70, 4.86 and $2.63 \text{ mL.100g}^{-1}$ during acute metabolic acidosis. The CBV/Paco_2 response showed a hockeystick configuration with the point of inflection around normocapnia. $\Delta\text{CBV}/\Delta\text{Paco}_2$ reactivity from normo- to hypercapnia and from normo- to hypocapnia was calculated; no significant differences in $\Delta\text{CBV}/\Delta\text{Paco}_2$ were found in both metabolic conditions. **Conclusion:** Cerebrovascular reactivity to CO_2 does not alter during acute metabolic acidosis.

KEYWORDS

Ammonium-Chloride-pharmacology, carbon dioxide, cerebral blood volume, NIRS.

INTRODUCTION

Metabolic acid-base disorders resulting from ingesting nonvolatile acid are buffered in both the extracellular (ECF) and intracellular fluid (ICF)^[1]. The $\text{CO}_2/\text{HCO}_3^-$ buffersystem is the principal ECF buffer. When a nonvolatile acid is added to the body, buffering of H^+ occurs by binding HCO_3^- resulting in a lowered plasma $[\text{HCO}_3^-]$. Respiratory compensation ensues, the induced metabolic acidosis is corrected by the kidneys by reabsorbing more HCO_3^- from the urine and increased ammonium excretion (enhanced production of new HCO_3^-). In the cerebral compartment, the shifts in acid-base balances depend mainly on the permeability of the blood brain barrier.

Ammoniumchloride (NH_4Cl) has been shown to be effective at inducing acute metabolic acidosis^[2,3]. The resultant decrease in pH causes a systemic (metabolic) acidosis. The blood-brain barrier will prevent $[\text{H}^+]$ ions to diffuse into the brain tissue and therefore the pH of the brain-ECF will not alter initially. Subsequently, the cerebral vessels hardly dilate in response to an increase in the hydrogen ion concentration of the arterial blood. However, they are very sensitive to CO_2 changes. Cerebrovascular reactivity can be measured as the change of cerebral blood volume (CBV) to a change of PaCO_2 ^[4,5]. We wanted to investigate respiratory changes with and without a metabolic acidosis induced by the administration of oral NH_4Cl and compare cerebrovascular reactivity, expressed as $\Delta\text{CBV}/\Delta\text{PaCO}_2$ during both conditions. The influence of an altered concentration of $[\text{H}^+]$ in arterial blood on the cerebrovascular autoregulation can be monitored and is valuable for understanding central chemoreceptor activity^[6-8].

METHODS

Subjects

Fifteen healthy volunteers –5 male and 10 female, mean age 56 years (range 46 to 68 years) –participated in the study. None of them were on medication. At least two hours prior to the experiments, they had to abstain from caffeinated drinks and cigarettes. A short physical examination was done with a supplementary brief dynamic lungfunction test. All volunteers gave informed consent. The study was approved by the ethical committee of the Department of Pulmonology Dekkerswald, University of Nijmegen.

Administration of NH₄Cl

The administered dose of NH₄Cl was based on the assumption that ECF varies between $23 \pm 3\%$ ^[9] and 30% ^[10] of total bodyweight. This value (in litres) multiplied by the target change in BE of 2 mEq provides the total amount of NH₄Cl necessary to reach the desired degree of acidification. Multiplication of this value by the molecular weight of NH₄Cl (53.5), gives the dosage in mg. The dose was given at $t=0$ and a repeated dose after 60 min to approximate the $t_{1/2}$ of 1 - 1½ hours^[11]. Taking these doses, an altered base-excess (Δ BE) of at least 2 mEq.L⁻¹ can be achieved for at least 3 hours^[12].

NIRS

NIRS is an optical technique, based on the principle that light in the NIR region is absorbed by chromophores of oxyhemoglobin [O₂Hb] and deoxyhemoglobin [HHb]^[13]. Changes in light absorption are converted into concentration changes of these chromophores. Owing to light scattering in the tissue, only changes in concentration in the chromophores from an arbitrary zero can be determined. A modified Lambert-Beer law gives the relationship between the changes in concentration and absorption. The difference between [O₂Hb] and [HHb] is defined as the oxygenation-index [OI].

The OXYMON (Departments of Physiology and Instrumentation, University of Nijmegen, the Netherlands) uses a high sampling rate of 10 Hz^[14]. Changes in light absorption are measured at three different wavelengths: 905, 845 and 770 nm. Optodes were strapped to the forehead, approximately 2 cm from the midline. A distance of 5.5 cm between transmitting and receiving fibers was used. This optode distance ensures deep enough penetration of the near infrared light into the brain to exclude most of the extra-cranial circulation from the detected signal^[15]. A pathlength factor of 6.0 was used. Data were stored on disk for off-line analysis and calculation of CBV.

CBV measurements

To quantify CBV, a slight decrease in arterial saturation (Δ SaO₂) of 5% is necessary. This was performed by lowering the FiO₂. Δ SaO₂ was measured with a pulse oximeter (N200 Nellcor Puritan Bennett, St. Louis, USA), in beat-to-beat mode. When stable readings of [O₂Hb], [HHb] and SaO₂ at a lowered saturation level were seen, a FiO₂ of 21% was given to restore normoxia. When the change in arterial SaO₂ was related to the change of [OI], absolute values of CBV can be calculated^[16,17], under the assumption that blood flow, blood volume and oxygen consumption remain constant. Steady state conditions are needed to obtain reproducible values of [OI] and SaO₂ at two levels. A steady state

condition was defined as a condition with stable values of $[O_2Hb]$, $[HHb]$ and $[tHb]$, SaO_2 , mean arterial pressure (MAP), endtidal CO_2 ($PETCO_2$), heart rate (HR) and respiratory rate (RR) for at least 1 minute. Values of $[OI]$ and SaO_2 at the applied desaturation level and during normoxia were obtained from a 20 s average.

Experimental setup

The setup was described previously^[4]. The subjects were in supine position during the whole experiment. SaO_2 and HR were monitored with a pulse-oximeter, with the sensor attached to the right-frontal forehead. The optodes of the NIRS were strapped to the left side of the forehead. The subjects breathed through a facemask with valves for in- and expiratory gasmixture. Changes in inspiratory gasmixture of oxygen, nitrogen and carbon dioxide were induced by means of a computer controlled massflow system (Bronckhorst Hitec, Veenendaal, The Netherlands). The FiO_2 was monitored continuously using an oxygen (OM-11 Beckman, Fullerton, CA., USA). Fast changes in inspiratory gasmixture could be performed; the aimed changes were reached within one breath. The expiratory port of the mask was connected to a capnograph (N200 Nellcor Puritan Bennett, St. Louis, USA) to monitor $PETCO_2$ (kPa), respiratory rate (min^{-1}). The analogue data were linked directly to the NIRS computer for real time display and storage simultaneously with the NIRS data.

Hypercapnia was induced by giving adequate amounts of CO_2 in the inspired air; hypocapnia could be performed by making the subject increase the respiratory rate and/or tidal volume. Arterialized capillary bloodgas samples were taken during (each) respiratory condition, from a warmed fingertip. Samples were analyzed within 2 minutes (Synthesis 25, Instrumentation Laboratory SpA, Italy). The $[Hb]$ was determined on the same analyzer. A metabolic control state is defined as a condition without applied metabolic changes. During control state, the three respiratory conditions were measured. A calculated dose of oral administrated NH_4Cl was given at the end of the control measurements. One hour after ingestion of the first dose of NH_4Cl and immediately after the second dose, respiratory changes were repeated to determine the same parameters during acute metabolic acidosis.

Statistical analysis

Each subject was measured twice during the respiratory conditions. Duplicate values of CBV and $Paco_2$ were averaged afterwards.

During the whole experiment, values of $PETCO_2$, HR, RR and MAP were recorded continuously; they were expressed as means with standard deviation during each CO_2

challenge. All former variables, including CBV and capillary PaCO_2 values measured during hyper- and hypocapnia, were compared to the values determined during normocapnia using a paired t -test. A paired t -test was also used to compare all variables (CBV, PaCO_2 , MAP, HR, RR) measured during acidification to variables measured during baseline (control) conditions. Linear regression analysis was performed on the slopes of CBV/ PaCO_2 of the individual subjects. The control state was compared to the results from acidification. The level of statistical significance was set at $p < 0.01$. All values were reported as mean \pm SD.

RESULTS

This study shows the cerebrovascular response to CO_2 exposed to two different metabolic conditions. Table 1 shows the results of the measurements of CBV, capillary PaCO_2 , HR and RR during metabolic baseline conditions (controls) and after administration of NH_4Cl .

The mean dose of NH_4Cl was 2250 (SD 400) mg. During the entire experiment of metabolic acidosis, capillary arterialized blood samples showed a mean ΔBE of -2.7 (1.3) $\text{mEq}\cdot\text{L}^{-1}$ and ΔpH of -0.04 (0.02) (both $p < 0.001$). Capillary PaCO_2 was significantly decreased (mean ΔPaCO_2 0.20 (0.17) kPa) after NH_4Cl ingestion ($p < 0.001$).

Table 1 Mean values (with SD) during normo-, hyper- and hypocapnia in baseline metabolic conditions (control) and after NH_4Cl administration.

n=15	Age (yrs)	CBV ^a (ml.100g ⁻¹) Mean(SD)	PaCO ₂ [^] (kPa) Mean(SD)	HR ^d (min ⁻¹) Mean(SD)	PetCO ₂ (kPa) Mean(SD)	RR [*] (min ⁻¹) Mean(SD)
control						
normocapnia	56(7)	3.51(0.71)	5.14(0.36)	72(10)	4.33(0.42)	14(3)
hypercapnia		4.82(1.12)	5.63(0.29)	71(10)	5.57(0.46)	15(4)
hypocapnia		2.55(0.72)	3.80(0.24)	70(8)	3.21(0.14)	19(4)
NH_4Cl						
normocapnia		3.65(0.56)	4.94(0.39)	73(10)	4.47(0.34)	15(3)
hypercapnia		4.86(0.70)	5.58(0.32)	74(11)	5.80(0.25)	16(3)
hypocapnia		2.67(0.85)	3.78(0.21)	73(9)	3.24(0.12)	18(5)

^aCerebral Blood Volume

[^] capillary PaCO_2

^{*} Heart rate

^d Respiratory Rate

During metabolic control state, an increase of PaCO_2 of $+0.52$ (0.27) kPa was reached during hypercapnia. Hypocapnia resulted in a decreased PaCO_2 of -1.35 (0.39) kPa. Similar

respiratory changes are effected in the course of metabolic acidosis eventuating in an increase of ΔPaCO_2 of 0.64 (0.25) and a decrease of 1.16 (0.43) kPa towards hyper- and hypocapnia, respectively. CBV values were increased during hypercapnia and decreased during hypocapnia ($p < 0.001$), compared to normocapnic values (table 1).

Figure 1 CBV/ PaCO_2 reactivity data of all subjects ($n=15$) during acute metabolic acidosis and control condition

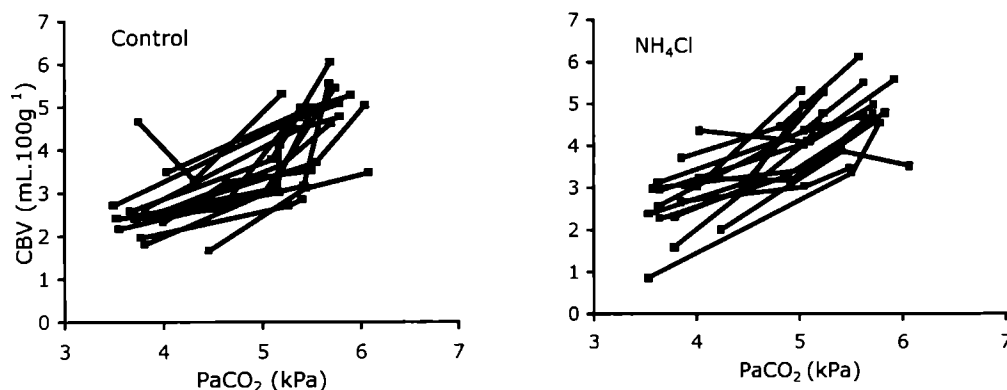
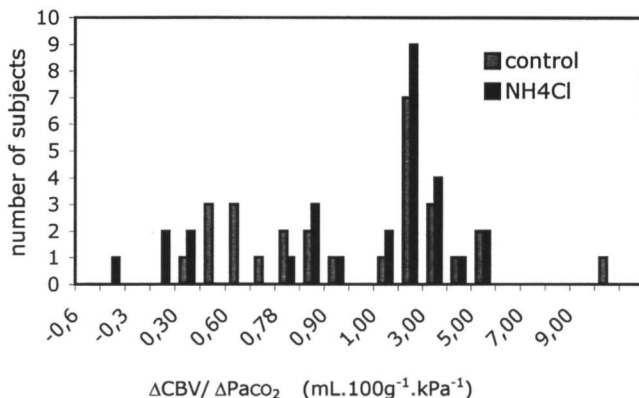


Figure 1 shows the data of all subjects in three different respiratory conditions; mean values of PaCO_2 during hypo-, normo- and hypercapnia with concomitant CBV values are plotted from each individual during both metabolic conditions. Linear regression analysis in the control condition gave the following equation: $\text{CBV} = 1.01 * \text{PaCO}_2 - 1.39$ ($r=0.69$, $p < 0.0001$). During the condition of metabolic acidosis, the CBV shows a not significant different slope and intercept: $\text{CBV} = 1.05 * \text{PaCO}_2 - 1.29$ ($r=0.67$, $P < 0.0001$) (CBV: mL.100g⁻¹.kPa⁻¹, PaCO_2 : kPa). Values of RR are the same in both metabolic conditions. In contrast, HR is significantly increased during acidosis ($P < 0.01$) compared to the control condition.

We determined the individual $\Delta\text{CBV}/\Delta\text{PaCO}_2$ reactivity for the hypercapnic and for the hypocapnic response, taking the normocapnic situation as a starting point (Figure 2). Due to one blood sample error to determine capillary arterialized PaCO_2 , the outcome of only 14 subjects is shown in this histogram. Mean slopes in the hyper- and hypocapnic ranges were calculated, 2.60 (1.32-3.88) mL.100g⁻¹.kPa⁻¹ and 0.83 (0.64-1.02) mL.100g⁻¹.kPa⁻¹ (95% confidence) respectively, during control condition. After NH_4Cl administration, mean hyper- and hypocapnic slopes show not significantly different, values of 2.07(1.39-2.76) and 0.72 (0.413-1.034) mL.100g⁻¹.kPa⁻¹, respectively.

Figure 2. Individual $\Delta\text{CBV}/\Delta\text{Paco}_2$ reactivity for the hypercapnic and hypocapnic response, during control condition and after oral administration of NH_4Cl .



DISCUSSION

We investigated cerebrovascular reactivity to acute changes in Paco_2 during metabolic control condition and acute metabolic acidosis using NIRS.

The use of CBV, instead of CBF, may be discussed. Using $[\text{O}_2\text{Hb}]$ as a tracer to measure CBF, NIRS cannot provide the standards to obtain reproducible values of CBF^[18,19]. CBV however, can be measured reproducible^[17,20]. Furthermore, the use of CBV instead of cerebral blood flow (CBF) eliminates the problems related to the mean transit time^[21]. CBV values of the present study during normocapnia ($3.51 \pm 0.71 \text{ mL.100g}^{-1}$) are in good agreement with other studies: $2.85 \pm 0.97 \text{ mL.100g}^{-1}$ ^[17].

In the present study, a linear relationship was seen to both hyper- and hypocapnia, but with significant different slopes. This confirms the findings of Edvinsson et al.^[22] cerebrovascular responsiveness to hypocapnia is much lower than to hypercapnia. Interpretation of the CBV response to Paco_2 may therefore be divided into a reactivity from normo- to hypercapnia and normo- to hypocapnia.

Absolute values of CBV and cerebrovascular ($\Delta\text{CBV}/\Delta\text{Paco}_2$) responsiveness did not differ significantly during both metabolic conditions. This means that possible ventilatory responses to non-volatile acid, will not be modulated by changes in cerebral blood volume or blood flow. So it seems that the location of the chemoreceptor modulating vasoresponsiveness is at the interstitial side of the blood brain barrier and in this way not sensitive to H^+ changes in the blood. These sensors are however sensitive to changes in

PaCO_2 since CO_2 easily diffuses through the blood brain barrier. In the present study, the small but significantly lowered PaCO_2 did not lead to a lowered CBV. This study does not allow conclusions whether the vascular response at the brain side is affected either by CO_2 itself, or by H^+ from the bicarbonate equilibrium.

Data on cerebrovascular reactivity, expressed as CBV/PaCO_2 , are scarce. Brun et al.^[5] used NIRS to study mechanically ventilated preterm infants and found a CBV/CO_2 reactivity in the same range ($0.89 \text{ mL} \cdot 100\text{g}^{-1}$, 95% CI 0.63-1.26) as in the present study.

Tojima et al.^[3] stated that NH_4Cl primarily stimulates the peripheral chemoreceptor as their results show an augmented hypoxic ventilatory response and an increased respiratory frequency at rest. They used a much higher dose (8 gr NH_4Cl daily in 6 subjects), during 3 days. Our study supports this notion that oral NH_4Cl does not penetrate the blood brain barrier.

The present study shows an increased heart rate during metabolic acidosis. As mentioned earlier, the carotid chemoreceptors are stimulated by NH_4Cl , resulting in a compensating hyperventilation. Both influences tend to depress the primary cardiac response to chemoreceptor stimulation and thereby accelerate the heart^[23].

We conclude that an acute metabolic acidosis, induced by oral administrated NH_4Cl , did not change absolute values of cerebral blood volume, nor cerebrovascular reactivity to CO_2 . It may hypothesised that cerebrovascular reactivity is not predominantly controlled by fixed $[\text{H}^+]$ -ions in the blood.

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Ventilatory response in metabolic acidosis and cerebral blood volume in humans

MJT van de Ven

WNJM Colier

MC van der Sluijs

B Oeseburg

H Folgering

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ABSTRACT

The relationship between alterations in cerebral blood volume (CBV) and central chemosensitivity regulation was studied under neutral metabolic conditions and during metabolic acidosis. Methods: 15 healthy subjects (56 ± 10 yrs) were investigated. To induce metabolic acidosis, ammoniumchloride (NH_4Cl) was given orally. CBV was measured using Near Infrared Spectroscopy during normo- and hypercapnia and related to inspired ventilation (\dot{V}_I). Results: A mean acute metabolic acidosis of $\Delta\text{pH} -0.04$ was realized with a mean decreased arterialized capillary P_{CO_2} (PC_{CO_2}) of 0.20 kPa (1.5 mmHg) (both $p < 0.001$). During normocapnia, CBV was 3.51 ± 0.71 and 3.65 ± 0.56 $\text{ml} \cdot 100\text{g}^{-1}$ (mean \pm SD), measured under neutral metabolic conditions and during acute metabolic acidosis, respectively (ns). Corresponding values of \dot{V}_I were 7.6 ± 1.4 and 10.0 ± 2.4 $\text{L} \cdot \text{min}^{-1}$ ($p < 0.01$), respectively. The slopes of the CO_2 -responsiveness ($\Delta\text{CBV}/\Delta\text{PC}_{\text{CO}_2}$ and $\Delta\dot{V}_I/\Delta\text{PC}_{\text{CO}_2}$), were not significantly different during both metabolic conditions. A significant correlation between $\Delta\text{CBV}/\Delta\text{PC}_{\text{CO}_2}$ and $\Delta\dot{V}_I/\Delta\text{PC}_{\text{CO}_2}$ was found during metabolic acidosis ($p < 0.01$), but not under neutral metabolic conditions. Conclusion: CBV does not contribute in a predictable way to the regulation of central chemoreceptors.

KEYWORDS

Ammoniumchloride, control of breathing, carbon dioxide, cerebral blood volume, metabolic acidosis, near infrared spectroscopy, humans

INTRODUCTION

Cerebral vaso-responsiveness as a controller for central chemosensitivity has been investigated in a number of laboratories^[1-3]. Patients with chronic obstructive pulmonary disease (COPD) are of particular interest, since abnormal cerebral blood flow regulation, especially in chronic hypercapnia, could lead to alterations in the relationship between arterial PCO_2 and extracellular fluid PCO_2 ; the latter being the actual stimulus for the central chemoreceptor. This could contribute to a lowered chemosensitivity to CO_2 in COPD patients with chronic hypercapnia.

It is well known that cerebral blood flow (CBF) and cerebral blood volume (CBV) are increased by hypercapnia^[4,5]. In healthy subjects, an increase in CBF and CBV would increase CO_2 washout and lead to central hypocapnia^[6]. The effect of CBV alterations on central chemosensitivity however, remains unclear. While Berkenbosch, Olievier and DeGoede^[7] predicted a substantial role for CBF on central chemosensitivity in anaesthetized cats, Poulin and Robbins^[7] suggested a "quantitatively insufficient" role in conscious humans. However, in contrast to their conclusions, the latter investigators noted a correlation between central ventilatory sensitivity to hypercapnia and the magnitude of hypoxic ventilatory decline ($r=0.89$; $P<0.05$), suggesting at least some influence of alterations in CBF on ventilation. In addition, Tuteur et al.^[1] found a significant inverse relationship between the rapidity of the ventilatory response to CO_2 and the clinical severity of the cerebrovascular disease.

As a chronic respiratory acidosis is usually compensated via metabolic pathways, in the present study we have investigated the effects of metabolic acidification on the control of cerebrovascular responses. To induce metabolic acidosis, ammoniumchloride (NH_4Cl) was given orally.

Cerebrovascular responses, expressed as a change of CBV (ΔCBV) to hypercapnia were studied for their relationship to ventilatory responses in normal healthy subjects in the age range of patients with COPD, using a non-invasive technique of Near Infrared Spectroscopy (NIRS). The purpose of the study was to quantify the effects of hypercapnia on CBV and ventilation under two different metabolic conditions. Cerebrovascular CO_2 -responsiveness was expressed as the slope of CO_2 -CBV ($\Delta\text{CBV}/\Delta\text{PC}_{\text{CO}_2}$) and ventilatory CO_2 -responsiveness was expressed as the slope of CO_2 -ventilation ($\Delta \dot{V}_I/\Delta\text{PC}_{\text{CO}_2}$). We hypothesized that when metabolic CO_2 -production in the brain remains unchanged, the mass balance for CO_2 flux from the brain predicts a low ventilatory response to P_{CO_2} when the CBV responses is high, and *vice versa*. During metabolic acidosis, a parallel shift of the CO_2 -ventilation curve to higher ventilation or lower CO_2 tension is assumed, according to Lerche et al.^[8]. A changed CBV during metabolic acidosis would support the

concept of a luminal site of cerebral reactivity to account for modifications in CBV. Subsequently, the increment in ventilation during NH_4Cl -acidosis could be explained, at least partly, by CBV adjustment.

MATERIALS AND METHODS

Subjects

Fifteen healthy volunteers (5 males, 10 females, aged 46 to 68 yrs) with a mean (\pm SD) body weight of 71.1 ± 12.1 kg participated in the study. None of them were on medication. At least two hours prior to the experiments, they had to abstain from caffeinated drinks and cigarettes. A physical examination was performed, with a supplementary brief dynamic lung function test (FEV_1 , FVC). All volunteers gave informed consent. The study was approved by the ethical committee of the Department of Pulmonology Dekkerswald, University of Nijmegen.

Administration of NH_4Cl

We induced a metabolic acid change by oral administration of NH_4Cl in order to induce a changed base excess (BE) of at least 2.0 mEq.L^{-1} , which is approximately a ten-fold shift of the intra individual variability ($\text{SD } 0.2 \text{ mEq.L}^{-1}$)^[9]. The administered dose of NH_4Cl was based on the assumption that ECF equals approximately 25%^[10] of total bodyweight in liters. This value multiplied by the target ΔBE of 2 mEq.L^{-1} provides the total amount of NH_4Cl necessary to reach the desired degree of acidification. With a molecular weight of NH_4Cl of 53.5 g.mol^{-1} , the calculated dose of NH_4Cl application (mg.kg^{-1}) was 26.75 mg.kg^{-1} ($0.25 * 2 * 53.5$).

The dose was given at $t=0$ and a repeated dose after 60 min, to approximate the half-life of NH_4Cl of 1 - $1\frac{1}{4}$ hours^[11]. Using this protocol, an acute metabolic acidosis (ΔBE of at least 2 mEq.L^{-1}) can be achieved for a minimum of 3 hours^[12].

NIRS

NIRS has been developed to monitor brain oxygenation and dynamics^[13]. The theory of NIRS has been described extensively^[14]. This technique is based on oxygenation-dependent absorption changes in the cerebral blood caused by chromophores, mainly oxy- and deoxyhemoglobin (O_2Hb and HHb). Changes in optical density in the near-infrared region at different wavelengths are converted into concentration *changes* of these chromophores^[15-17].

Near-infrared light was carried to and from a pulsed continuous-wave NIRS instrument (OXYMON, Department of Physiology and Department of Biomedical Engineering, University of Nijmegen, the Netherlands) through two fiber optic bundles (optodes) which were placed on the left side of the forehead. One optode emits near infrared light at three different wavelengths. The receiving optode is positioned 5.5 cm apart from the emitting optode. This distance ensures penetration of 3-5 cm³ of the brain tissue and excludes most of the extracranial circulation from the detected signal^[18].

CBV measurements

A slight change of saturation (~5%) is necessary to quantify CBV. Assuming a constant CBF, CBV and oxygen consumption during the short maneuver, absolute values of CBV can be calculated^[19,20], according to the following equation:

$$CBV \text{ (ml.100g}^{-1}\text{)} = \frac{K * \Delta[OI]}{(Hb) * \Delta Sa_{O_2}}$$

where K is a constant, which accounts for metric conversions to convert from mmol.L⁻¹ to mL.100g⁻¹, cerebral tissue density (1.06 g.mL⁻¹) and the cerebral-to-large vessel hematocrit ratio (0.69)^[21]. (Hb) represents the hemoglobin concentration of whole blood (mmol.L⁻¹), measured from a blood sample. Sa_{O₂} represents arterial saturation, measured by pulse-oximetry. [OI] is the oxygenation-index, which is the difference between [O₂Hb] and [HHb]. The slight desaturation was obtained by lowering the Fio₂. The sum of [O₂Hb] and [HHb], total hemoglobin [tHb], was continuously visualised and is an indicator of changes of total blood volume present in the investigated tissue. Experiments in which [tHb] was not constant during the desaturation maneuver were rejected. When stable readings of [O₂Hb], [HHb], [tHb] and Sa_{O₂} at a lowered saturation level of ~5% were obtained, resaturation followed by giving a Fio₂ of 21% in the inspiratory air. Values of [OI] and Sa_{O₂} at the applied desaturation level were obtained in a 20 s time span prior to resaturation. A steady state condition was predefined as a condition with stable values of NIRS, Sa_{O₂}, mean arterial blood pressure (MAP), endtidal P_{CO₂} (PETCO₂), cardiac frequency (fH) and respiratory frequency (fR) for at least 60 s.

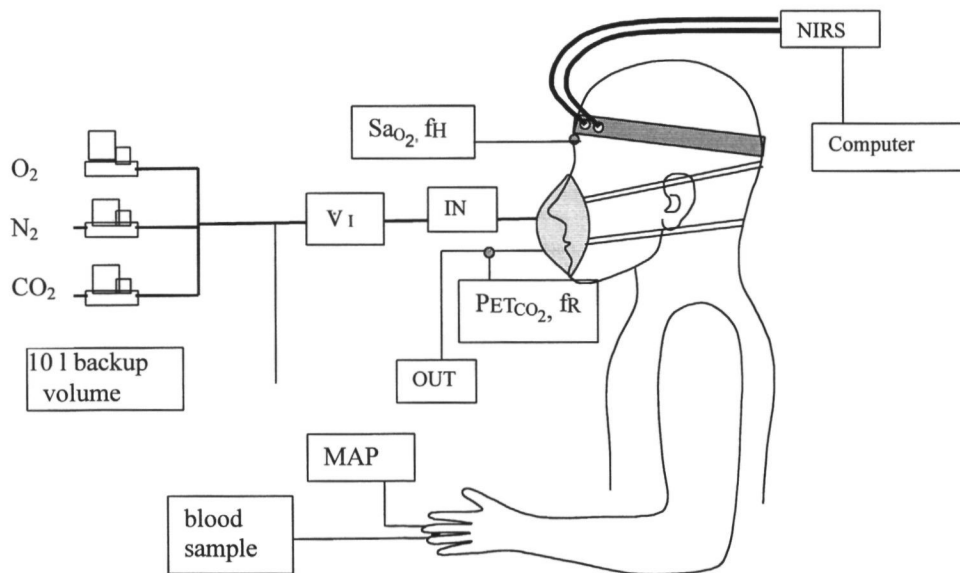
Experimental setup

Figure 1 shows an outline of the experimental setup, which was described previously^[22]. During the whole experiment, the subjects were resting, in the supine position. Sa_{O₂} and fH were monitored with a pulse-oximeter (N200 Nellcor Puritan Bennett, St. Louis, USA),

with the sensor attached to the right-frontal forehead. The probe of the pulse-oximeter and the optodes of the NIRS were held in position by an elastic band around the head. They were not disconnected until the experiment was completed.

MAP was measured continuously from the middle finger of the warmed right hand with a finger plethysmographic device (Finapres, Ohmeda, USA). The subjects were breathing through a facemask with valves for in- and expiratory gas mixture. Changes in inspiratory gas mixture of oxygen (O_2), nitrogen (N_2) and CO_2 were induced by means of a computer controlled massflow Bronckhorst Hitec, Veenendaal, The Netherlands). The FiO_2 was monitored continuously using an oxygen analyzer (OM-11 Beckman, Fullerton, CA., USA). Fast changes in inspiratory gas mixture could be performed and the desired changes were reached within one breath. The inspiratory port of the mask was connected to a Fleisch pneumotachograph with a differential pressure transducer to measure steady state inspired ventilation (\dot{V}_I). The expiratory port of the mask was connected to a capnograph (N1000 Nellcor Puritan Bennett, St. Louis, USA) to monitor PET_{CO_2} (kPa) and fr (min^{-1}). The data were linked directly to the NIRS computer for real time display and simultaneous storage with the NIRS data.

Figure 1. Flow of oxygen (O_2), nitrogen (N_2), and carbon dioxide (CO_2), regulated with massflow controller. Sa_{O_2} and fh , arterial oxygen saturation and cardiac frequency, measured with pulse-oximetry. NIRS: Near Infrared Spectroscopy. \dot{V}_I : inspired ventilation, measured by pneumotachography. MAP: mean arterial pressure, measured by Finapres. PET_{CO_2} and fr : endtidal CO_2 and respiratory frequency measured by capnograph. For safety reasons, a backup volume of 10 l was created.



Experimental protocol

Hypercapnia (mean $\Delta P_{ETCO_2} + 1.2$ kPa (9 mmHg)) was induced by giving adequate amounts of CO_2 (3-5%) in the inspired air. Arterialized capillary blood gas samples were analyzed within 2 minutes (Synthesis 25, Instrumentation Laboratory SpA, Italy). When the measurements during neutral metabolic condition were completed, a calculated dose of oral administrated NH_4Cl was given. One hour after ingestion of the first dose of NH_4Cl and immediately after the second dose, respiratory changes were repeated to determine the same parameters during acute metabolic acidosis. The CBV- and \dot{V}_I -measurements during normo- and hypercapnia were duplicated.

Statistical analysis

Duplicated measurements of CBV and \dot{V}_I were averaged for every CO_2 condition. During the whole experiment, time-averaged values of \dot{V}_I , P_{ETCO_2} , fH, fR and MAP were recorded continuously; they were expressed as mean with standard deviation during each CO_2 challenge. Values obtained during normocapnia were compared to values during hypercapnia using a Wilcoxon matched-paired signed-ranks test. The same statistical test was used to compare all variables measured during acidification with those measured under neutral metabolic conditions. After individual linear regression analysis of both CBV and \dot{V}_I as a function of P_{CCO_2} , individual values of CBV and \dot{V}_I could be calculated around a control P_{CCO_2} of 5.14 kPa (39 mmHg) ($CBV_{5.14}$ and $\dot{V}_{I5.14}$). This control P_{CCO_2} (5.14 kPa (39mmHg)) was obtained by averaging all the subjects' P_{CCO_2} during normocapnia under neutral metabolic conditions. As the statistical method of Kolmogorov and Smirnov, as described in GraphPad InStat® software, showed a gaussian distribution, a paired *t*-test could be used to compare the slopes and intercepts of the linear regression equations in both metabolic conditions. Individual ventilatory and cerebrovascular CO_2 -responsiveness were correlated using linear regression analysis. The level of statistical significance was set at $p < 0.01$. All values were reported as mean \pm SD.

RESULTS

Table 1 shows the results of the measurements of CBV, P_{CCO_2} , \dot{V}_I , MAP, fH, fR and tidal volume (V_T) during neutral metabolic condition and after administration of NH_4Cl .

Table 1. Mean values \pm SD during normocapnia and hypercapnia in 15 healthy subjects, under neutral metabolic conditions and after administration of NH_4Cl .

		CBV (ml.100g ⁻¹)	P _{CCO₂} (kPa) (mmHg)	\dot{V}_I (L.min ⁻¹)	MAP (mmHg)	f _H (min ⁻¹)	f _R (min ⁻¹)	V _T (ml)
control	NC	3.51 \pm 0.71	5.14 \pm 0.36 39 \pm 3	7.61 \pm 1.4	85 \pm 11	72 \pm 10	14 \pm 3	0.62 \pm 0.20
	HC	4.82 \pm 1.12 [†]	5.63 \pm 0.29 [†] 43 \pm 2 [†]	15.9 \pm 8.3 [†]	93 \pm 12 [†]	71 \pm 10	15 \pm 4	1.13 \pm 0.52 [†]
NH ₄ Cl	NC	3.65 \pm 0.56	4.94 \pm 0.39* 38 \pm 3*	10.0 \pm 2.4*	86 \pm 15	74 \pm 10	15 \pm 3	0.68 \pm 0.27*
	HC	4.86 \pm 0.70 [†]	5.58 \pm 0.32 [†] 43 \pm 2 [†]	18.6 \pm 5.9 [†]	94 \pm 12 [†]	74 \pm 11	16 \pm 3	1.18 \pm 0.45 [†]

*p<0.01: neutral metabolic condition vs acute metabolic acidosis

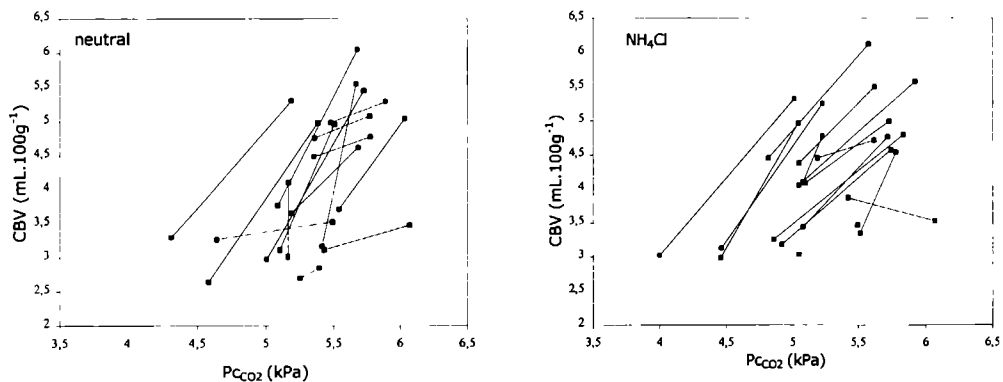
[†]p<0.01: normocapnia vs hypercapnia

Mean values \pm SD for cerebral blood volume (CBV), arterialized capillary P_{CO₂} (P_{CCO₂}), inspired ventilation (\dot{V}_I), mean arterial pressure (MAP), cardiac frequency (f_H), respiratory frequency (f_R) and tidal volume (V_T) during normocapnia (NC) and hypercapnia (HC) in 15 healthy subjects, under neutral metabolic conditions and after administration of NH_4Cl .

Effects of NH_4Cl

The mean dose of NH_4Cl was 2250 ± 400 mg. During the entire experiment of metabolic acidosis, capillary arterialized blood samples showed a mean ΔBE of -2.7 ± 1.3 mEq.L⁻¹ ($p < 0.001$). PC_{CO_2} was significantly decreased (mean $\Delta\text{PC}_{\text{CO}_2}$ 0.20 ± 0.17 kPa (2 ± 1 mmHg) after NH_4Cl ingestion ($p < 0.001$). In addition, pH decreased from 7.45 ± 0.03 to 7.41 ± 0.03 ($p < 0.001$). The small, but significantly lowered PC_{CO_2} resulted from a significant increase of \dot{V}_I ($p < 0.01$). Additional determination of respiratory parameters showed an unchanged fr with a significant increase of V_T ($p < 0.01$). In addition, CBV did not change significantly during metabolic acidosis (Table 1).

Figure 2. Cerebral blood volume (CBV, mL.100g⁻¹) responses to arterialized capillary P_{CO_2} (PC_{CO_2} , kPa) under neutral metabolic conditions (neutral, upper diagram) and during acute metabolic acidosis (NH_4Cl , lower diagram) for each individual. Intra-individual average values of duplicate measurements of CBV- PC_{CO_2} are shown.



Effects of hypercapnia

Hypercapnia was induced by means of a $\Delta\text{PC}_{\text{CO}_2}$ of $+0.52 \pm 0.27$ kPa ($+4 \pm 2$ mmHg) and $+0.64 \pm 0.25$ kPa ($+5 \pm 2$ mmHg) during neutral metabolic state and acidification, respectively. CBV and \dot{V}_I were increased during hypercapnia ($p < 0.001$), compared to normocapnic values (Table 1). The individual responses of CBV to hypercapnia during both metabolic conditions are shown in figure 2. Individual linear regression analysis of CBV and \dot{V}_I as a function of PC_{CO_2} was performed for each subject, based on 4 measurements

(duplicated normocapnia, duplicated hypercapnia, Table 2 and 3). The mean slope and intercept of the lines describing CBV and P_{CO_2} (Table 2) and \dot{V}_I and P_{CO_2} (Table 3) did not differ during both metabolic conditions.

Table 2. Individual linear regression equations relating CBV to P_{CO_2} .

Subject	Neutral metabolic condition			NH ₄ Cl		
	Slope ml.100g ⁻¹ .kPa ⁻¹	CBV ₀ ml.100g ⁻¹	CBV ₅₋₁₄ ml.100g ⁻¹	Slope ml.100g ⁻¹ .kPa ⁻¹	CBV ₀ ml.100g ⁻¹	CBV ₅₋₁₄ ml.100g ⁻¹
1	0.79	-0.86	3.20	3.12	-10.86	5.18
2	1.88	-6.703	2.96	3.27	-14.53	2.28
3	3.33	-12.55	4.56	2.84	-9.59	5.01
4	1.41	-4.18	3.06	0.55	-1.59	1.24
5	3.71	-15.87	3.20	2.29	-6.62	5.16
6	3.69	-14.48	4.49	1.4	-3.09	4.11
7	2.34	-6.77	5.25	2.13	-5.46	5.49
8	3.19	-12.60	3.79	1.77	-4.55	4.55
9	4.11	-18.50	2.63	1.27	-2.19	4.34
10	0.86	-1.82	2.60	-0.46	6.34	3.98
11	§			0.23*	2.014	3.20
12	0.05†	4.70	4.96	1.00	-1.32	3.82
13	1.84	-5.53	3.93	4.49	-18.68	4.40
14	1.97	-6.69	3.44	1.56	-4.36	3.66
15	0.21†	3.60	4.68	1.13	-2.17	3.64
Mean	2.10	-7.02	3.77	1.93	-5.11	4.00
SD	1.35	7.09	0.89	1.29	6.32	1.13

§ failure to reach hypercapnia: $\Delta P_{\text{CO}_2} + 0.2$ kPa (+1.5 mmHg)

* : $r < 0.40$

Linear regression analysis of cerebral blood volume (CBV) as a function of arterialized capillary P_{CO_2} (P_{CO_2}) was performed on each subject, based on 4 measurements (duplicated normocapnia, duplicated hypercapnia). The y-intercept is the CBV value at a $P_{\text{CO}_2} = 0$ kPa (CBV₀).

$\text{CBV}_{5-14} = \text{slope} * P_{\text{CO}_2} + \text{CBV}_0$. Coefficient of determination (r^2) was 0.44 under neutral metabolic conditions and 0.49 during metabolic acidosis.

Correlation between ventilatory and cerebrovascular CO₂-responses

The slopes of the cerebrovascular ($\Delta \text{CBV} / \Delta P_{\text{CO}_2}$) and ventilatory ($\Delta \dot{V}_I / \Delta P_{\text{CO}_2}$) CO₂-responses were related by linear regression analysis, and showed only poor correlations (Figure 3) under neutral metabolic conditions (upper diagram) and a significant correlation (lower diagram) during metabolic acidosis ($p < 0.01$). As r^2 was 0.04 under neutral metabolic conditions, and 0.51 during metabolic acidosis, changes of ventilation

can not be explained consistently by changes of CBV under neutral metabolic conditions (4%), but may contribute to the changes observed during metabolic acidosis (51%).

Table 3. Individual linear regression equations relating \dot{V}_I to P_{CCO_2} .

Subject	Neutral metabolic condition			NH ₄ Cl		
	Slope L.min ⁻¹ .kPa ⁻¹	\dot{V}_{I0} L.min ⁻¹	$\dot{V}_{I5.14}$ L.min ⁻¹	Slope L.min ⁻¹ .kPa ⁻¹	\dot{V}_{I0} L.min ⁻¹	$\dot{V}_{I5.14}$ L.min ⁻¹
1	21.3	-91.13	18.35	23.58	-92.71	28.49
2	11.97	-60.87	0.66	9.03	-40.13	6.28
3	20.65	-86.87	19.27	24.22	-95.37	29.12
4	18.39	-89.72	4.78	10.26	-46.28	6.46
5	5.21	-16.54	10.24	8.66	-33.3	11.21
6	8.78	-36.78	8.35	17.36	-79.6	9.63
7	9.86	-33.7	16.98	11.15	-34.87	22.44
8	16.22	-74.17	9.20	21.61	-97.29	13.79
9	24.43	-124.32	1.25	4.99	-15.88	9.77
10	5.39	-23.08	4.62	6.29	-26.75	5.58
11	§			11.5	-45.53	13.58
12	17.78	-87.85	3.54	4.1	-8.26	12.81
13	18.52	-90.35	4.83	45.37	-218.01	15.18
14	15.74	-72.08	8.82	6.26	-14.98	17.20
15	0.25†	7.128	8.41	5.019	-15.71	10.09
Mean	13.89	-62.88	8.52	13.95	-57.64	14.11*
SD	7.11	36.82	6.01	11.05	54.00	7.17

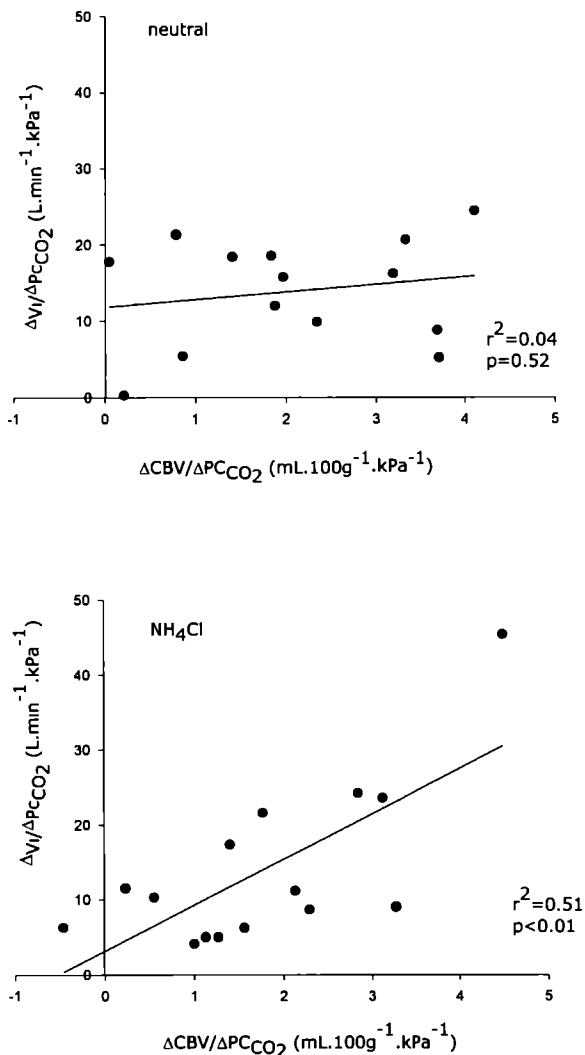
*p<0.01, neutral metabolic condition vs acute metabolic acidosis

† r<0.40

§ failure to reach hypercapnia: ΔP_{CCO_2} +0.2 kPa (+1.5 mmHg)

Linear regression analysis of inspired ventilation (\dot{V}_I) as a function of arterialized capillary P_{CO_2} (P_{CCO_2}) was performed on each subject, based on 4 measurements (duplicated normocapnia, duplicated hypercapnia). Individual \dot{V}_I values at the mean normocapnic P_{CCO_2} value of 5.14 kPa (39 mmHg) were calculated ($\dot{V}_{I5.14}$). The y-intercept is the \dot{V}_I value at a P_{CCO_2} = 0 kPa (\dot{V}_{I0}). $\dot{V}_{I5.14}$ = slope * P_{CCO_2} + \dot{V}_{I0} . Coefficient of determination (r^2) was 0.72 under neutral metabolic conditions and 0.83 during metabolic acidosis.

Figure 3. The relation between the individual ventilatory ($\Delta \dot{V}_I / \Delta P_{\text{CO}_2}$) and cerebrovascular ($\Delta \text{CBV} / \Delta P_{\text{CO}_2}$) CO_2 -responsiveness under neutral metabolic conditions (neutral, left-hand diagram) and during metabolic acidosis (NH_4Cl , lower diagram). The regression equation, describing the interindividual relationship between $\Delta \dot{V}_I / \Delta P_{\text{CO}_2}$ and $\Delta \text{CBV} / \Delta P_{\text{CO}_2}$ is $\Delta \dot{V}_I / \Delta P_{\text{CO}_2} = 0.99 * \Delta \text{CBV} / \Delta P_{\text{CO}_2} + 11.80$ (upper diagram) and $\Delta \dot{V}_I / \Delta P_{\text{CO}_2} = 6.09 * \Delta \text{CBV} / \Delta P_{\text{CO}_2} + 3.16$ (right-hand diagram).



DISCUSSION

This study showed a wide inter-individual variability of cerebrovascular and ventilatory reactivity to acute changes in P_{CO_2} under neutral metabolic conditions and during acute metabolic acidosis. Furthermore, orally administered NH_4Cl increased ventilation, but did not change CBV. However, the present study showed a tendency of high cerebrovascular responses being accompanied by high ventilatory responses to CO_2 during metabolic acidosis, thus refuting the hypothesis of an inverse relationship between slopes of $\Delta\text{CBV}/\Delta P_{\text{CO}_2}$ and $\Delta V/\Delta P_{\text{CO}_2}$.

Critique of methods

Prior to this study, the reproducibility of CBV measurements during resting conditions using NIRS was evaluated; an intra-individual coefficient of variation of $\pm 10\%$ was found^[23]. These results are in agreement with others^[19,20]. CBV values of the present study during normocapnia ($3.51 \pm 0.71 \text{ mL} \cdot 100\text{g}^{-1}$) are consistent with other studies using NIRS: $2.85 \pm 0.97 \text{ mL} \cdot 100\text{g}^{-1}$ ^[20].

We sampled arterialized capillary blood to measure the P_{CO_2} and BE changes. As Sauty et al.^[24] pointed out, the arterial-arterialized P_{CO_2} difference is negligible (0.067 kPa ($0.5 \pm 1.5 \text{ mmHg}$)), because of the arterio-venous P_{CO_2} difference at rest being very small: 0.8 kPa (6.0 mmHg). Therefore, P_{CO_2} can be considered as an equivalent to P_{aCO_2} .

It is important to consider the advantages of measurements of CBV for CBF measurements. Firstly, there is a close relationship ($r=0.9$) between CBV and CBF that has been extensively investigated by Grubb et al.^[5] and by Van Zijl et al.^[25]. Secondly, the use of CBV instead of CBF eliminates the problems related to the mean cerebral transit time^[26]. Finally, near infrared absorption changes reflect changes in the oxygenation of the microvasculature, and thus the CBV of the brain tissue^[27]. Changes of CBV reflect capillary recruitment, which by some are considered a better reflection of cerebrovascular responses than CBF responses to acid-base stimuli^[26]. We measured CBV in the frontal cortex region as present techniques do not allow measures of CBV or CBF in the brainstem of conscious humans. Moreover, Hida et al.^[28] could not find any differences in CO_2 responses between the brain stem artery and the middle cerebral artery, supporting the assumption that our measurements of CBV are a good reflection of overall CBV changes.

Ventilatory effects of NH_4Cl

In the present study, a relatively low dose of NH_4Cl caused a significant increase of \dot{V}_I . However, the slope of the curves describing $\dot{V}_I/\text{PC}_{\text{CO}_2}$ was not significantly altered during acidosis. This is in agreement with the study of Lerche et al.^[8]. They administered an almost ten-fold oral dose of NH_4Cl (a total amount of $0.3 \text{ g} \cdot \text{kg}^{-1} \text{ NH}_4\text{Cl}$, the day before investigation), resulting in a mean ΔBE of $-6.0 \text{ mEq} \cdot \text{L}^{-1}$. In their study, similar slopes were found under neutral metabolic conditions and during acidosis. The acidosis in their study also resulted in a parallel shift of the curve towards higher ventilation and lower CO_2 tension.

Cerebrovascular effects of NH_4Cl

Absolute values of CBV and cerebrovascular CO_2 -responsiveness ($\Delta\text{CBV}/\Delta\text{PC}_{\text{CO}_2}$) did not differ significantly during both metabolic conditions. As a result, the ventilatory response to non-volatile acid, appears not to be modulated by changes in CBV or CBF. Thus it seems that the location of the chemoreceptor modulating vasoresponsiveness is at the interstitial side of the BBB and in this way is not sensitive to H^+ changes in the blood. These sensors are however sensitive to changes in PC_{CO_2} , since CO_2 easily diffuses through the BBB^[29]. This study does not allow conclusions as to whether the vascular response at the brain site is affected either by CO_2 itself, or by H^+ from the bicarbonate equilibrium. The findings of Hida et al.^[28] suggest that CO_2 rather than ventilation per se is the important stimulus to change brain blood flow velocity. Apart from changes of PC_{CO_2} and pH, other parameters which control cerebrovascular responsiveness, were measured. Sympathetic activity, represented by fh and MAP, remained the same during both metabolic conditions. However, a primary respiratory change of CO_2 (hypercapnia) induced an increase of MAP of about 10 mm Hg. This small rise is completely within the range of cerebral autoregulation^[30].

It is possible that the dose of NH_4Cl could be too low to cause CBV alterations. However, Tojima et al.^[31] administered a four times higher dose of NH_4Cl (8 gr daily in 6 subjects), over 3 days. These doses corresponded with significant decreases in PC_{CO_2} of $0.48 \pm 0.33 \text{ kPa}$ ($4 \pm 3 \text{ mmHg}$) ($p < 0.02$). A predominant stimulus of the peripheral chemoreceptor was assumed, as their results showed an augmented hypoxic ventilatory response and an increased respiratory frequency at rest. Their study does not disagree with our notion that oral NH_4Cl does not penetrate the BBB. Furthermore, our study suggests that the induced metabolic acidosis has an additive effect to the CO_2 stimulus at the peripheral chemoreceptor.

Correlation between ventilatory and cerebrovascular CO₂-responsiveness

There was a mild, significant positive correlation between ventilatory and cerebrovascular CO₂-responsiveness ($\Delta\text{CBV}/\Delta\text{PcCO}_2$ and $\Delta\dot{V}_I/\Delta\text{PcCO}_2$) during metabolic acidosis. However, no correlation was found under neutral metabolic conditions. It might be conceivable that there is a relatively high level of noise of various non-chemical ventilatory drives in that condition. The effect of CBV on ventilatory output would therefore tend to be of more importance during metabolic acidosis, as compared to neutral metabolic conditions. However, the power of the study should be increased for this final conclusion. The present study shows no consistent influence of CBV on ventilation in various metabolic situations.

CONCLUSIONS

We conclude that ventilatory responses are not consistently correlated to CBV responses in various metabolic acid-base conditions. An acute metabolic acidosis, induced by orally administered NH₄Cl, has different effects on CBV and ventilation. NH₄Cl did not change absolute values of CBV, nor cerebrovascular reactivity to CO₂. Inspired ventilation however, was augmented by NH₄Cl, but this substance did not affect ventilatory reactivity to CO₂. The control of CBV did not seem to be affected by intraluminal changes in pH thus refuting the hypothesis that a negative correlation exists between ventilatory and cerebrovascular CO₂-responsiveness ($\Delta\text{CBV}/\Delta\text{PcCO}_2$ and $\Delta\dot{V}_I/\Delta\text{PcCO}_2$) during various metabolic conditions. The increased influence of CBV changes during metabolic acidosis might be suggestive for similar tendencies in CO₂ sensitivities of CBV and \dot{V}_I in this condition.

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**Ventilatory and cerebrovascular responses in
normocapnic and hypercapnic COPD patients**

MJT van de Ven

WNJM Colier

MC van der Sluijs

BTP Kersten

B Oeseburg

H Folgering

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ABSTRACT

This study investigated the hypothesis that hypercapnia in some COPD-patients may be related to a high cerebrovascular response to CO_2 .

The relationship between responses of ventilation and of cerebral blood volume, to acute changes in arterial PaCO_2 , was measured in 17 chronic hypercapnic ($\text{PaCO}_2 > 6.0 \text{ kPa}$) and 16 normocapnic ($\text{PaCO}_2 \leq 6.0 \text{ kPa}$) COPD-patients, who were matched for degree of airway obstruction (FEV_1 27%pred). Results were compared with 15 age-matched healthy subjects. CBV was measured using Near Infrared Spectroscopy during normo- and hypercapnia and related to inspired minute ventilation (V'_I) and mouth occlusion pressure ($P_{0.1}$). Hypercapnia ($\Delta \text{PET}_{\text{CO}_2} > 1 \text{ kPa}$) was induced by giving adequate amounts of CO_2 in the inspired air.

During normocapnia, CBV ($\text{mL } 100\text{g}^{-1}$) was 2.41 ± 0.66 and 2.90 ± 0.60 (mean \pm SD) in the normocapnic and chronic hypercapnic patients, respectively, which was significantly lower compared to healthy subjects (3.53 ± 0.77). All slopes of CO_2 -responsiveness ($\Delta \text{CBV}/\Delta \text{PaCO}_2$, $\Delta V'_I/\Delta \text{PaCO}_2$, $\Delta P_{0.1}/\Delta \text{PaCO}_2$) were significantly lower in both COPD groups relative to healthy subjects, but were not significantly different between the COPD groups. A poor, but positive correlation between ventilatory and cerebrovascular CO_2 -responsiveness ($\Delta \text{CBV}/\Delta \text{PaCO}_2$ and $\Delta V'_I/\Delta \text{PaCO}_2$) was found in COPD patients and healthy subjects.

The findings do not support the hypothesis of abnormal cerebrovascular responses to CO_2 in hypercapnic COPD-patients.

KEYWORDS

chemoresponsiveness; chronic obstructive pulmonary disease; control of breathing; mouth occlusion pressure; near infrared spectroscopy; cerebral blood volume

INTRODUCTION

Patients with chronic obstructive pulmonary disease (COPD) frequently show a blunted ventilatory response to hypercapnia. This diminished response has been ascribed to either mechanical limitations imposed by the disease process itself ("can't breathe") or to reduced sensitivity of the respiratory centers to the CO_2 stimulus ("won't breathe")^[1]. Both will result in CO_2 retention and hypercapnia. Furthermore, arterial PCO_2 values are often used as the input parameter for measuring ventilatory responsiveness. As the central chemoreceptors represent approximately 80% of the total CO_2 -chemosensitivity^[2], it might be conceived that the stimulus to these central chemoreceptors, brain interstitial fluid (ISF)-pH, neither is adequately processed, nor adequately reflected by the arterial CO_2 tension (Paco_2) value in the hypercapnic COPD-patients. The latter may occur when the control of cerebral blood flow (CBF) and cerebral blood volume (CBV) and their responses to changes in PCO_2 are abnormal.

The importance of CBF as a crucial link in stimulus-response studies of ventilatory control was first pointed out by the classical study of KETY and SCHMIDT^[3]. Since then, CBF was thought to modify the apparent ventilatory responses to changes in arterial PCO_2 . Variations in blood flow will alter the relationship between Paco_2 (the stimulus that can be measured) and the CO_2 tension of brain tissue at the central chemoreceptors (the true stimulus). Simultaneous measurements of cerebrovascular and of ventilatory reactivity are therefore important^[4].

As reviewed by FEIHL and PERRET^[5], both cerebral resistance vessels (arterioles) and capillaries/venules are dilated by hypercapnia. However, a *chronic* hypercapnia is associated with a blunted cerebrovascular reactivity to acute PCO_2 variations^[6]. As a result, only minor alterations in CBF and CBV can be expected, not able to attenuate the acute hypercapnic stimulus to the central chemoreceptors, and leading to an elevated PCO_2 in the ISF (true stimulus). Consequently, an elevated ventilatory drive could be expected. However, the opposite, a lowered ventilatory drive is found^[7,8].

According to PONTEN and SIESJÖ^[9] and other's^[4], a high CBF (and CBV) washes out tissue- CO_2 and lowers PCO_2 in the ISF, leading to a low chemoreceptor stimulus and a low ventilatory drive. We hypothesized an inverse relationship between cerebrovascular and ventilatory responsiveness to acute hypercapnia in COPD patients. Relatively high vasodilating cerebrovascular responses were hypothesized in hypercapnic patients, leading to a wash-out from CO_2 and a lowered PCO_2 in the ISF. This would result in a low chemoreceptor stimulus and a low ventilatory drive and sustained systemic hypercapnia. Normocapnic patients, however, may be thought to show a lowered cerebrovascular response and thus an adequate ventilatory drive, leading to systemic normocapnia.

In the present study, cerebrovascular CO₂-responsiveness was expressed as the slope of CBV-CO₂ plot ($\Delta\text{CBV}/\Delta\text{Paco}_2$) and ventilatory CO₂-responsiveness was expressed as the slope of V_I-CO₂ ($\Delta\text{V}'_I/\Delta\text{Paco}_2$). CBV was measured using a non-invasive technique of Near Infrared Spectroscopy (NIRS). Mouth occlusion pressure (P_{0.1}) and its response to changes of PCO₂ ($\Delta\text{P}_{0.1}/\Delta\text{Paco}_2$) were measured in order to approximate the ventilatory drive independent of airway resistance.

MATERIALS AND METHODS

Subjects

The study was performed on 33 patients with COPD as defined by the American Thoracic Society. Ten males and six females, aged (mean \pm SD) 60 \pm 11 yrs, were normocapnic (Paco₂ \leq 6.0 kPa); and 15 males and 2 females, aged 63 \pm 8 yrs were hypercapnic (Paco₂ >6.0 kPa). Patients were excluded if they 1) had evidence of obstructive sleep disorders or restrictive pulmonary function, 2) had an exacerbation in the 6 weeks before enrollment, 3) had a history of cardiopulmonary, cerebrovascular or other chronic diseases, 4) took other medications other than pulmonary bronchodilating agents, theophyllines and (systemic) corticosteroids. Three normocapnic and two hypercapnic patients were current smokers, all other patients stopped smoking for more than 6 months. An age-matched healthy control group, (56 \pm 10 yrs, 6 m, 10 f), was also studied. None of them were on medication. A description of the patients is presented in table 1.

At least two hours prior to the experiments, all participants were asked to abstain from caffeinated drinks and cigarettes, but were allowed to continue their pulmonary medication. All volunteers gave informed consent. The study was approved by the ethical committee of the Department of Pulmonology Dekkerswald, University of Nijmegen.

Measurements

Ventilation measurements. The subjects were in comfortable, reclining position. They were breathing through a facemask with low-resistance valves for in- and expiratory gasmixture. First, dead space ventilation (V_D/V_T) was measured using the Bohr equation. Expiratory air was collected in a Douglas bag for 10 minutes for measurements of expiratory P_{CO₂} (capnograph N1000, Nellcor Puritan Bennett, USA). Next, the inspiratory port of the mask was connected via a Fleisch pneumotachograph (No.3) to an inspiratory reservoir (figure 1). The flow signal was integrated into inspired minute ventilation (V_I). Air was sampled from the expiratory port of the mask to a capnograph to monitor endtidal CO₂ (PET_{CO₂}) (kPa) and respiratory rate (RR, min⁻¹). Changes in inspiratory

gasmixture of O_2 , N_2 , CO_2 were induced by means of a computer controlled massflow system (Bronckhorst, Hitec, The Netherlands). The fraction of inspired O_2 (FI_{O_2}) was monitored continuously using an O_2 analyzer (OM-11, Beckman Inc., USA). Fast changes in inspiratory gasmixture could be induced; the aimed changes were reached within one breath. Hypercapnia ($\Delta PET_{CO_2} > 1$ kPa) was induced by giving adequate amounts of CO_2 (FI_{CO_2} 3-5%) in the inspired air.

CBV measurements. Near Infrared Spectroscopy (NIRS) has been developed to monitor brain oxygenation and dynamics^[10]. The theory of NIRS has been described extensively^[11]. The technique is based on oxygenation-dependent absorption changes in the blood caused by chromophores, mainly oxy- and deoxyhaemoglobin ($[O_2Hb]$ and $[HHb]$, respectively). Near-infrared light was carried to and from a pulsed continuous-wave NIRS instrument (OXYMON, Departments of Physiology and Instrumentation, University of Nijmegen, the Netherlands) through two fiber optic bundles (optodes) on the left side of the forehead. One optode emits near infrared light at three different wavelengths, which penetrates through the skull/brain. The receiving optode is positioned at a distance of 5.5 cm apart from the emitting optode. This distance ensures that most of the extracranial circulation is excluded from the detected signal^[12].

Calculation of CBV was described by ELWELL^[13]. A slight change of saturation ($\sim 5\%$) is necessary to quantify CBV. The change of saturation is related to the difference of concentration of hemoglobin chromophores at two levels of saturation. CBV can be calculated taken the individual hemoglobin concentration into account and a fixed constant. The constant accounts for molecular weight of hemoglobin, cerebral tissue density and the cerebral-to-large vessel hematocrit ratio.

Mouth occlusion pressure measurements. Ventilatory effort during inspiration was determined by occlusion pressure at 0.1 second after the start of inspiration. A solenoid valve was positioned in the inspiratory line of the circuit^[14]. Closure of the valve during expiration was manually controlled, and the valve automatically opened after the first 100 ms of the occluded inspiration. Five repeated measurements of $P_{0.1}$ were averaged during each CO_2 condition. $P_{0.1}$ was expressed both as absolute value (cmH₂O) and as percentage of maximal inspiratory pressure (MIP), in order to normalize $P_{0.1}$ for the individual differences in inspiratory muscle strength^[15].

Protocol

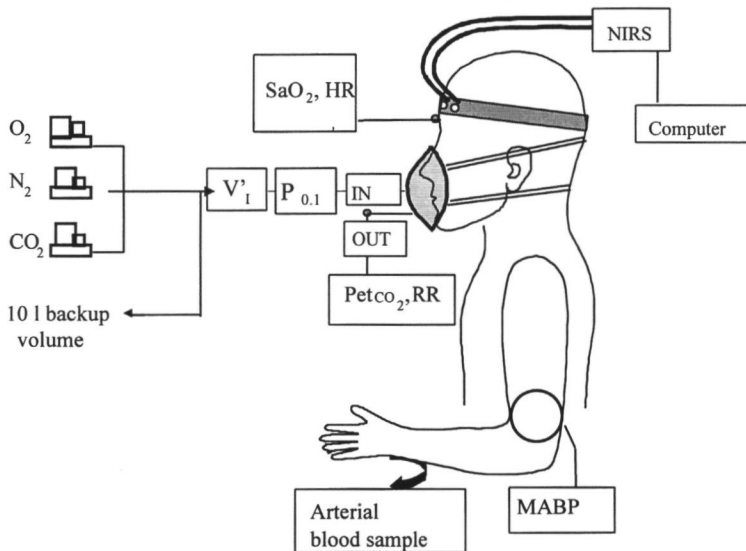
All patients underwent routine spirometry and blood analysis of hemoglobin, hematocrit and resting arterial blood gases to assign the individual patients into the normocapnic and chronic hypercapnic COPD group. A canula was introduced in the left brachial artery to collect arterial blood samples. SaO_2 and heart rate (HR) were monitored with a pulse-

oximeter (N200, Nellcor Puritan Bennet, USA), with the sensor attached to the right-frontal forehead (figure 1).

Hypercapnia was induced by giving adequate amounts of CO_2 in the inspired air. Duplicate measurements of CBV and $P_{0.1}$ during normo- and hypercapnia were performed after a period of 10 min equilibration. Arterial pressure was measured manually during each CO_2 condition. Mean arterial blood pressure (MABP) was calculated as: diastolic pressure + $1/3 \times (\text{systolic-diastolic})$ pressure. All data (except MABP) were linked directly to the NIRS computer for real time display and simultaneous storage with the NIRS data.

Figure 1. *Experimental setup*

Flow of oxygen (O_2), nitrogen (N_2), and carbon dioxide (CO_2) are regulated with a massflow controller. SaO_2 and HR, arterial oxygen saturation and heart rate are measured with pulse-oximetry. NIRS: Near Infrared Spectroscopy. V'_I : inspired minute ventilation, measured by pneumotachography. $P_{0.1}$: mouth occlusion pressure. MABP: mean arterial blood pressure. PET_{CO_2} and RR: endtidal CO_2 and respiratory rate measured by capnograph. For safety reasons, a backup volume of 10 l was created. Arterial blood was sampled from left brachial artery.



Statistics

During the whole experiment, time-averaged values of V'_I , PET_{CO_2} , SaO_2 , HR and RR were recorded, expressed as mean \pm SD during each CO_2 challenge. Anthropometric characteristics, pulmonary function, MABP and arterial blood gas values were compared

between the three groups by the Mann-Whitney test for two independent samples. Within the groups, values during normocapnia were compared to values during hypercapnia using a Student's paired t-test. Between the groups, unpaired t-tests were used to compare outcome variables. For each individual, CBV, V'_I and $P_{0.1}$ was plotted against corresponding $Paco_2$ values and subjected to linear regression analysis. The individual slopes of CBV, V'_I and $P_{0.1}$ responses to acute CO_2 -changes were calculated using linear regression analysis. Mean slopes of the three groups were compared with a Student's unpaired test. The level of statistical significance was set at $p < 0.05$. All tests should be regarded as explorative due to the multiplicity of tests.

Table 1. Characteristics of normocapnic patients, chronic hypercapnic patients and control subjects, mean \pm SD

Variable	Normocapnic COPD	Hypercapnic COPD	Controls [†]
Age, yr	59.8 \pm 10.7	62.8 \pm 8.4*	56 \pm 1
BMI, kg m ⁻²	22.9 \pm 2.54	21.5 \pm 2.4**	24.2 \pm 3.1
FEV ₁ , %pred	28.8 \pm 9.6***	24.3 \pm 7.5***	88.7 \pm 8.8
MIP, %pred	87.9 \pm 39.9	77.2 \pm 29.8	
MEP, %pred	69.6 \pm 28.7	59.4 \pm 25.7	
V _D /V _T , %	48 \pm 11	53 \pm 7	
Ht, vol%	41.6 \pm 3.6	42.0 \pm 3.4	40.8 \pm 3.1
Paco ₂ , kPa	5.26 \pm 0.27	6.27 \pm 0.45*** ^{§§}	5.14 \pm 0.36
Pao ₂ , kPa	9.05 \pm 0.59	8.31 \pm 0.99 ^{§§}	
pH	7.42 \pm 0.02***	7.39 \pm 0.02*** ^{§§}	7.45 \pm 0.03
HCO ₃ ⁻ , mEq·L ⁻¹	25.2 \pm 1.2	28.0 \pm 1.6*** ^{§§}	25.6 \pm 1.8
BE, mEq L ⁻¹	0.8 \pm 1.2***	2.4 \pm 1.3 ^{§§}	2.1 \pm 1.7

Definition of abbreviations: BMI = body mass index; MVV = maximal voluntary ventilation; MIP = maximal inspiratory pressure; MEP = maximal expiratory pressure; Ht = hematocrit; BE = base excess.

^{§§}: $p < 0.01$; ^{§§§}: $p < 0.001$ normocapnic COPD-group compared to hypercapnic COPD-group

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ COPD-group compared to control group

[†]control group: arterialized capillary blood gas sampling

RESULTS

The anthropometric characteristics and respiratory function data of the patients are summarized in table 1. Both COPD groups showed the same degree of airway obstruction (FEV₁), maximal voluntary ventilation (MVV) and MIP. Acute hypercapnia was

attained by means of a ΔPaco_2 of $+0.83\pm0.19$, $+0.65\pm0.18$ and $+0.52\pm0.27$ kPa in the normocapnic, chronic hypercapnic and healthy (control) group, respectively. The degree of (necessary) transient desaturation to measure absolute values of CBV was 6 ± 2 , 7 ± 2 and 8 ± 2 % for the same groups.

Hypercapnia induced significant changes ($p<0.01$) in all variables except for HR, RR and MABP, within the three groups (table 2). In addition, only normocapnic COPD patients showed a different RR during hypercapnia ($p<0.01$), compared to the other two groups. MABP was significantly higher in COPD patients, compared to healthy subjects ($p<0.001$), but did not further increase during hypercapnia. In healthy subjects, MABP increased during induction of hypercapnia ($p<0.001$).

Ventilation and ventilatory responses

Both COPD groups had significantly higher resting values of V'_I compared to healthy subjects (table 2). The ventilatory response to CO_2 ($\Delta V'_I/\Delta\text{Paco}_2$), was lower in both COPD groups from controls, but only in the hypercapnic group significantly different ($p<0.01$) compared to controls (table 3). When both COPD groups were compared and the median rather than the average value of $\Delta V'_I/\Delta\text{Paco}_2$ was taken, a significant different slope of $\Delta V'_I/\Delta\text{Paco}_2$ between the two COPD groups was found ($p<0.05$). Average responses (V'_I : L min^{-1} ; Paco_2 : kPa) are displayed in figure 2 (lower diagram).

In contrast to COPD patients, V'_I is a good parameter for neuromuscular output of ventilatory drive in healthy subjects. Both absolute values of $P_{0.1}$ (table 2) and its reactivity ($\Delta P_{0.1}/\Delta\text{Paco}_2$, table 3) were not significantly different between the COPD groups, even after correction for MIP.

CBV and cerebrovascular responses

Twelve out of 192 measurements were rejected and excluded from the CBV calculations, because of inadequate measurements. Values of CBV were lower in the normocapnic patients as compared to the hypercapnic patients ($p<0.01$) (table 2). Both COPD groups showed a significant lower value of CBV compared to the healthy subjects. The cerebrovascular response to CO_2 , $\Delta\text{CBV}/\Delta\text{Paco}_2$, was lower in both COPD groups compared to controls, however, only in the hypercapnic group significantly lower ($p<0.05$). (table 3). Average equations of CBV as a function of Paco_2 are displayed in figure 2 (upper diagram).

Table 2. Outcome parameters before (start) and during hypercapnia when taking CO₂ responses in normocapnic patients, chronic hypercapnic patients and control subjects, mean ± SD

	Normocapnic COPD		Hypercapnic COPD		Controls	
Variable	start	hypercapnia	start	hypercapnia	start	hypercapnia
CBV mL 100g ⁻¹	2.41 ± 0.66 ^{***}	3.36 ± 0.75 ^{***}	2.90 ± 0.60 ^{§§}	3.76 ± 0.71 ^{***}	3.53 ± 0.77	4.82 ± 1.12
V _I L min ⁻¹	9.7 ± 2.5 ^{**}	16.6 ± 4.2 ^{***}	9.0 ± 2.0 [*]	12.5 ± 2.2 ^{***}	7.7 ± 1.4	15.9 ± 8.3 ^{***}
P ₀₁ cmH ₂ O	5.12 ± 2.57	7.14 ± 3.44 ^{***}	5.31 ± 2.89	6.76 ± 3.78 ^{***}		
P ₀₁ %MIP	7.72 ± 4.53	11.42 ± 7.19 [†]	8.14 ± 4.38	10.44 ± 5.17 ^{††}		
Paco ₂ kPa	5.26 ± 0.27	6.08 ± 0.24 ^{***}	6.27 ± 0.45 ^{**}	6.92 ± 0.39 ^{***}	5.14 ± 0.36	5.63 ± 0.29 ^{***}
Pao ₂ kPa	9.05 ± 0.59	10.16 ± 1.36 ^{††}	8.31 ± 0.99	8.94 ± 0.81 ^{††}		
MVV %pred	33.1 ± 12.7	55.0 ± 16.7	33.3 ± 10.2	45.6 ± 11.9		
RR min ⁻¹	16 ± 4	19 ± 4 ^{††}	19 ± 5 [*]	19 ± 4	14 ± 3	15 ± 4
V _T mL	640 ± 210	920 ± 225 ^{***}	520 ± 200	710 ± 200 ^{***}	610 ± 200	1130 ± 20 ^{***}
HR min ⁻¹	83 ± 11 ^{**}	79 ± 19	82 ± 12 ^{**}	83 ± 12	72 ± 10	71 ± 10
MABP mmHg	106 ± 12 ^{***}	108 ± 18	111 ± 14 ^{***}	110 ± 13	85 ± 11	93 ± 12 ^{***}

Definition of abbreviations: CBV = cerebral blood volume; V_I = inspired ventilation; P₀₁ = mouth occlusion pressure; MIP = maximal inspiratory pressure; MVV = maximal voluntary ventilation, RR = respiratory rate; V_T = tidal volume; HR = heart rate; MABP = mean arterial blood pressure.

† : p<0.05; †† : p<0.01; ††† : p<0.001 normocapnia compared to acutely induced hypercapnia within the group

§§ : p<0.01; §§§ : p<0.001 normocapnic COPD-group compared to hypercapnic COPD-group

* : p<0.05; ** : p<0.01; *** : p<0.001 COPD-group compared to control group

Table 3. Linear regression of CBV, V'_I and $P_{0.1}$ to hypercapnia in normocapnic patients, chronic hypercapnic patients and control subjects, mean \pm SD

	Normocapnic COPD			Hypercapnic COPD			Controls		
	Slope	y-intercept	r	Slope	y-intercept	r	Slope	y-intercept	r
CBV-PaCO ₂ mL·100g ⁻¹ kPa ⁻¹	1.59 \pm 0.91	-5.18 \pm 5.27	0.9	1.23 \pm 0.67*	-5.16 \pm 3.56	0.77	2.10 \pm 1.35	-7.02 \pm 7.1	0.66
V' _I -PaCO ₂ L·min ⁻¹ kPa ⁻¹	8.2 \pm 4.9*	-33.4 \pm 26.2*	0.96	5.5 \pm 4.4***	-26.7 \pm 34.3**	0.93	13.9 \pm 7.1	-62.9 \pm 36.8	0.85
P _{0.1} -PaCO ₂ cm H ₂ O·kPa ⁻¹	2.65 \pm 1.84	-8.67 \pm 9.3	0.81	2.83 \pm 1.50	-12.38 \pm 9.18	0.80			

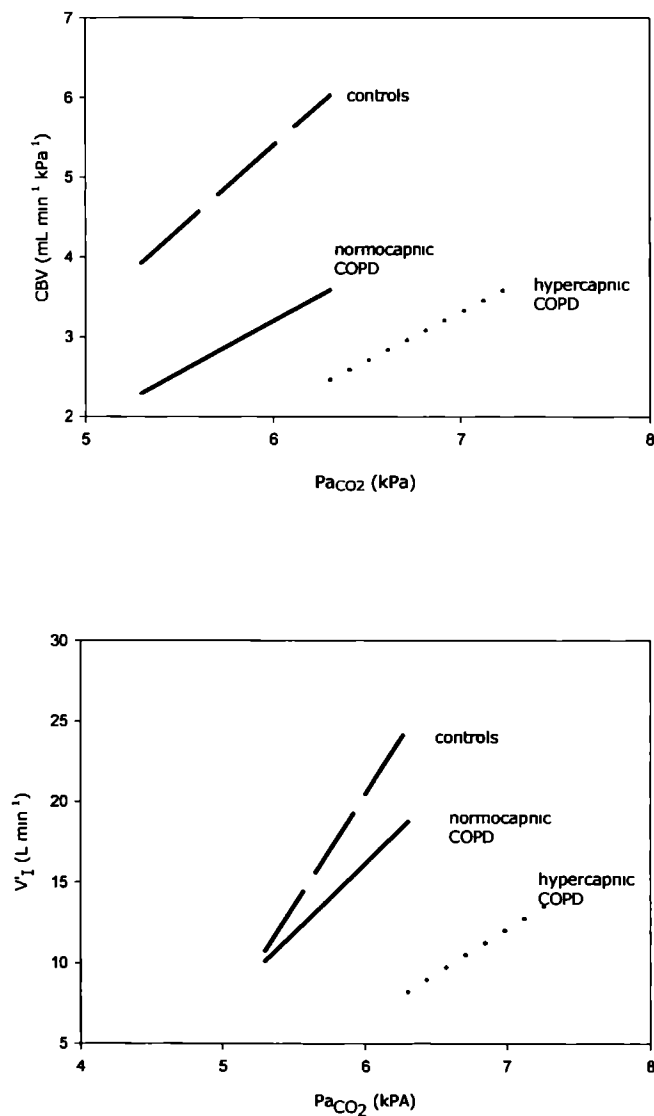
For *abbreviations*, see legend to table 1 and 2.

*: p<0.05; **: p<0.01; ***: p<0.001 COPD-group compared to control group

y-intercept: CBV, V'_I and P_{0.1} value at a PaCO₂ of 0 kPa

Figure 2. Ventilatory and cerebrovascular responses to CO_2

Regression equations were obtained for data from each individual subject and averaged afterwards for the group. All values of cerebral blood volume (CBV, upper diagram) and inspired minute ventilation (V_I , lower diagram) were indexed to similar PaCO_2 values to show average group results.



Correlation between the different outcome parameters

A weak poor correlation was seen in the control group between the individual CBV and V'_I responses to acute hypercapnia ($\Delta\text{CBV}/\Delta\text{Paco}_2$ and $\Delta V'_I/\Delta\text{Paco}_2$). A low, but significant correlation was found for all COPD patients (figure 3). When the COPD group was subdivided, the normocapnic patients tended to show a steeper slope ($\Delta V'_I/\Delta\text{Paco}_2/\Delta\text{CBV}/\Delta\text{Paco}_2$) as compared to the hypercapnic patients (figure 4). However, the latter correlations were poor and not significant. In addition, when V'_I was related to %MVV and $V'_I/(\%\text{MVV})/\Delta\text{Paco}_2$ was correlated to $\Delta\text{CBV}/\Delta\text{Paco}_2$ (not displayed), correlations remained poor. Nevertheless, the slope of these correlations was positive, showing that high cerebrovascular responses were accompanied by high ventilatory responses to CO_2 .

Correlations between the individual CBV and $P_{0.1}$ slopes ($\Delta\text{CBV}/\Delta\text{Paco}_2$ and $\Delta P_{0.1}/\Delta\text{Paco}_2$) were poor and not significant in both COPD groups ($r=0.28$ and 0.04 in the normocapnic and hypercapnic COPD-group, respectively). In order to evaluate CBV with respect to parasympathetic tone, we correlated CBV and HR. There was no correlation between absolute values of CBV and HR in normocapnic ($r=0.1$) and hypercapnic COPD-patients ($r=0.2$) and controls ($r<0.1$).

DISCUSSION

Cerebrovascular responses were studied and correlated with ventilatory reactivity in healthy subjects and both normo- and hypercapnic COPD patients. Acute hypercapnia gave rise to significant changes of cerebrovascular (CBV) and ventilatory (V'_I and $P_{0.1}$) outcome parameters in all investigated subjects. Healthy subjects showed the highest CBV- and V'_I -responsiveness, whereas hypercapnic COPD patients showed the poorest responsiveness among the three groups. A wide inter-individual variability of cerebrovascular and ventilatory reactivity to acute changes in Paco_2 was found between the investigated subjects. However, the present study showed a tendency of high cerebrovascular responses being accompanied by high ventilatory responses to CO_2 , thus refuting the hypothesis of an inverse relationship between $\Delta\text{CBV}/\Delta\text{Paco}_2$ and $\Delta V'_I/\Delta\text{Paco}_2$ in COPD-patients.

Ventilation and ventilatory responses

Ventilatory responsiveness to CO_2 was highest in healthy subjects and lowest in hypercapnic COPD patients. The results are in line with those of others^{-(8) [1,16]}, although the present study measured higher absolute values of responsiveness. The latter can be

Figure 3. Correlation between ventilatory and cerebrovascular CO_2 -responsiveness in healthy subjects (control) and COPD patients

The relationship between the individual ventilatory ($\Delta V'_i/\Delta \text{PaCO}_2$) and cerebrovascular ($\Delta \text{CBV}/\Delta \text{PaCO}_2$) CO_2 -responsiveness in healthy subjects (control, upper diagram) and in normo- and hypercapnic COPD patients (COPD, lower diagram). The regression equation, describing the interindividual relationship between $\Delta V'_i/\Delta \text{PaCO}_2$ and $\Delta \text{CBV}/\Delta \text{PaCO}_2$ is $\Delta V'_i/\Delta \text{PaCO}_2 = 0.99 * \Delta \text{CBV}/\Delta \text{PaCO}_2 + 11.80$ (upper diagram) and $\Delta V'_i/\Delta \text{PaCO}_2 = 2.36 * \Delta \text{CBV}/\Delta \text{PaCO}_2 + 3.99$ (lower diagram). V'_i = inspired minute ventilation ($\text{L}\cdot\text{min}^{-1}$); PaCO_2 : arterial Pco_2 (kPa); CBV : cerebral blood volume ($\text{mL}\cdot 100\text{g}^{-1}$)

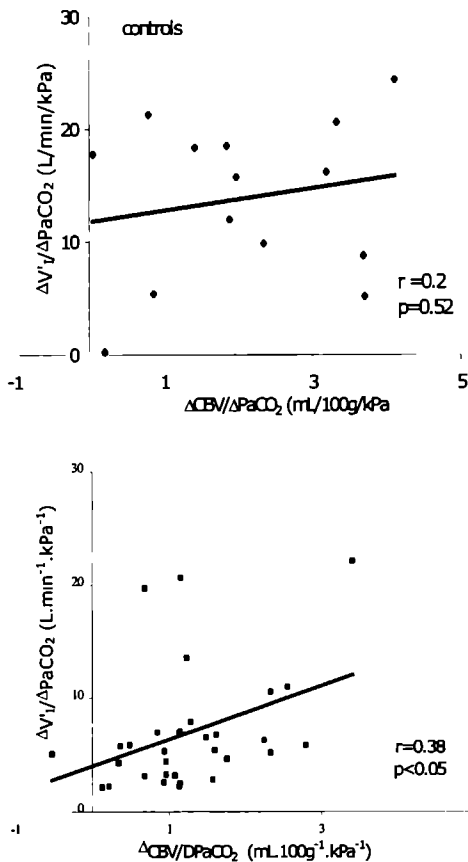
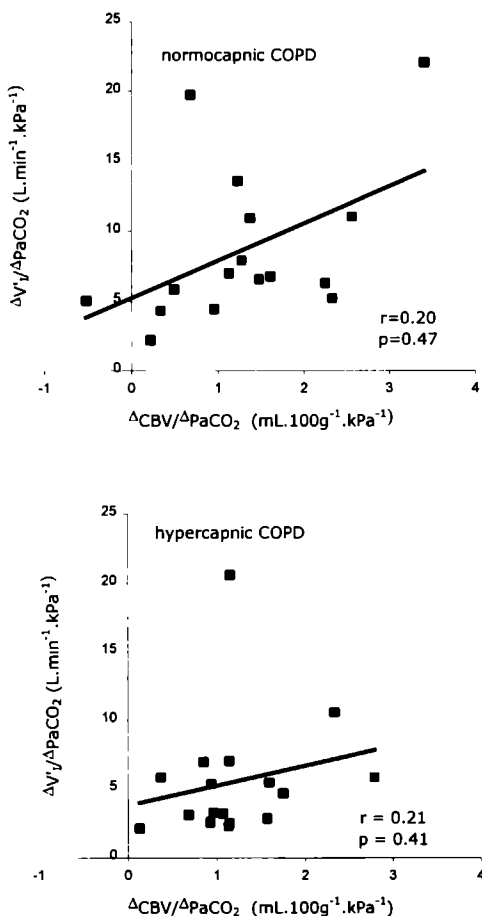


Figure 4. Correlation between ventilatory and cerebrovascular CO_2 -responsiveness in normocapnic and chronic hypercapnic COPD patients

The relation between the individual ventilatory ($\Delta V'_i/\Delta \text{PaCO}_2$) and cerebrovascular ($\Delta \text{CBV}/\Delta \text{PaCO}_2$) CO_2 -responsiveness in normocapnic (upper diagram) and chronic hypercapnic patients (lower diagram). The regression equation, describing the interindividual relationship between $\Delta V'_i/\Delta \text{PaCO}_2$ and $\Delta \text{CBV}/\Delta \text{PaCO}_2$ is $\Delta V'_i/\Delta \text{PaCO}_2 = 2.66 * \Delta \text{CBV}/\Delta \text{PaCO}_2 + 5.22$ (upper diagram) and $\Delta V'_i/\Delta \text{PaCO}_2 = 1.43 * \Delta \text{CBV}/\Delta \text{PaCO}_2 + 3.79$ (lower diagram). V'_i = inspired ventilation ($\text{L} \cdot \text{min}^{-1}$); PaCO_2 : arterial PCO_2 (kPa); CBV: cerebral blood volume ($\text{mL} \cdot 100\text{g}^{-1}$)



explained by various causes. Firstly, the present study used PaCO_2 instead of PETCO_2 as independent variable. Secondly, the (significantly) increased value of PaO_2 during hypercapnic challenge may have result in an overestimated value of ventilatory CO_2 -

responses as previous reports found a reduced CO₂ sensitivity during hyperoxia in healthy subjects^[17]. Thirdly, chronic hypercapnic patients were exposed to chronic hypoxemia (mean Pao₂ value 8.31 kPa). Superimposed desaturation changes to obtain absolute CBV values may have led to greater values of ventilation under both baseline conditions and during hypercapnic challenge, possibly leading to higher values of ventilatory slopes. Finally, all COPD patients have inhaled beta-2 adrenergic agonists, resulting in additional increases of ventilatory responses to hypercapnia, presumably by central chemoreceptor stimulation^[18].

The sex ratio in healthy controls and patients' groups is different. As all investigated subjects were postmenopausal, this study is not predominantly biased by gender.

To ascertain whether and to what extent the reduced ventilatory response to a hypercapnic stimulus in chronic obstructive pulmonary disease (COPD) patients depends on a blunted chemoresponsiveness of central origin or to mechanical impairment, SCANO *et al.*^[8] measured rebreathing CO₂-responses in normocapnic and hypercapnic COPD patients with similar degrees of airway obstruction and hyperinflation. Their study population was comparable to the present investigated group on pulmonary mechanics and arterial blood gas parameters. In contrast to GELB *et al.*^[16] and the present study, he found a lower P_{0.1} responsiveness (cmH₂O·kPa⁻¹) in hypercapnics (1.08±0.43) relative to normocapnics (2.72±2.08) and healthy controls (2.57 ± 0.49). On the other hand, when the normalization of P_{0.1} for individual differences in muscle strength was performed by expressing P_{0.1} as percentage of MIP^[15], a significant difference between the two groups did not occur, which is in line with our results.

CBV and cerebrovascular responses

Cerebrovascular responsiveness was expressed as a change of CBV over change in Paco₂. It is important to consider the advantages of measurements of CBV over CBF measurements. Firstly, there is a close relationship (r=0.9) between CBV and CBF that has been extensively investigated^[19]. Secondly, the use of CBV instead of CBF eliminates the problems related to the mean cerebral transit time^[20]. Finally, near infrared absorption changes reflect changes in the oxygenation of blood in the microvasculature, and thus the CBV of the brain tissue^[21]. Changes of CBV also reflects capillary recruitment, which are considered a better reflection of cerebrovascular responses than CBF responses to acid-base stimuli^[20].

We measured CBV in the frontal cortex, that may not react in the same way as the brain stem region, where the central chemoreceptors are located^[22]. However, Hida *et al.*^[23] used transcranial Doppler to determine changes in blood flow velocity and could not find any differences in CO₂ responses between the brain stem artery and the middle cerebral

artery using transcranial Doppler. The latter paper would support our measurements of frontal lobe vasoresponsiveness to be representative of overall CBV changes. However, since there is no general agreement on cerebrovascular CO₂ responsiveness, one has to be cautious to draw this conclusion.

Prior to this study, the reproducibility of CBV measurements during resting conditions using NIRS was evaluated; an intra-individual coefficient of variation of $\pm 10\%$ was found^[24]. These results are in agreement with others^[25]. CBV values of the present study during normocapnia (range 1.60-4.30 mL 100g⁻¹) are consistent with other studies using NIRS: 2.85 ± 0.97 mL 100g⁻¹^[25].

Absolute values of CBV were lower in both COPD groups, relative to healthy subjects. Increased age^[26], hematocrit^[27] and heart rate may lower CBF and therefore CBV. Heart rate was significantly increased in both COPD groups. Heart rate responses primarily tests the parasympathetic system. STEWART *et al.*^[28] showed a parasympathetic autonomic dysfunction in 93% (28 out of 30) of severe hypoxaemic, hypercapnic COPD-patients and in 65% (39 out of 60) of moderately to severely hypoxic, normocapnic COPD-patients. Only 18% (4 out of 22) of the control group had evidence of an age-related autonomic dysfunction. However, a correlation between absolute values of CBV and HR was not found in the present study. Additionally, no correlation was found between absolute values of CBV and age.

Medication, like theophyllines and systemic corticosteroids, may reduce CBV^[29,30]. Theophylline was chronically used by 9 of 16 (56%) normocapnic and 11 of 17 (64%) hypercapnic patients, which may have contributed to the low CBV in both COPD groups. Systemic corticosteroids were used in 4 out of 16 normocapnic and 4 out of 17 hypercapnic patients. To assess the effect of medical intervention, average CBV was recalculated after subdividing both COPD groups in users and non-users of theophyllines and/or oral corticosteroids. In contrast to others^[29,30], CBV was slightly, but not significantly higher in our group of theophylline-users, relative to the non-users in both COPD groups. In addition, CBV values measured in corticosteroid-users and non-users were not different. Both the small size of the subgroups and the high variation of CBV values among our subjects may mask the well documented effects of theophyllines. Intravenous salbutamol (1 µg/kg) leads to an increased CBV in rats^[31]. Since the present study showed a low CBV in all COPD patients, it is unlikely that the inhaled salbutamol ($\leq 400\mu\text{g}$) of our patients affected the CBV values substantially.

MABP was relatively higher in all COPD patients and remained unchanged during hypercapnia. Although their MABP values fell well within the range of autoregulation, pressure-dependent sensors may dominate flow-dependent sensors in the cerebral circulation during chronic elevated blood pressure, leading to a lowering of CBF and CBV.

This might partly explain a blunted cerebrovascular responsiveness in both COPD groups, compared to healthy subjects.

There are only a few studies, describing CBV reactivity in adults using NIRS. GUPTA *et al.*^[32] used the same method as the present study to calculate CBV and found a higher mean CBV of 5.38 mL·100g⁻¹ and a lower CBV reactivity of 1.25 mL·100g⁻¹·kPa⁻¹, in young adults. However, they induced deeper desaturations ($\Delta 10$ -15%, instead of $\sim 5\%$), assumed equal Hb values for each individual and used the $FE'CO_2$ to correlate with CBV. As they suggested, a deeper desaturation possibly gives rise to concomitant hypoxic vasodilation and thereby a higher CBV and different CBV reactivity. Other studies applied the same "O₂-desaturation-method" in neonates^[33], or used O₂Hb derived reactivity values, and are therefore not comparable with this study^[34].

CONCLUSIONS

Normocapnic and chronic hypercapnic COPD patients had lower absolute values of CBV relative to healthy subjects; autonomic dysfunction was suggested as a possible reason for this difference. There was a poor, but positive correlation between ventilatory and cerebrovascular CO₂-responsiveness ($\Delta CBV/\Delta PaCO_2$ and $\Delta V'_I/\Delta PaCO_2$) in COPD patients and healthy subjects, thus refuting our hypothesis concerning an *inverse* relationship between cerebrovascular and ventilatory responses to PaCO₂.

As compared to healthy subjects, both COPD groups showed lower ventilatory as well as cerebrovascular CO₂-responses, with significantly lower responses in the chronic hypercapnic group. Since similar P_{0.1} reactivity was measured in both COPD groups and CBV- and V'_I-reactivity were not significantly different, the present study was not able to elucidate why some patients with COPD become hypercapnic, whereas others with the same degree of airway obstruction remain normocapnic.

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Effects of acetazolamide and furosemide on ventilation and cerebral blood volume in normocapnic and hypercapnic COPD patients

MJT van de Ven

WNJM Colier

MC van der Sluijs

B Oeseburg

A Vis

H Folgering

Accepted for publication in: *Chest*

ABSTRACT

Effects of chronic metabolic alkalosis and acidosis and their relation to central chemoregulation may differ between normocapnic and chronic hypercapnic patients with chronic obstructive lung disease (COPD). The relationship between responses of ventilation (V'_I), mouth occlusion pressure ($P_{0.1}$) and of cerebral blood volume (CBV), to acute changes in arterial PCO_2 , was measured. Seventeen chronic hypercapnic ($Paco_2 > 6.0$ kPa) and sixteen normocapnic ($Paco_2 \leq 6.0$ kPa) COPD-patients (FEV_1 27%pred), under baseline metabolic conditions and after one-week treatment with oral furosemide (40 mg, daily) or acetazolamide (500 mg, daily) were studied. Hypercapnia ($\Delta P_{ETCO_2} > 1$ kPa) was induced by giving adequate amounts of CO_2 in the inspired air. CBV was measured using Near Infrared Spectroscopy.

Compared to baseline metabolic condition, chronic metabolic acidosis and alkalosis did not change ventilatory ($\Delta V'_I / \Delta Paco_2$) and cerebrovascular ($\Delta CBV / \Delta Paco_2$) reactivity. Base excess (BE) decreased by $\Delta 6.8 \pm 1.1$ and $\Delta 6.9 \pm 1.6$ mEq.L⁻¹ in the normocapnic and chronic hypercapnic COPD group during metabolic acidosis, resulting in a, not quite significant, leftward shift of both ventilatory and cerebrovascular CO_2 response curve. BE increased by $\Delta 2.3 \pm 1.2$ and $\Delta 1.2 \pm 1.3$ mEq.L⁻¹ during chronic metabolic alkalosis in both COPD groups respectively, without concomitant shift. Poor correlations between ventilatory and cerebrovascular CO_2 -responsiveness ($\Delta CBV / \Delta Paco_2$ and $\Delta V'_I / \Delta Paco_2$, $\Delta CBV / \Delta Paco_2$ and $\Delta P_{0.1} / \Delta Paco_2$) were found irrespective of baseline, respiratory condition and induced metabolic state.

Conclusions: Normocapnic and chronic hypercapnic COPD-patients have the same ventilatory and cerebrovascular CO_2 -responsiveness irrespective of induced metabolic state.

KEYWORDS

near infrared spectroscopy; cerebral blood volume; acid-base; metabolic acidosis; metabolic alkalosis; central chemosensitivity; control of breathing; humans; chronic obstructive pulmonary disease; mouth occlusion pressure

INTRODUCTION

The contribution of cerebral blood flow (CBF) on ventilation was studied in healthy humans^[1-3] and laboratory animals^[4]. Patients with chronic obstructive pulmonary disease (COPD) are of particular interest. It is poorly understood why CO₂-chemoresponsiveness between normocapnic and chronic hypercapnic COPD patients differ. Despite similar degrees of airway obstruction, different measures of mouth occlusion pressure ($P_{0.1}$) and electromyographic parameters of respiratory muscle activity are found^[5], suggesting different central control of CO₂ regulation in both COPD groups.

In healthy subjects, an increase in CBF and cerebral blood volume (CBV) would increase CO₂ washout and lead to central hypocapnia^[6]. In addition, as reviewed by Feigl and Perret^[7], both cerebral resistance vessels (arterioles) and capillaries/venules are dilated by hypercapnia. *Chronic* hypercapnia, however, is associated with a blunted cerebrovascular reactivity to acute PCO₂ alterations^[8,9]. As a result, only minor changes in CBF and CBV can be expected during the latter condition, less able to attenuate the acute hypercapnic stimulus to the central chemoreceptors, leading to an elevated PCO₂ in the cerebral interstitial fluid (see Appendix). Consequently, an elevated ventilatory drive could be expected during chronic hypercapnia. However, the opposite, a lowered ventilatory drive is found^[5,10].

Cerebrovascular responses to hypercapnia, expressed as a change of CBV (Δ CBV) were studied in their relationship to ventilatory responses in normocapnic and chronic hypercapnic COPD patients, using a non-invasive technique of Near Infrared Spectroscopy (NIRS). We hypothesised an inverse relationship between cerebrovascular (Δ CBV/ Δ Paco₂) and ventilatory reactivity ($\Delta V'_{I}/\Delta$ Paco₂); chronic hypercapnic patients are thought to have a high vasodilatory response to PCO₂/pH, keeping the brain ECF less hypercapnic, thus keeping the central chemoreceptor mediated ventilatory drive relatively low, resulting in systemic hypercapnia. In the normocapnic group, the cerebrovascular response to CO₂ might be less, leading to a higher ECF PCO₂ and resulting in a normal (high) ventilatory drive.

As a chronic respiratory acidosis is usually compensated via metabolic pathways, in the present study we have investigated the effects of superimposed chronic metabolic acid-base changes on the control of cerebrovascular and ventilatory responses. Therefore, a chronic metabolic acidosis and alkalosis was induced by orally administrated acetazolamide and furosemide, respectively. Mouth occlusion pressure ($P_{0.1}$) and its response to changes of PCO₂ ($\Delta P_{0.1}/\Delta$ Paco₂) were measured in order to approximate the ventilatory drive independent of airway resistance, and related to CBV responsiveness.

MATERIAL AND METHODS

Subjects

The study was performed on 33 patients with COPD as defined by the American Thoracic Society. Ten males and six females, aged 60 ± 11 yrs, were normocapnic ($P_{aCO_2} \leq 6.0$ kPa); and 15 males and 2 females, aged 63 ± 8 yrs were hypercapnic ($P_{aCO_2} > 6.0$ kPa). Patients were excluded if they 1) had evidence of obstructive sleep disorders or restrictive pulmonary function or had a history of cardiopulmonary, cerebrovascular or other chronic diseases, 2) had an exacerbation in the 6 weeks before enrollment, 3) took other medications other than pulmonary bronchodilating agents, theophyllines and (systemic) corticosteroids. Three normocapnic and two hypercapnic patients were current smokers, all other patients stopped smoking for more than 6 months. A description of the patients is presented in Table 1.

At least two hours prior to the experiments, all participants had to abstain from caffeinated drinks and cigarettes, but were allowed to continue their pulmonary medication. All volunteers gave informed consent. The study was approved by the ethical committee of the Department of Pulmonology Dekkerswald, University Medical Center Nijmegen.

Patients were studied on three separate days, during induced acidosis, alkalosis and baseline (control) condition, in random order. Metabolic acidosis was induced by orally administrated acetazolamide (ACET) (Diamox®), 250 mg every 12 h during one week. Metabolic alkalosis was induced by orally administrated furosemide, 40 mg daily, during one week.

Measurements

Ventilation measurements. The subjects were in comfortable, reclining position. They were breathing through a facemask with low-resistance valves for in- and expiratory gasmixture. First, dead space ventilation (V_D/V_T) was measured using the Bohr equation. Expiratory air was collected in a Douglas bag for 10 minutes for measurements of mean expiratory P_{CO_2} (capnograph N1000, Nellcor Puritan Bennett, St. Louis, MO, U.S.A). Next, the inspiratory port of the mask was connected via a Fleisch pneumotachograph (No.3) to an inspiratory reservoir. The flow signal was electrically integrated into volume to calculate inspired ventilation (V'_I). End-tidal CO_2 (P_{ETCO_2}) (kPa) and respiratory rate (RR, min^{-1}) were measured at the expiratory port of the mask. Changes in inspiratory gasmixture of O_2 , N_2 , CO_2 were induced by means of a computer controlled massflow system (Bronckhorst-Hitec, Veenendaal, The Netherlands). The fraction of inspired O_2 (FiO_2) was monitored continuously using an O_2 analyzer (OM-11, Beckmann, Fullerton,

CA, U.S.A.). Fast changes in inspiratory gasmixture could be induced; the aimed changes were reached within one breath. Hypercapnia ($\Delta\text{PETCO}_2 > 1$ kPa) was induced by giving adequate amounts of CO_2 (FiCO_2 3-5%) in the inspired air.

Table 1. Characteristics of normocapnic and chronic hypercapnic patients, mean \pm SD

Variable	Normocapnic COPD	Hypercapnic COPD
Age, yr	59.8 \pm 10.7	62.8 \pm 8.4
BMI, kg.m ⁻²	22.9 \pm 2.54	21.5 \pm 2.4
FEV ₁ , %pred	28.8 \pm 9.6	24.3 \pm 7.5 ^{§§§}
IVC, %pred	79.3 \pm 13.5	64.1 \pm 9.3 ^{§§§}
TLC, %pred	109.9 \pm 20.1	97.8 \pm 16.4
FRC, %pred	147.6 \pm 38.2	137.4 \pm 27.3
RV, %pred	171.3 \pm 47.2	167.2 \pm 39.4
MIP, %pred	87.9 \pm 39.9	77.2 \pm 29.8
MEP, %pred	69.6 \pm 28.7	59.4 \pm 25.7

Definition of abbreviations: BMI = body mass index; TLC = total lung capacity; RV = residual volume; FRC = functional residual capacity; MIP = mean inspiratory pressure; MEP = mean expiratory pressure

^{§§§}: $p < 0.001$ normocapnic COPD-group compared to chronic hypercapnic COPD-group

CBV measurements. Near Infrared Spectroscopy (NIRS) has been developed to monitor brain oxygenation and dynamics^[11]. The theory of NIRS has been described extensively^[12]. The technique is based on oxygenation-dependent absorption changes in the blood caused by chromophores, mainly oxy- and deoxyhaemoglobin (O_2Hb and HHb), respectively. Near-infrared light was carried to and from a pulsed continuous-wave NIRS instrument (OXYMON, Artinis Medical Systems, the Netherlands) through two fiber optic bundles (optodes) on the left side of the forehead. One optode emits near infrared light at three different wavelengths, which penetrates through skull and brain. The receiving optode is positioned at a distance of 5.5 cm apart from the emitting optode. This distance ensures that most of the extracranial circulation is excluded from the detected signal^[13].

Calculation of CBV was described by Elwell.^[14,15] A slight change of saturation ($\sim 5\%$) is necessary to quantify CBV. The change of saturation is related to the difference of concentration of hemoglobin chromophores at two levels of saturation. CBV can be calculated taken the individual hemoglobin concentration into account and a fixed constant. This constant accounts for the molecular weight of hemoglobin, the cerebral tissue density and the cerebral-to-large vessel hematocrit ratio.

Mouth occlusion pressure measurements. Ventilatory effort during inspiration was determined by occlusion pressure at 0.1 second after the start of inspiration. A solenoid valve was positioned in the inspiratory line of the circuit^[16]. Closure of the valve during expiration was manually controlled, and the valve automatically opened after the first 100 ms of the occluded inspiration. Five repeated measurements of $P_{0.1}$ were averaged during each CO_2 condition. $P_{0.1}$ was expressed both as absolute value (cmH₂O) and as percentage of maximal inspiratory pressure (MIP), in order to normalize $P_{0.1}$ for the individual differences in inspiratory muscle strength^[17].

Protocol

All patients underwent routine spirometry and blood analysis of hemoglobin, hematocrit and resting arterial blood gases to assign the individual patients into the normocapnic and chronic hypercapnic COPD group. On each of three study days, a canula was introduced in the left brachial artery to collect arterial blood samples during normcapnia and to control the level of acutely induced respiratory hypercapnia (Chiron Diagnostics Cooperation Rapid Lab 855, East Walpole MA, U.S.A). SaO_2 and heart rate (HR) were monitored with a pulse-oximeter (N200, Nellcor Puritan Bennett, U.S.A.), with the sensor attached to the right-frontal forehead.

Duplicate measurements of CBV, and $P_{0.1}$ during normo- and hypercapnia were performed after a period of 10 min equilibration. Arterial pressure was measured manually during each CO_2 condition. Mean arterial blood pressure (MABP) was calculated as: diastolic pressure + $1/3 \times (\text{systolic} - \text{diastolic})$ pressure. All data (except MABP) were linked directly to the NIRS computer for real time display and simultaneous storage with the NIRS data.

Statistics

During the whole experiment, time-averaged values of V'_I , SaO_2 , HR and RR were recorded, expressed as mean \pm SD during each CO_2 challenge. The latter parameters, anthropometric characteristics, pulmonary function, MABP and arterial blood gas values under control condition were compared between the two COPD groups by the Mann-Whitney test for two independent samples. Within the COPD groups, values measured under control condition were compared to values during chronic metabolic acidosis (ACET) and during chronic metabolic alkalosis (furosemide) using the Wilcoxon matched-paired signed-ranks test. For each individual, CBV, V'_I and $P_{0.1}$ was plotted against corresponding PaCO_2 values during each metabolic condition and subjected to linear regression analysis. As the statistical method of Kolmogorov and Smirnov, as described in GraphPad InStat@

software, showed a gaussian distribution, a paired *t*-test could be used to compare the slopes and intercepts of the linear regression equations during control and both metabolic conditions. The level of statistical significance was set at $p < 0.05$. All tests should be regarded as explorative due to the multiplicity of tests.

RESULTS

Comparison between the normocapnic and hypercapnic COPD group:

control condition

The anthropometric characteristics and respiratory function data of the patients are summarized in Table 1. The degree of airway obstruction was the same in both COPD groups. Other ventilatory parameters (V'_I , RR, V_T , MVV, V_D/V_T) were similar in both groups (Table 2). Mean values (\pm SD) of P_{aCO_2} were 5.26 ± 0.27 kPa and 6.27 ± 0.45 kPa in the normocapnic and chronic hypercapnic COPD group, respectively, under baseline metabolic conditions. The hypercapnic COPD group showed significantly lower resting P_{aO_2} values. Values of CBV were lower in the normocapnic patients relative to chronic hypercapnic patients ($p < 0.01$) (Table 2).

Both COPD groups were equally using inhaled salbutamol (5 /16 normocapnic and 6/17 chronic hypercapnic COPD). To account for medical intervention, average CBV and V_I was recalculated after subdividing both COPD groups in users and non-users of theophyllines and/or oral corticosteroids. Theophylline was chronically used by 9 of 16 (56%) normocapnic and 11 of 17 (64%) hypercapnic patients. Systemic corticosteroids were used in 4 out of 16 normocapnic and 4 out of 17 hypercapnic patients. In both COPD groups, V_I -values ($\text{mL} \cdot \text{min}^{-1}$) were not significantly different in users and non-users of theophylline, 10.3 and 8.9 in the normocapnic, and 8.5 and 9.8 in the chronic hypercapnic group, respectively. In addition, values of V_I were not significantly different in users and non-users of corticosteroids, 11.2 and 9.3 in the normocapnic, and 8.8 and 10.0 in the chronic hypercapnic group, respectively. CBV was not significantly different in our group of theophylline-users, relative to the non-users in both COPD groups. Furthermore, CBV-values measured in corticosteroid-users and non-users were not different.

Both cerebrovascular and ventilatory response to CO_2 ($\Delta CBV/\Delta P_{aCO_2}$ and $\Delta V'_I/\Delta P_{aCO_2}$) were the same in the chronic hypercapnic group as in the normocapnic group (Table 3, Figure 1, Figure 2). Both absolute values of P_{O_1} (Table 2) and its reactivity ($\Delta P_{O_1}/\Delta P_{aCO_2}$, Table 3, Figure 1, Figure 2) were the same in both COPD groups, even after correction

Table 2. Outcome parameters during three metabolic conditions in normocapnic and chronic hypercapnic patients, mean \pm SD

Variable	Normocapnic COPD			Hypercapnic COPD		
	control	ACET	furosemide	control	ACET	furosemide
Paco ₂ , kPa	5.26 \pm 0.27	4.69 \pm 0.35 ^{***}	5.43 \pm 0.30 ^{***^{¶¶¶}}	6.27 \pm 0.45 ^{§§§}	5.95 \pm 0.71 ^{**}	6.37 \pm 0.61 ^{¶¶¶}
PaO ₂ , kPa	9.05 \pm 0.59	9.50 \pm 0.75 [*]	8.81 \pm 0.48	8.31 \pm 0.99 ^{§§}	8.67 \pm 0.75	7.81 \pm 0.56 ^{*^{¶¶¶}}
pH	7.42 \pm 0.02	7.35 \pm 0.02 ^{***}	7.44 \pm 0.02 ^{***^{¶¶¶}}	7.39 \pm 0.02 ^{§§}	7.32 \pm 0.02 ^{***}	7.40 \pm 0.03 ^{¶¶¶}
HCO ₃ ⁻ , mEq.L ⁻¹	25.2 \pm 1.2	18.96 \pm 1.19 ^{***}	27.35 \pm 1.28 ^{**^{¶¶¶}}	28.0 \pm 1.6 ^{§§}	22.64 \pm 2.13 ^{***}	29.12 \pm 1.83 ^{*^{¶¶¶}}
BE, mEq.L ⁻¹	0.8 \pm 1.2	-6.0 \pm 1.1 ^{***}	3.1 \pm 1.3 ^{***^{¶¶¶}}	2.4 \pm 1.3 ^{§§}	-3.5 \pm 1.7 ^{***}	3.7 \pm 1.8 ^{*^{¶¶¶}}
CBV, mL.100g ⁻¹	2.41 \pm 0.66	2.95 \pm 0.80	2.60 \pm 0.84	2.90 \pm 0.60 ^{§§}	3.04 \pm 0.8	2.79 \pm 0.61
V _I , L.min ⁻¹	9.7 \pm 2.5	11.1 \pm 1.8 ^{**}	9.4 \pm 1.7 ^{¶¶¶}	9.0 \pm 2.0	9.3 \pm 1.8	8.4 \pm 2.8
P ₀₁ , cmH ₂ O	5.12 \pm 2.57	5.12 \pm 2.48	5.11 \pm 2.11	5.44 \pm 2.96	5.34 \pm 5.70	5.70 \pm 3.14
P ₀₁ , %MIP	7.72 \pm 4.53	7.90 \pm 3.00	7.52 \pm 4.62	8.14 \pm 4.38	8.47 \pm 3.95	9.82 \pm 6.47
MVV, % pred	33.1 \pm 12.7	37.4 \pm 13.1	29.9 \pm 11.6 ^{¶¶}	33.3 \pm 10.2	33.9 \pm 9.0	31 \pm 11.9
V _D /V _T , %	48 \pm 11	52 \pm 5	51 \pm 7	53 \pm 7	55 \pm 7	54 \pm 6
RR, L.min ⁻¹	16 \pm 4	16 \pm 4	16 \pm 4	19 \pm 5	18 \pm 5	17 \pm 5
V _T , mL	648 \pm 200	710 \pm 140	610 \pm 204 [*]	520 \pm 200	552 \pm 150	534 \pm 194
HR, L.min ⁻¹	83 \pm 11	82 \pm 14	85 \pm 11	82 \pm 12	82 \pm 13	86 \pm 13 ^{**}
MABP, mmHg	106 \pm 12	111 \pm 15	107 \pm 13	111 \pm 14	106 \pm 14	106 \pm 9

Definition of abbreviations: CBV = cerebral blood volume; V_I = inspired ventilation; P₀₁ = mouth occlusion pressure; MIP = maximal inspiratory pressure; MVV = maximal voluntary ventilation, RR = respiratory rate; V_T = tidal volume; HR = heart rate; MABP = mean arterial blood pressure.

§§: p<0.01; §§§: p<0.001 normocapnic COPD-group compared to chronic hypercapnic COPD-group

Within the subgroups of COPD:

*=p<0.05, **= p<0.01, ***= p<0.001, no medication compared to chronic ACET or furosemide, Mann-Whitney test

¶¶p<0.01, administration of chronic ACET compared to chronic furosemide, Mann-Whitney test

Table 3. Linear regression of CBV, V'_I and $P_{0.1}$ to hypercapnia in normocapnic and chronic hypercapnic patients, mean \pm SD

Variables	Normocapnic COPD			Hypercapnic COPD		
	control	ACET	furosemide	control	ACET	furosemide
CBV						
slope (ml.100g ⁻¹ .kPa ⁻¹)	1.59 \pm 0.91	1.61 \pm 0.90	1.03 \pm 0.49	1.23 \pm 0.67	1.32 \pm 0.79	1.21 \pm 0.77
CBV ₀ (kPa)	3.30 \pm 1.41	1.99 \pm 2.27	2.15 \pm 2.29	3.60 \pm 1.45	2.43 \pm 2.88	3.92 \pm 2.27
r	0.90	0.90	0.82	0.84	0.89	0.74
V'_I						
slope (L.min ⁻¹ .kPa ⁻¹)	8.2 \pm 4.9	7.5 \pm 3.1	7.4 \pm 3.5	5.5 \pm 4.4	7.3 \pm 6.2	6.0 \pm 4.2
V'_{I0} (kPa)	3.63 \pm 1.12	2.99 \pm 0.57	3.87 \pm 0.57	3.91 \pm 1.56	3.77 \pm 1.66	3.84 \pm 2.74
r	0.96	0.96	0.92	0.90	0.93	0.92
$P_{0.1}$						
slope (cmH2O ⁻¹ .kPa ⁻¹)	3.02 \pm 1.73	4.51 \pm 3.19	3.09 \pm 2.16	3.02 \pm 1.50	5.39 \pm 3.97	5.72 \pm 2.28*
$P_{0.10}$ (kPa)	2.89 \pm 1.41	3.32 \pm 1.12	2.69 \pm 2.13	4.29 \pm 1.07 [§]	4.07 \pm 2.42	4.83 \pm 1.40
r	0.66	0.90	0.53	0.85	0.83	0.74

Linear regression analysis of cerebral blood volume (CBV, V'_I and $P_{0.1}$ value) as a function of P_{aCO_2} was performed on each subject, based on 4 measurements (duplicated normocapnia, duplicated hypercapnia). Mean (\pm SD) slopes, x-intercepts and coefficient of correlation (r) of the groups are displayed. The x-intercept is the P_{aCO_2} value at zero CBV, zero V'_I and zero $P_{0.1}$ (CBV₀, V'_{I0} , $P_{0.10}$).

[§]p<0.05, normocapnic COPD-group compared to chronic hypercapnic COPD-group

*p<0.05; COPD-group compared to control group

for MIP. However, the x-intercept (P_{aCO_2} at zero $P_{0.1}$) was higher ($p < 0.05$) in the hypercapnic COPD group.

Figure 1. Ventilatory and cerebrovascular responses to CO_2 . Regression equations were obtained for data from each individual subject and averaged afterwards for the group. All values of cerebral blood volume (CBV, first diagram), inspired minute ventilation (V_I , second diagram) and mouth occlusion pressure ($P_{0.1}$, third diagram) were indexed to similar P_{aCO_2} values to show average group results.

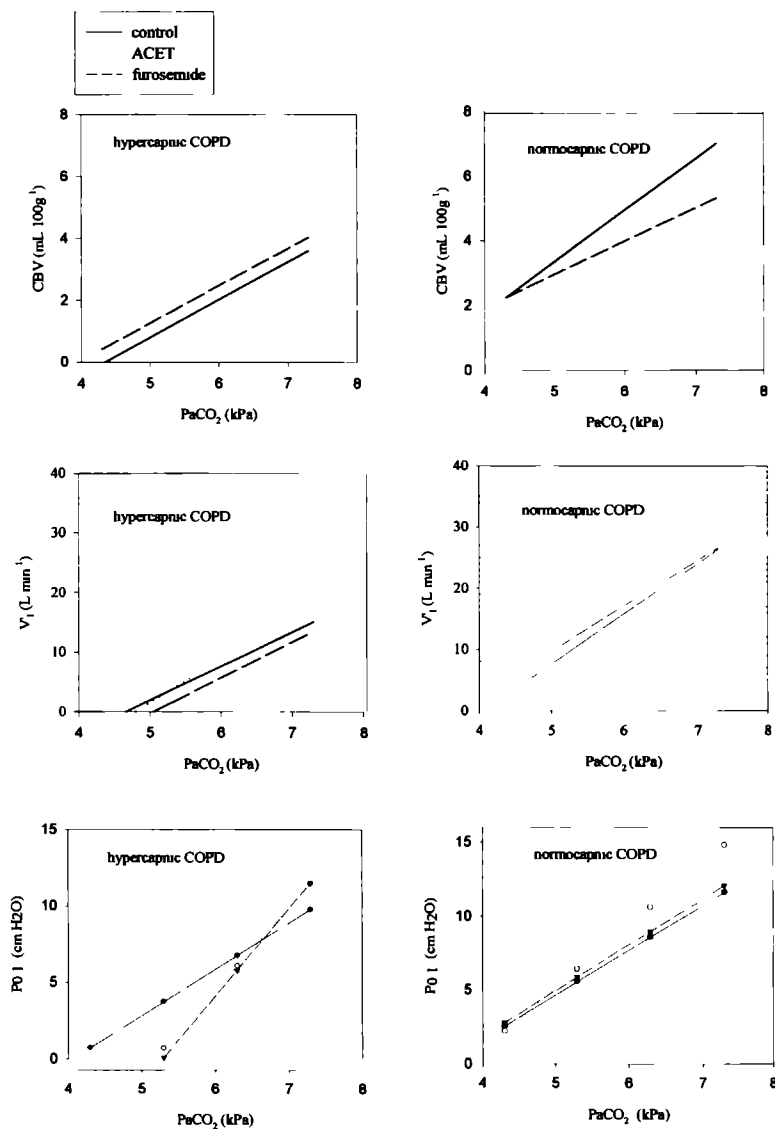
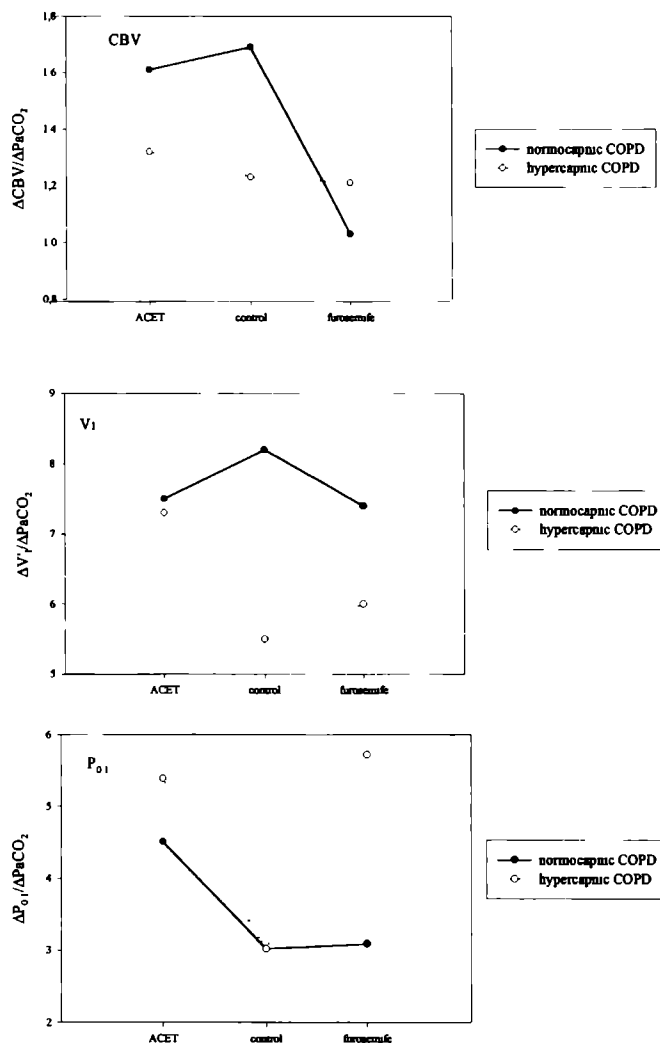


Figure 2. Slopes of cerebrovascular and ventilatory responsiveness to CO_2 during three metabolic conditions. The mean values of slopes of $\Delta\text{CBV}/\Delta\text{PaCO}_2$, $\Delta\dot{V}'_I/\Delta\text{PaCO}_2$ and $\Delta\text{P}_{0.1}/\Delta\text{PaCO}_2$ were displayed to clarify differences between the slopes in three metabolic conditions in normocapnic and chronic hypercapnic COPD-patients.



Effects of chronic metabolic acidosis

The degree of metabolic acidosis was reflected in a mean decrease of base-excess (BE) of $-6.8 \pm 1.1 \text{ mEq.L}^{-1}$ in the normocapnic COPD group and $-5.9 \pm 1.6 \text{ mEq.L}^{-1}$ in the chronic

hypercapnic COPD group (both $p < 0.001$). Oral ACET induced significant changes of P_{aCO_2} as compared to control condition in both COPD groups in spite of unchanged ventilation in the hypercapnic COPD group. In addition, only the normocapnic COPD group showed a simultaneously significant increased P_{aO_2} value ($p < 0.05$). Both ventilatory ($\Delta V'_I/\Delta P_{aCO_2}$), mouth pressure ($\Delta P_{0.1}/\Delta P_{aCO_2}$) and cerebrovascular ($\Delta CBV/\Delta P_{aCO_2}$) reactivity and corresponding intercepts did not change significantly during metabolic acidosis (Table 3, Figure 1, Figure 2) in both COPD groups.

Effects of chronic metabolic alkalosis

Orally administrated furosemide induced a chronic metabolic alkalosis, with a mean increased ΔBE of 2.3 ± 1.2 mEq.L⁻¹ ($p < 0.001$) in the normocapnic COPD group and 1.2 ± 1.3 mEq.L⁻¹ ($p < 0.05$) in the chronic hypercapnic COPD group (Table 2). Mean value of P_{aCO_2} increased ($p < 0.01$) in the normocapnic group in spite of unchanged ventilation. Furosemide lowered P_{aO_2} ($p < 0.05$) in the hypercapnic COPD group. Normocapnic COPD patients showed the same reactivity of both $\Delta V'_I/\Delta P_{aCO_2}$ and $\Delta CBV/\Delta P_{aCO_2}$ as the chronic hypercapnic group (Table 3, Figure 1, Figure 2). Absolute values of $P_{0.1}$ did not differ between both control condition and metabolic alkalosis, however, its reactivity ($\Delta P_{0.1}/\Delta P_{aCO_2}$) was significantly higher ($p < 0.05$) during metabolic alkalosis in the chronic hypercapnic COPD group.

Correlation between the different reactivity parameters

Poor, not significantly different correlations were found between the individual CBV and V_I responses to acute hypercapnia ($\Delta CBV/\Delta P_{aCO_2}$ and $\Delta V'_I/\Delta P_{aCO_2}$, $r \leq 0.44$, $p > 0.1$) for both COPD patients (Figure 3) under control condition and during metabolic acidosis, and a weak, but significant correlation during metabolic alkalosis in both COPD groups ($r = 0.58$, $p < 0.05$). Correlations between the individual CBV and $P_{0.1}$ slopes ($\Delta CBV/\Delta P_{aCO_2}$ and $\Delta P_{0.1}/\Delta P_{aCO_2}$) were poor and not significant in both COPD groups in three metabolic conditions (Figure 4).

DISCUSSION

Cerebrovascular responses were studied and correlated with ventilatory reactivity in normocapnic and chronic hypercapnic COPD patients. Chronic hypercapnic patients showed the same CBV- and V'_I -reactivities to acute CO_2 changes under baseline metabolic conditions as normocapnic patients. Mouth occlusion pressure was the same in both COPD groups, even after correction for MIP. The influence of superimposed chronic

Figure 3. Correlations between $\Delta CBV/\Delta PaCO_2$ and $\Delta V'_I/\Delta PaCO_2$ in normocapnic and chronic hypercapnic COPD patients. Hypercapnic COPD - patients: control: $\Delta V'_I/\Delta PaCO_2 = 1.34^* \Delta CBV/\Delta PaCO_2 + 4.08$ ($r=0.20$; $p=0.46$). ACET: $\Delta V'_I/\Delta PaCO_2 = 0.09^* \Delta CBV/\Delta PaCO_2 + 7.19$ ($r=0.01$; $p=0.96$). Furosemide: $\Delta V'_I/\Delta PaCO_2 = 2.89^* \Delta CBV/\Delta PaCO_2 + 2.09$ ($r=0.54$; $p<0.05$). Normocapnic COPD - patients: control: $\Delta V'_I/\Delta PaCO_2 = 2.39^* \Delta CBV/\Delta PaCO_2 + 4.70$ ($r=0.44$; $p=0.09$). ACET: $\Delta V'_I/\Delta PaCO_2 = -0.9^* \Delta CBV/\Delta PaCO_2 + 8.96$ ($r=-0.27$; $p=0.3$). Furosemide: $\Delta V'_I/\Delta PaCO_2 = 3.75^* \Delta CBV/\Delta PaCO_2 + 3.30$ ($r=0.58$; $p<0.05$).

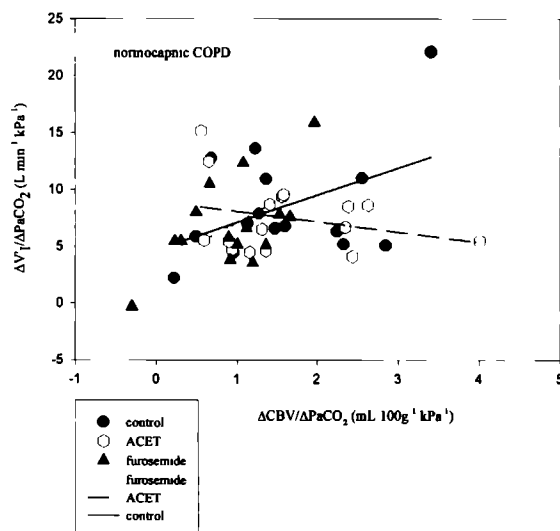
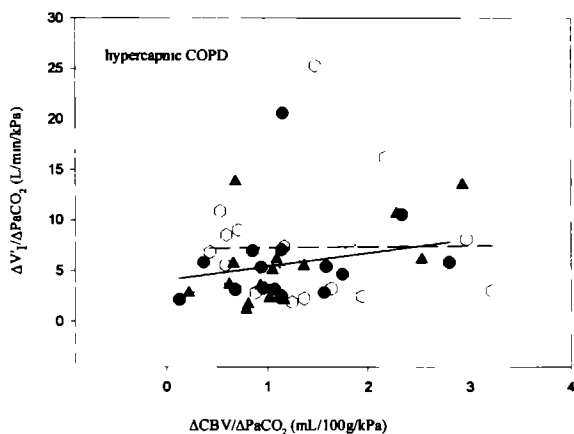
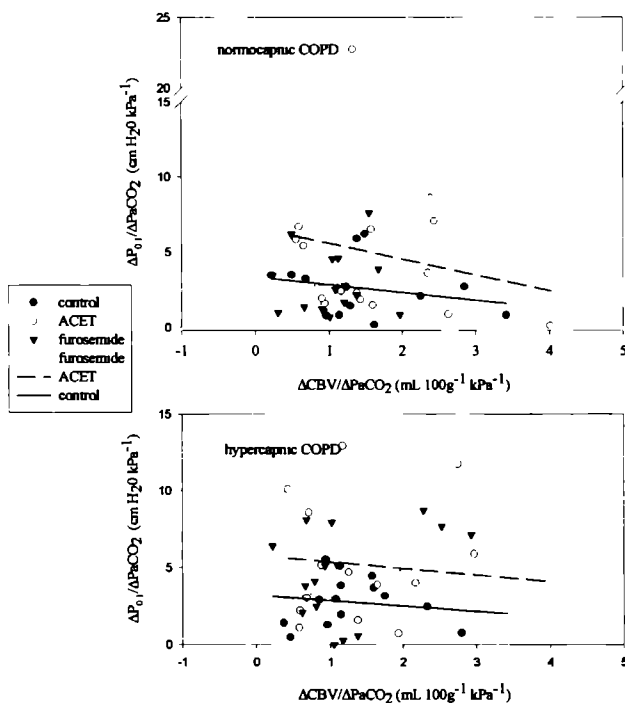


Figure 4. Correlations between $\Delta P_{0.1}/\Delta P_{aCO_2}$ and $\Delta CBV/\Delta P_{aCO_2}$ in normocapnic and chronic hypercapnic COPD patients. Hypercapnic COPD - patients: control: $\Delta P_{0.1}/\Delta P_{aCO_2} = -0.35 * \Delta CBV/\Delta P_{aCO_2} + 3.20$ ($r=0.16$; $p>0.05$). ACET: $\Delta P_{0.1}/\Delta P_{aCO_2} = -0.42 * \Delta CBV/\Delta P_{aCO_2} + 5.77$ ($r=0.07$; $p>0.05$). Furosemide: $\Delta P_{0.1}/\Delta P_{aCO_2} = 1.26 * \Delta CBV/\Delta P_{aCO_2} + 2.32$ ($r=0.29$; $p>0.05$). Normocapnic COPD - patients: control: $\Delta P_{0.1}/\Delta P_{aCO_2} = -5.15 * \Delta CBV/\Delta P_{aCO_2} + 3.33$ ($r=0.25$; $p>0.05$). ACET: $\Delta P_{0.1}/\Delta P_{aCO_2} = -1.05 * \Delta CBV/\Delta P_{aCO_2} + 6.62$ ($r=0.17$; $p>0.05$). Furosemide: $\Delta P_{0.1}/\Delta P_{aCO_2} = 0.29 * \Delta CBV/\Delta P_{aCO_2} + 2.54$ ($r=0.06$; $p>0.05$)



metabolic acidosis on CBV- and V'_{I-} and $P_{0.1}$ -reactivity was not significantly different in both COPD groups. Effects of superimposed chronic metabolic alkalosis however, were more pronounced in the normocapnic COPD group, with (tendency to) lower ventilatory and cerebrovascular CO_2 -responses in the latter group. In addition, $P_{0.1}$ -reactivity was significantly increased in the chronic hypercapnic group. Ventilatory and cerebrovascular CO_2 -responsiveness were correlated and showed a wide inter-individual variability of cerebrovascular and ventilatory reactivity to acute changes in P_{aCO_2} , thus refuting the

hypothesis of an inverse relationship between $\Delta\text{CBV}/\Delta\text{Paco}_2$ and $\Delta V'/\Delta \text{Paco}_2$ in COPD-patients.

Critique of methods

Prior to this study, the reproducibility of CBV measurements during resting conditions using NIRS was evaluated; an intra-individual coefficient of variation of $\pm 10\%$ was found^[19]. These results are in agreement with others^[14,15]. CBV values of the present study under baseline metabolic conditions in both COPD groups (2.41 ± 0.66 and 2.90 ± 0.60 ml.100g⁻¹, respectively) are consistent with other investigators using NIRS in healthy subjects: 2.85 ± 0.97 ml 100g⁻¹^[14].

It is important to consider the advantages of measurements of CBV over CBF measurements. Firstly, there is a close relationship ($r=0.9$) between CBV and CBF that has been extensively investigated by Grubb et al[20] and by Van Zijl et al^[21]. Secondly, the use of CBV instead of CBF eliminates the problems related to the mean cerebral transit time^[22]. Finally, near infrared absorption changes reflect changes in the oxygenation of the microvasculature, and thus the CBV of the brain tissue^[23]. Changes of CBV reflect capillary recruitment, which by some are considered a better reflection of cerebrovascular responses than CBF responses to acid-base stimuli^[22]. We measured CBV in the frontal cortex region as present techniques do not allow measures of CBV or CBF in the brainstem of conscious humans. Moreover, Hida et al.^[24] could not find any differences in CO₂ responses between the brain stem artery and the middle cerebral artery, supporting the assumption that our frontal lobe CBV measurements may be a good reflection of overall CBV changes in the brain.

Baseline metabolic (control) conditions

Absolute values of CBV were lower in the normocapnic COPD group. Age^[25], hematocrit^[26], MABP and heart rate^[27] are established factors to affect CBV. However, both COPD groups were age-matched, and all other parameters were not significantly different. The influence of medication was evaluated in order to find an explanation for the differences of CBV-values between the COPD groups. Theophylline and corticosteroids are known to lower CBV^[18,28]. To account for medical intervention, average CBV and V_i was recalculated after subdividing both COPD groups in users and non-users of theophyllines and/or oral corticosteroids. In contrast to others^[18,28], CBV was not significantly different in our group of theophylline-users, relative to the non-users in both COPD groups. In addition, no differences were seen in ventilation. Furthermore, CBV- and V_i -values measured in corticosteroid-users and non-users were

not different. It is described that *intravenous* salbutamol ($1 \mu\text{g/kg}$) leads to an increased CBV in rats^[29]. In addition, the influence of *inhaled* salbutamol is likely to be less important on CBV regulation. As both COPD groups were equally using inhaled salbutamol, it is unlikely to account for salbutamol as important interfering factor for CBV differences between the groups. This study did not show any significant effect of inhaled salbutamol in both COPD groups on both CBV and V_t .

CBV responsiveness to CO_2 was not reduced in the chronic hypercapnic COPD group as compared to the normocapnic group. This is probably due to the power of the study. A reduced cerebral vascular responsiveness to a CO_2 challenge is in line with others^[8,9] and is suggested to be the resultant of several factors as 1: a reduced increase in tissue H^+ concentration secondary to an increased buffering capacity of the brain substance 2: changes in the chemical composition of the CSF bathing the cerebral vessels (arterioles), involving an adjustment in the concentration of bicarbonate ions,^[8] 3: changes in neurotransmitter production secondary to chronic hypercapnia, 4: a chronic increase in interstitial fluid, 5: increase venous resistance to venous return, and 6: inability to increase cardiac output.

Ventilatory responsiveness to CO_2 was highest in normocapnic COPD patients, compared to chronic hypercapnic COPD patients. This result was expected, and is in agreement with results of others^[5,10,30,31]. As serum bicarbonate levels are higher in patients with chronic hypercapnia than the normocapnic ones, pH changes, due to acute respiratory hypercapnia, at the central chemoreceptor are lower for a given increment in PaCO_2 . This could explain the lower ventilatory responses in the first group.^[32] The lack of statistical significance may be explained by the relatively small size of the group.

In line with Gelb et al.^[30] and Montes de Oca^[10], but in contrast to others^[5,32], the present study found the same values of $P_{0.1}$ responsiveness ($\text{cmH}_2\text{O}\cdot\text{kPa}^{-1}$) in hypercapnics relative to normocapnics. The present study agreed with the results of Scano et al.^[5] that even after normalization of $P_{0.1}$ for individual differences in muscle strength was performed ($P_{0.1}$ as percentage of MIP), no differences between both COPD groups were seen.

Effects of chronic metabolic acidosis

Acetazolamide is used in patients with COPD to improve blood gas values, especially in cases with a metabolic alkalosis related to the use of steroids and diuretics.^[33] The beneficial effect of ACET in these patients is probably primarily due to an increase in ventilatory drive, secondary to a metabolic acidosis induced by effective inhibition of renal carbonic anhydrase^[34]. A clinical dose of ACET (250 mg p.o., every 8 h for three days) leads to increased ventilation, resulting in a lowered PaCO_2 ^[35] In the present study

however, results of ventilation after ACET administration differed in normocapnic patients relative to chronic hypercapnic patients. This might be due to the relatively flat CO₂ response curve, which is a common observation in the latter group. A change in base excess would shift the CO₂ response curve leftwards, without hardly any measurable change in ventilation and in ventilatory responsiveness ($\Delta V'_{\text{I}}/\Delta \text{Paco}_2$)^[35]. Earlier studies found different effects of ACET on the ventilatory CO₂ sensitivity in man with variations from no change^[35,36] to an increase^[37] after chronic application. It is suggested that differences in dose regimens and methodology to determine slopes of CO₂ responses curves (e.g. steady state methods vs. rebreathing) may account for these variable study outcomes^[35].

The increase in ventilation caused a rise in Pao₂ in the normocapnic group. The presence of many lung regions with low ventilation-perfusion ratios may have mainly contributed to the lack of increase of ventilation and increase of Pao₂ in the chronic hypercapnic group^[35]. However, the degree of ventilation-perfusion mismatch (Vd/Vt) was only slightly higher in the latter group.

Due to its physical-chemical properties, acetazolamide does not easily cross the blood brain barrier^[38], even at higher doses. However, even after one low-dose of ACET (4 mg.kg⁻¹), a decrease of CO₂ sensitivity of the central chemoreflex loop was found in carotid body-denervated cats, and the investigators reasoned an altered relationship between brain blood flow and brain tissue PCO₂^[39]. The present study however, could not support differences in cerebrovascular reactivity and thus altered relationships between cerebral blood volume and Paco₂ after chronic ACET administration in both COPD groups.

Effect of chronic metabolic alkalosis

It is interesting to note different effects of furosemide in both COPD groups, with reduced effects on ΔBE in the hypercapnic COPD group. This may be due to the preexisting metabolic compensated alkalosis in the latter group, relative to the normocapnic group (mean control value of BE 2.4 vs. 0.8 mEq.L⁻¹) and therefore difficulties to induce a further metabolic alkalosis. Ventilatory and cerebrovascular slopes were not different in the normocapnic group, after inducing metabolic alkalosis. Values of Paco₂ (in spite of unchanged ventilation) are only significantly elevated in the normocapnic group. Despite minor BE changes, Pao₂ deteriorated significantly in the chronic hypercapnic group. The higher P_{0.1} slope with concomitant unchanged ventilation slope in chronic hypercapnic COPD patients is probably caused by an ensuing increased airway resistance as seen during alkalosis^[40].

Mean values of CBV did not alter during metabolic alkalosis. Earlier studies suggest lower cerebral blood flows during maintained steady chronic metabolic alkalosis^[41] in healthy

humans. Assuming similarities in CBF between healthy subjects and normocapnic COPD patients, the present study suggest only a tendency to a lower CBV reactivity in normocapnic COPD patients.

Correlation between the different reactivity parameters

Other investigators pointed out the importance to measure ventilation and cerebrovascular reactivity simultaneously^[34,42]. The present study showed a wide variety in ventilatory and cerebrovascular CO₂-responsiveness, albeit showing some positive correlation and thereby refuting the hypothesised inverse relationship.

In conclusion, chronic hypercapnic patients showed the same CBV- and V'_I-reactivities under baseline metabolic conditions compared to normocapnic patients. The effect of superimposed chronic metabolic acidosis, on mean CBV- and V'_I-reactivity was not significantly different in both COPD groups. Different effects on arterial blood gas values however, were seen between the COPD groups. In addition, superimposed chronic metabolic alkalosis was more obvious in the normocapnic COPD group and led to some tendency to lower ventilatory and cerebrovascular CO₂-responses in the latter group. Mouth occlusion pressure was similar in both COPD groups, even after correction for MIP during control condition and metabolic acidosis. The increased P_{O₁}-reactivity during superimposed chronic metabolic alkalosis in the chronic hypercapnic group was probably due to increased airway resistance. The poor, but positive correlation between ventilatory and cerebrovascular CO₂-responsiveness ($\Delta\text{CBV}/\Delta\text{Paco}_2$ and $\Delta\text{V}'_I/\Delta\text{Paco}_2$) during all metabolic conditions argued against our hypothesis concerning 1. an *inverse* relationship between cerebrovascular and ventilatory responses to Paco₂ and 2. differences in neuroventilatory reactivity between normocapnic and hypercapnic COPD groups.

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Appendix

We describe a simple linearized static model of the interaction between cerebral blood flow (CBF) and ventilation (V) in response to changes in arterial PCO_2 (PaCO_2). Consider a certain volume of brain tissue with metabolic rate M L (STPD) $\text{CO}_2 \cdot \text{min}^{-1}$ and perfused by Q L $\cdot \text{min}^{-1}$ of blood entering the tissue with an arterial partial CO_2 pressure PaCO_2 .

According to Fick's law, the venous partial CO_2 pressure in steady state then is

$$\text{PvCO}_2 = \text{PaCO}_2 + M / (c \cdot Q) \quad (1)$$

where c is solubility.

For reasons of simplicity the index CO_2 will now be dropped.

We now make the simplifying assumption that during normoxia perfusion around the operating point is linearly dependent on Pa , so

$$Q = Q_0 + a \cdot \text{Pa} \quad (2)$$

We next assume that the normoxic central ventilatory drive is a simple linear combination of Pa and Pv :

$$V = V_0 + \alpha \cdot \text{Pa} + \beta \cdot \text{Pv} \quad (3)$$

Substituting eq(1) and eq(2) in eq(3) yields

$$V = V_0 + (\alpha + \beta) \cdot \text{Pa} + \beta M / \{c(Q_0 + a \text{Pa})\} \quad (4)$$

Equation 4 consists of 3 terms. Since the ventilatory drive $V=0$ at some non zero, positive value of Pa , term 1 must be a negative constant.

Term 2 increases linearly with Pa . Term 3 is the interesting part. It represents the larger part of the interaction between cerebral blood flow and ventilatory drive and has an inverse response ($\Delta V / \Delta \text{Pa} < 0$). It decreases with increasing Pa thus damping the ventilatory response to an increase in Pa . At lower values of Pa this term is the more important one, increasing with decreasing values of Pa . Depending on the balance of term 2 and term 3, term 3 may even induce hyperventilation.

The blunted response of COPD patients can be modelled here with relative small values for α and β in eq(3) making term 3 more important.

CHAPTER 10

Summary and conclusions

In the group of patients with chronic obstructive pulmonary diseases (COPD), two extreme forms of the control of breathing exist: normocapnic and hypercapnic patients. There are indications that the CO_2 -chemoresponsiveness of both patient groups differ and this cannot be explained simply by pulmonary mechanics. The central chemoreceptor drive to ventilation is controlled by the acid-base composition of the extracellular fluid of the brain stem, which in turn is partly regulated by cerebral blood flow (CBF). Variations in blood flow will alter the relationship between Paco_2 (the stimulus that can be measured) and the CO_2 tension of brain ECF at the central chemoreceptors (the true stimulus). Simultaneous measurements of cerebrovascular and of ventilatory reactivity to CO_2 may be relevant in understanding the control of breathing in COPD patients. We hypothesised that, in hypercapnic patients, there is a high cerebral vasodilatory response to PCO_2/pH , keeping the brain ECF less hypercapnic, thus keeping the central chemoreceptor mediated ventilatory drive relatively low, and eventually resulting in systemic hypercapnia. We supposed that in normocapnic patients this autoregulatory system of CBF is less reactive. The hypothesis predicts an *inverse* relationship between the ventilatory- and CBF-responses to changes in Paco_2 .

A highly sensitive continuous wave near infrared spectrometer (NIRS, OXYMON) was used to determine cerebrovascular reactivity. Invasiveness was not necessary, as an intravascular tracer, $[\text{O}_2\text{Hb}]$, was used. By monitoring changes in $[\text{O}_2\text{Hb}]$ in response to changes in inspired oxygen, CBF and cerebral blood volume (CBV) could be measured and quantified (Chapter 1).

Prior to all experiments, the reproducibility and sensitivity to measure CBF and CBV using NIRS needed to be tested. In spite of improved technical properties in comparison to other NIRS-instruments, CBF could not be measured reproducibly under predefined well-controlled conditions in 27 subjects, as described in Chapter 2. Although the measured values of CBF were in the same range as measured by other investigators, the within subject coefficient of variation (CV) for CBF measurements was unacceptable high (22.3 to 92.4% (mean 42.4%). Considering methodological and physiological problems, the heart-rate limited frequency of the pulse oximetry remained an important limitation to the use of O_2Hb as an indicator. We abandoned CBF measurements with NIRS.

However, CBV could be measured reproducibly (mean within-subject CV 11.7%) in the same population (Chapter 3). As CBV is closely related to CBF, it is allowed to measure CBV instead of CBF under controlled circumstances. CBV ranged from 1.19 to 6.57 $\text{mL} \cdot 100\text{g}^{-1}$ between individuals. We adapted the initial research question from CBF into CBV measurements.

A study was performed to induce acute metabolic acid/base changes of $\geq 2 \text{ mEq.L}^{-1}$ change in base excess (BE) in the perspective of future investigations of respiratory parameters in these conditions (Chapter 4). Ammoniumchloride (NH_4Cl) was given for acidification, and furosemide for alkalization. Nine healthy volunteers ingested a calculated amount of NH_4Cl at $t=0$, and a repeated dose after 60 min. Eight healthy volunteers took 40 mg of furosemide. Arterialized capillary blood gases were measured at $t=0$, 30, 60, 90, 120 and 180 min. The aimed acidification for acute metabolic acidosis was attained after 30 min and reached the greatest change at 90 min ($\Delta\text{BE} -4.9 \pm 2.2 \text{ mEq.L}^{-1}$). In addition, the arterialized capillary PCO_2 (Pc_{CO_2}) was decreased by an average value of $-0.30 \pm 0.5 \text{ kPa}$, which was not significant. The aimed alkalization for acute metabolic alkalosis was seen between 120 and 180 min and reached the greatest change at 180 min ($\Delta\text{BE} +2.2 \pm 1.4 \text{ mEq.L}^{-1}$). A simultaneous average value of $\Delta\text{Pc}_{\text{CO}_2} -0.01 \pm 0.43 \text{ kPa}$ was measured, which was not significant compared with baseline. We concluded that NH_4Cl and furosemide induced a steady state of pure metabolic acid/base conditions in humans, that were buffered in an isocapnic way.

First, CBV responses to acute changes in PaCO_2 were measured in healthy subjects. Fifteen subjects, aged 21-54 (mean 30) years, were investigated during respiratory hyper- and hypocapnia under baseline metabolic conditions. We concluded that CBV responses to acute PaCO_2 changes in humans could adequately be assessed by NIRS: hypercapnia results in an increase of CBV, whereas hypocapnia decreases CBV. A linear relationship between CBV and PaCO_2 ($\text{CBV} = 1.01 * \text{Pc}_{\text{CO}_2} - 1.39$, $r=0.69$) (CBV: mL.100g^{-1} ; $\text{PaCO}_2: \text{kPa}$) was found. This was in agreement with other studies in human neonates using NIRS. The individual $\Delta\text{CBV}/\Delta\text{PaCO}_2$ reactivity for the hypercapnic and for the hypocapnic response, taking the normocapnic situation as a starting point was determined. Mean slopes in the hypercapnic and hypocapnic ranges were 2.60 ($1.32\text{--}3.88$) $\text{mL.100g}^{-1}.\text{kPa}^{-1}$ and 0.83 ($0.64\text{--}1.02$) $\text{mL.100g}^{-1}.\text{kPa}^{-1}$ (95% confidence interval), respectively ($p < 0.01$) (Chapter 5).

Apart from baseline metabolic acid-base conditions (Chapter 5), each healthy subject was also measured during acute metabolic acidosis (Chapter 6). A calculated dose of ammoniumchloride (NH_4Cl) was given, using the protocol as presented in Chapter 4. A mean $\Delta\text{BE} -2.7 \text{ mEq.L}^{-1}$ ($p < 0.001$) with a mean decreased Pc_{CO_2} of -0.2 kPa ($p < 0.001$) was attained. During normo-, hyper- and hypocapnia, CBV values (mL.100g^{-1}) of 3.51, 4.82 and 2.55 were calculated during baseline metabolic conditions. Corresponding CBV values yielded 3.70, 4.86 and 2.63 during acute metabolic acidosis. During metabolic acidosis, a linear relationship between CBV and PaCO_2 ($\text{CBV} = 1.05 * \text{PaCO}_2 - 1.29$ ($r=0.67$)) was found. The individual $\Delta\text{CBV}/\Delta\text{PaCO}_2$ reactivity for the hypercapnic and for the

hypocapnic response, taking the normocapnic situation as starting point, was determined. After NH_4Cl administration, mean hyper- and hypocapnic slopes were $2.07(1.39-2.76)$ and $0.72(0.413-1.034) \text{ mL} \cdot 100\text{g}^{-1} \cdot \text{kPa}^{-1}$ (95% confidence interval), respectively. This was not significantly different compared to baseline metabolic acid-base conditions.

We concluded that an acute metabolic acidosis, induced by oral administrated NH_4Cl , did not change absolute values of CBV, nor cerebrovascular reactivity to CO_2 .

Inspired minute ventilation (V'_I) was measured during normocapnia and hypercapnia (not hypocapnia) and related to CBV under neutral metabolic conditions and during metabolic acidosis in 15 healthy humans (Chapter 7). NH_4Cl was given orally to induce metabolic acidosis. The results of CBV were the same results as described in Chapter 6. During normocapnia, V'_I was 7.6 ± 1.4 and $10.0 \pm 2.4 \text{ L} \cdot \text{min}^{-1}$ ($p < 0.01$), under neutral metabolic conditions and during metabolic acidosis, respectively. The slopes of the CO_2 -responsiveness ($\Delta\text{CBV}/\Delta\text{PC}_{\text{CO}_2}$ and $\Delta V'_I/\Delta\text{PC}_{\text{CO}_2}$), were not significantly different during both metabolic conditions. A significant positive correlation between $\Delta\text{CBV}/\Delta\text{PC}_{\text{CO}_2}$ and $\Delta V'_I/\Delta\text{PC}_{\text{CO}_2}$ was found during metabolic acidosis ($r^2 = 0.51$, $p < 0.01$), but not under neutral metabolic acid-base conditions ($r^2 = 0.04$, $p = 0.52$). The control of CBV did not seem to be affected by intraluminal changes in pH. The hypothesis that a negative correlation exists between ventilatory and cerebrovascular CO_2 -responsiveness ($\Delta\text{CBV}/\Delta\text{PC}_{\text{CO}_2}$ and $\Delta V'_I/\Delta\text{PC}_{\text{CO}_2}$) during various metabolic conditions had to be refuted in normal subjects. The increased correlation between CBV changes and ventilatory changes during metabolic acidosis might be suggestive for similarities in CO_2 sensing mechanisms for cerebrovascular –as well as for ventilatory– control.

After completing the experiments with healthy subjects, measurements of COPD patients were started. Seventeen chronic hypercapnic ($\text{PaCO}_2 > 6.0 \text{ kPa}$) and sixteen normocapnic ($\text{PaCO}_2 \leq 6.0 \text{ kPa}$) COPD-patients, who were matched for degree of airway obstruction (FEV_1 27%pred) were measured and compared with 15 age-matched healthy subjects during rest and acutely induced respiratory hypercapnia (Chapter 8). CBV was related to V'_I and mouth occlusion pressure ($P_{0.1}$). In the control condition, CBV ($\text{mL } 100\text{g}^{-1}$) was 2.41 ± 0.66 and 2.90 ± 0.60 (mean \pm SD) in the normocapnic and chronic hypercapnic patients, respectively, which was significantly lower compared to healthy subjects (3.51 ± 0.77). All slopes of CO_2 -responsiveness ($\Delta\text{CBV}/\Delta\text{PaCO}_2$, $\Delta V'_I/\Delta\text{PaCO}_2$) were significantly lower in both COPD groups relative to healthy subjects, probably due to autonomic dysfunction in the COPD groups. Since similar $P_{0.1}$ reactivity ($\Delta P_{0.1}/\Delta\text{PaCO}_2$) was measured in both COPD groups, the present study was not able to elucidate why some patients with COPD become hypercapnic, whereas others with the same degree of airway obstruction

remain normocapnic. A poor, but positive correlation ($r \leq 0.52$) between ventilatory and cerebrovascular CO_2 -responsiveness ($\Delta\text{CBV}/\Delta\text{Paco}_2$ and $\Delta V'_I/\Delta\text{Paco}_2$) was found in both COPD groups and healthy subjects. Consequently, the difference in Paco_2 in the two COPD groups could not be explained by differences in cerebrovascular control.

The same groups of COPD patients were studied during chronic metabolic alkalosis and chronic metabolic acidosis, induced by one-week treatment with oral furosemide (40 mg, daily) and acetazolamide (500 mg, daily), respectively (Chapter 9). Compared to baseline metabolic conditions, neither chronic metabolic acidosis nor alkalosis changed ventilatory ($\Delta V'_I/\Delta\text{Paco}_2$) and cerebrovascular ($\Delta\text{CBV}/\Delta\text{Paco}_2$) reactivity in both COPD groups. Furthermore, there were no differences in reactivity between the hypercapnic and normocapnic COPD groups. Again poor, but positive correlations ($r \leq 0.58$) between ventilatory and cerebrovascular CO_2 -responsiveness ($\Delta\text{CBV}/\Delta\text{Paco}_2$ and $\Delta V'_I/\Delta\text{Paco}_2$, $\Delta\text{CBV}/\Delta\text{Paco}_2$ and $\Delta\text{P}_{0.1}/\Delta\text{Paco}_2$) were found irrespective of baseline, respiratory condition and metabolic acid-base state. Thus it seems that low or high CO_2 -responsiveness is a generalised characteristic of the individual patient. This is seen in chemosensitivity of the ventilatory system, as well as in the cerebral vasomotor responses.

In conclusion, the hypothesis that a *negative* correlation exists between ventilatory and cerebrovascular CO_2 -responsiveness had to be rejected and the present study did not show abnormal cerebrovascular responses to CO_2 in hypercapnic COPD-patients.

CHAPTER 11

Samenvatting en conclusies

Sommige patiënten met een ernstig chronisch obstructief longlijden (COPD) hebben een normaal ademminuutvolume en een normaal koolzuurgehalte (CO_2) van het arteriele bloed (chronische normocapnie), andere patiënten ademen te weinig waardoor ze een verhoogd CO_2 gehalte (chronische hypercapnie) in het bloed hebben. Er zijn aanwijzingen dat beide groepen verschillen in de mate waarin de ademhaling wordt gestimuleerd vanuit de hersenen. Hierbij is de zuurgraad van de hersenvloeistof rond de centrale chemoreceptoren bepalend, welke wordt beïnvloed door de hersendoorbloeding (CBF). De mate van doorbloeding wordt bepaald door het CO_2 gehalte en/of de zuurgraad van de hersenvloeistof: hoe zuurder, hoe sterker de doorbloeding en hoe sneller het overtollige zuur wordt afgevoerd. Veranderingen van CBF beïnvloeden de relatie tussen het arteriele CO_2 (PaCO_2) gehalte van het bloed (wat meetbaar is) en de CO_2 spanning van het hersenweefsel, daar waar de centrale chemoreceptoren zijn gelokaliseerd (niet meetbaar, de "echte" stimulus). Simultane metingen van de reactiviteit van de ademhaling en CBF op acute CO_2 -verhoging kunnen derhalve belangrijk zijn om meer te weten te komen over de mechanismen van hypercapnie of normocapnie bij COPD patiënten.

De volgende hypothese werd getoetst: COPD-patiënten met een chronische hypercapnie hebben een sterkere reactie van de CBF op veranderingen in het CO_2 gehalte. Een lichte toename van de hoeveelheid CO_2 in de hersenvloeistof zou resulteren in een versterkte doorbloeding, leidend tot afvoer van CO_2 uit de hersenen. Hierdoor blijft de zuurgraad van de hersenvloeistof rond de centrale chemoreceptoren relatief constant, en blijft de prikkel voor de ademhaling laag. Hierdoor blijft er in de rest van het lichaam een verhoogd CO_2 gehalte van het bloed. Dus hoe groter het vermogen om de CBF te regelen, hoe lager de prikkel voor de ademhaling zal zijn. In COPD-patiënten met een chronische normocapnie zou de CBF minder sterk op een CO_2 toename reageren, wat zou resulteren in een verhoogde ademhalingsprikkel om zo het CO_2 in het bloed te verlagen. Er zou dus een *omgekeerde* relatie kunnen bestaan tussen de ventilatoire en cerebrovasculaire CO_2 reactiviteit?

Near Infrared Spectroscopy (NIRS) meet met behulp van (bijna)infrarood laserlicht op een onbloedige wijze de CBF en het bloedvolume van de hersenen (CBV), waarbij gebruik wordt gemaakt van een lichaamseigen intravasculaire tracer, O_2Hb . Veranderingen van ingeademde zuurstof (O_2) leiden tot veranderingen van O_2Hb ; hierdoor kan CBF en CBV worden gemeten en berekend (Hoofdstuk 1).

Voordat de hypothese kon worden getoetst, moest eerst de reproduceerbaarheid en gevoeligheid van de NIRS-metingen van CBF en CBV worden bepaald. Bij 27 mensen werd de CBF gemeten onder gestandaardiseerde omstandigheden (Hoofdstuk 2);

Alhoewel de absolute waarden van de CBF overeenkwamen met waarden van andere onderzoekers, bleek de reproduceerbaarheid slecht, met een variatie coëfficiënt van 22.3 tot 92.4% (gemiddeld 42.4%). De begrensde (cardiale) frequentie van de saturatie meter bleek de belangrijkste oorzaak van de slechte reproduceerbaarheid, wanneer O_2Hb als intravasculaire tracer werd gebruikt. Ondanks het onderzochte, zeer sensitieve karakter van de NIRS bleek deze niet bruikbaar om reproduceerbare onbloedige CBF metingen mee te verrichten.

CBV kon wel reproduceerbaar worden gemeten (gemiddelde variatie coëfficiënt 11.7%) in dezelfde onderzoeksgroep (Hoofdstuk 3). CBV is nauw gerelateerd aan CBF; derhalve is het toegestaan om CBV in plaats van CBF te meten onder gecontroleerde omstandigheden. De absolute waarde van CBV varieerde van 1.19 tot 6.57 mL.100g⁻¹ in de onderzoeksgroep.

We pasten onze initiële onderzoeksvraag aan en onderzochten CBV i.p.v. CBF.

Een volgend onderzoek had als doel een acute metabole zuur/base verandering van ≥ 2 mEq.L⁻¹ van base-excess (ΔBE) te bewerkstelligen om later metingen van CBV- en ademhalings-parameters tijdens deze metabole condities te kunnen doen (Hoofdstuk 4). Ammoniumchloride (NH_4Cl) werd gebruikt voor het induceren van metabole acidose, furosemide voor inductie van metabole alkalose. Negen gezonde vrijwilligers werden gevraagd om een berekende dosis NH_4Cl op tijdstip $t=0$, en een zelfde dosis op tijdstip $t=60$ min. in te nemen. Acht vrijwilligers namen 40 mg furosemide in op $t=0$. Capillaire bloedgasen werden gemeten op $t=0$, 30, 60, 90, 120 en 180 min. De gewenste verzuring voor acute metabole acidose was bereikt na 30 min. en bereikte een maximale verzuring op 90 min ($\Delta BE -4.9 \pm 2.2$ mEq.L⁻¹). Hierbij daalde de capillaire PCO_2 (PC_{CO_2}) gemiddeld -0.30 ± 0.5 kPa (niet significant). De gewenste alkalose werd gezien tussen 120 en 180 min. en bereikte een maximale metabole alkalose na 180 min. ($\Delta BE +2.2 \pm 1.4$ mEq.L⁻¹). De corresponderende ΔPC_{CO_2} was -0.01 ± 0.43 kPa (niet significant). Wij concludeerden dat NH_4Cl en furosemide een steady state van metabole zuur/base condities in gezonde vrijwilligers kon induceren, zonder significante CO_2 veranderingen.

De CBV responsen op acute PCO_2 -veranderingen werden gemeten in vijftien gezonde proefpersonen, variërend in leeftijd van 21 tot 54 (gemiddeld 30) jaar. Zij werden onderzocht tijdens respiratoire hyper- en hypocapnie onder basale metabole condities. Hypercapnie werd geïnduceerd door het toedienen van CO_2 in de inademiingslucht; hypocapnie werd geïnduceerd door de ademhalingsfrequentie te verdubbelen (hyperventilatie) of de ademteugen te vergroten. De conclusie van deze metingen liet zien dat CBV veranderingen als gevolg van acute $Paco_2$ veranderingen ($\Delta CBV/\Delta Paco_2$)

door NIRS reproduceerbaar kon worden gemeten: een hypercapnie resulteerde in een toename van CBV, en een hypocapnie in een afname. Een lineaire relatie tussen CBV en P_{aCO_2} ($CBV = 1.01 * P_{aCO_2} - 1.39$, $r = 0.69$) (CBV : $mL \cdot 100g^{-1}$; P_{aCO_2} : kPa) werd gevonden. De resultaten van deze reactiviteit waren gelijk aan bekende resultaten uit de literatuur, gemeten bij neonaten. Als CBV responsen werden opgesplitst van normo- naar hypercapnie en van normo- naar hypocapnie, werd een lineaire relatie gevonden met significant verschillende hellingen; van normo- naar hypercapnie werd een helling gevonden van 2.60 ($1.32-3.88$) $mL \cdot 100g^{-1} \cdot kPa^{-1}$ en van normo- naar hypocapnie van 0.83 ($0.64-1.02$) $mL \cdot 100g^{-1} \cdot kPa^{-1}$ (95% betrouwbaarheidsinterval) ($p < 0.01$) (Hoofdstuk 5).

Behalve tijdens rust onder basale zuur-base metabole condities (Hoofdstuk 5), werd iedere gezonde vrijwilliger gemeten tijdens acute metabole acidose na inname van NH_4Cl , met een protocol zoals beschreven in Hoofdstuk 4. In deze groep werd een metabole acidose geïnduceerd met een gemiddelde daling van BE van 2.7 $mEq \cdot L^{-1}$ ($p < 0.001$) en een (nu wel) gedaalde capillaire PCO_2 (P_{cCO_2}) van -0.2 kPa ($p < 0.001$). Tijdens normo-, hyper- en hypocapnia, CBV waarden ($mL \cdot 100g^{-1}$) van 3.51 , 4.82 and 2.55 werden gemeten. Tijdens acute metabole acidose waren deze respectievelijk 3.70 , 4.86 and 2.63 (niet significant). Verder werd een lineaire relatie tussen CBV en P_{aCO_2} ($CBV = 1.05 * P_{aCO_2} - 1.29$ ($r = 0.67$)) gevonden. Afzonderlijke evaluaties van CBV reactiviteit van normo- naar hypercapnie en van normo- naar hypocapnie ($\Delta CBV / \Delta P_{aCO_2}$) lieten waarden zien van 2.07 ($1.39-2.76$) and 0.72 ($0.413-1.034$) $mL \cdot 100g^{-1} \cdot kPa^{-1}$ (95% betrouwbaarheidsinterval) tijdens acute metabole acidose. Dit was niet significant anders dan metingen tijdens basale zuur-base metabole condities. Concluderend liet een acute metabole acidose, door NH_4Cl geïnduceerd, geen verandering zien van zowel de absolute waarde van CBV als wel de CBV reactiviteit ($\Delta CBV / \Delta P_{aCO_2}$) (Hoofdstuk 6).

Het ingeademde ademminuutvolume (V'_I) werd gemeten tijdens normocapnie en hypercapnie (geen hypocapnie) en gerelateerd aan CBV tijdens basale zuur-base condities en tijdens acute metabole acidose bij 15 gezonde vrijwilligers (Hoofdstuk 7). NH_4Cl werd gegeven, om een acute metabole acidose te verkrijgen. De resultaten van CBV waren deels overlappend met resultaten beschreven in Hoofdstuk 6. Tijdens normocapnie was V'_I 7.6 ± 1.4 $L \cdot min^{-1}$ tijdens basale metabole condities, en 10.0 ± 2.4 $L \cdot min^{-1}$ ($p < 0.01$) tijdens metabole acidose. De hellingen van CO_2 reactiviteit ($\Delta CBV / \Delta P_{cCO_2}$ en $\Delta V'_I / \Delta P_{cCO_2}$) waren niet verschillend tijdens beide metabole condities. Een significante positieve relatie werd gevonden tussen $\Delta CBV / \Delta P_{cCO_2}$ en $\Delta V'_I / \Delta P_{cCO_2}$ tijdens metabole acidosis ($r^2 = 0.51$, $p < 0.01$), maar niet tijdens basale zuur-base condities ($r^2 = 0.04$, $p = 0.52$). CBV leek niet te worden beïnvloed door intraluminale veranderingen

van zuurgraad (pH). De hypothese, dat er een negatieve correlatie bestaat tussen ventilatoire en cerebrovasculaire CO_2 reactiviteit moest bij gezonde proefpersonen verworpen worden.

Zeventien chronische hypercapnische ($\text{Paco}_2 > 6.0 \text{ kPa}$) en 16 normocapnische COPD patiënten ($\text{Paco}_2 \leq 6.0 \text{ kPa}$) met een vergelijkbare ernstige luchtwegvernauwing (FEV_1 27% van voorspeld), werden gemeten en vergeleken met 15 gezonde vrijwilligers van dezelfde leeftijd (Hoofdstuk 8). In rust werd een gemiddelde CBV waarde van $2.90 \text{ mL} \cdot 100\text{g}^{-1}$ bij de eerstgenoemde patiëntengroep en $2.41 \text{ mL} \cdot 100\text{g}^{-1}$ bij de tweede COPD groep gemeten, wat in beide groepen significant lager was dan bij de gezonde vrijwilligers ($3.51 \text{ mL} \cdot 100\text{g}^{-1}$). De metingen werden herhaald, nadat CO_2 in de inademenslucht werd toegediend (acute hypercapnie). Veranderingen van CBV ($\Delta\text{CBV}/\Delta\text{Paco}_2$) werden gecorreleerd aan veranderingen van V'_1 ($\Delta V'_1 / \Delta\text{Paco}_2$) en mond occlusie drukken ($P_{0.1}$, $\Delta P_{0.1}/\Delta\text{Paco}_2$). Hellingen van CO_2 -reactiviteit ($\Delta\text{CBV}/\Delta\text{Paco}_2$, $\Delta V'_1 / \Delta\text{Paco}_2$) waren significant lager in beide COPD groepen t.o.v. de gezonde vrijwilligers, mogelijk als gevolg van autonome dysfunctie in beide COPD groepen. In beide COPD groepen werd een gelijke $P_{0.1}$ reactiviteit ($\Delta P_{0.1}/\Delta\text{Paco}_2$) gemeten; derhalve kan deze studie niet verklaren waarom sommige COPD patiënten chronisch hypercapnisch worden en sommigen, ondanks *dezelfde* mate van luchtwegobstructie, niet. Een slechte, maar positieve correlatie ($r \leq 0.52$) tussen ventilatoire en cerebrovasculaire CO_2 -reactiviteit ($\Delta\text{CBV}/\Delta\text{Paco}_2$ and $\Delta V'_1 / \Delta\text{Paco}_2$) werd gevonden bij beide COPD groepen en gezonde vrijwilligers.

Bij dezelfde COPD patiënten werden de metingen herhaald tijdens chronische metabole acidose en chronische metabole alkalose en vergeleken met hun uitgangs ("basis"-) zuurgraad (Hoofdstuk 9). De chronische metabole verzuring werd verkregen door dagelijks acetazolamide (500 mg) in te nemen gedurende één week; een chronische metabole alkalose werd verkregen door dagelijks furosemide (40 mg) in te nemen, eveneens gedurende één week. Tijdens de geïnduceerde chronische metabole acidose en alkalose werden vergelijkbare, niet significant verschillende, resultaten gezien van de reactiviteit van CBV en V'_1 , als tijdens de uitgangs zuurgraad. Verder werd er geen verschil gezien in reactiviteit tussen de beide COPD groepen. Er werd wederom een slechte, maar positieve correlatie gevonden ($r \leq 0.58$) tussen de cerebrovasculaire en ventilatoire en CO_2 -reactiviteit ($\Delta\text{CBV}/\Delta\text{Paco}_2$ and $\Delta V'_1 / \Delta\text{Paco}_2$, $\Delta\text{CBV}/\Delta\text{Paco}_2$ and $\Delta P_{0.1}/\Delta\text{Paco}_2$), welke onafhankelijk was van de uitgangssituatie van (respiratoire) arteriële CO_2 conditie en geïnduceerde metabole omstandigheden. Mogelijk bestaat er in aanleg een lage of hoge CO_2 gevoeligheid, welke individueel bepaald is. Dit is zichtbaar als specifieke

individuele CO₂ gevoeligheid voor ventilatoire reactiviteit ($\Delta V'_I / \Delta \text{PaCO}_2$) (chemoreceptoren) en cerebrale vasomotore reactiviteit ($\Delta \text{CBV} / \Delta \text{PaCO}_2$).

Concluderend is de regulatie van de ademhaling en het bloedvolume van de hersenen niet verschillend bij COPD patiënten met een chronische hypercapnie in vergelijking met patiënten met een chronische normocapnie. De hypothese, dat er een *negatieve* correlatie bestaat tussen ventilatoire en cerebrovasculaire CO₂ reactiviteit, dient te worden verworpen.

List of Abbreviations

ACET	Acetazolamide
BBB	blood brain barrier
BE	base excess
CBF	cerebral blood flow
CBV	cerebral blood volume
COPD	chronic obstructive pulmonary disease
ECF	extracellular fluid
FEV ₁	forced expiratory volume in one second
fr	respiratory frequency
fH	cardiac frequency
F _{IO₂}	fraction of oxygen in inspiratory gas
[HHb]	concentration of deoxyhemoglobin
MIP	maximal inspiratory pressure
MVV	maximal voluntary ventilation
NIRS	near infrared spectroscopy
MABP	mean arterial blood pressure
NH ₄ Cl	ammoniumchloride
[O ₂ Hb]	concentration of oxyhemoglobin
OD	optical density
OI	oxygenation index
P _{0.1}	mouth occlusion pressure
PaCO ₂	partial carbon dioxide pressure in arterial blood
PcCO ₂	partial carbon dioxide pressure in arterialized capillary blood
PETCO ₂	partial carbon dioxide pressure in the end tidal expiratory air
PO ₂	partial oxygen pressure in arterial blood
%pred	per cent of predicted value
SaO ₂	arterial oxygen saturation
SD	standard deviation
[tHb]	concentration of total hemoglobin
V _I or V _I	inspired minute ventilation
V _T	tidal volume

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Curriculum Vitae

MJT (Marjo) van de Ven, de auteur van dit proefschrift werd op 7 oktober 1962 geboren te Eindhoven. In 1981 behaalde zij het diploma V.W.O.B aan het Anton van Duinkerkencollege te Veldhoven. Van 1981 tot 1988 studeerde zij geneeskunde aan de Katholieke Universiteit te Nijmegen. De eerste wetenschappelijke ervaring was in (het toenmalige West-) Berlijn op het gebied van kinderoncologie (Universitätskinderklinik Berlin, Prof. Dr. G. Henze, 1996). Aansluitend aan haar artsexamen werkte ze een half jaar als assistent niet-in-opleiding op de afdeling Keel- Neus- en Oorheelkunde, Academisch Ziekenhuis Nijmegen, St. Radboud (hoofd: Prof. Dr. P. van den Broek).

In 1989 startte zij de opleiding tot internist, eveneens in het Academisch Ziekenhuis Nijmegen, St. Radboud (opleiders Prof. Dr. A. van 't Laar en Prof. Dr. J.W.M. van der Meer) en werd zij in 1995 geregistreerd als internist.

Vanaf het laatste jaar van de opleiding tot internist (1994) startten haar werkzaamheden als arts-assistent longziekten in het Universitair Longcentrum Dekkerswald, te Groesbeek, waar per 1 december 1996 de opleiding tot longarts officieel begon (opleider: Prof. Dr. C.L.A. van Herwaarden).

Deze opleiding werd tijdelijk onderbroken door het onderzoek, dat in dit proefschrift is beschreven. Het werd ten dele uitgevoerd op de afdeling Fysiologie, Faculteit Medische Wetenschappen, Universiteit Nijmegen (hoofd: Prof. Dr. B. Oeseburg) en ten dele op de afdeling Longfunctie (hoofd: Prof. Dr. H. Folgering) van het Universitair Longcentrum Dekkerswald. Het laatste jaar van haar opleiding (1 mei 2000 tot 1 mei 2001) werkte zij grotendeels in het Academisch Ziekenhuis Nijmegen, St. Radboud. Vanaf 1998 tot 2001 was zij voorzitter van de Sectie Assistenten van de Nederlandse Vereniging voor artsen van Longziekten en Tuberculose (NVALT).

Vanaf 1 mei 2001 werkt zij als longarts in het Rijnstate Ziekenhuis te Arnhem en het Streekziekenhuis Zevenaar.

Zij is gehuwd met Bart van den Berg. Samen hebben zij twee kinderen: Maike (1993) en Karlijn (1996).

STELLINGEN

behorende bij het proefschrift

Cerebral blood volume and ventilation in severe COPD

M.J.T. van de Ven

11 december 2001

1. Door metingen van het cerebrale bloed volume kan men niet verklaren waarom sommige COPD patienten chronisch hypercapnisch worden en sommigen, ondanks dezelfde mate van luchtwegobstructie, niet (*Dit proefschrift*).
2. De cerebrale bloed flow kan (momenteel) niet reproduceerbaar met Near Infrared Spectroscopie worden gemeten; het cerebrale bloed volume wel (*Dit proefschrift*).
3. Er is een slechte (maar positieve) correlatie tussen de cerebrovasculaire en ventilatoire CO₂-reactiviteit, welke onafhankelijk is van de uitgangssituatie van respiratoire arteriele CO₂ conditie (*Dit proefschrift*).
4. De invloed van acute respiratoire hypercapnie moet invasief worden gecontroleerd bij ernstige COPD patienten ten gevolge van grote dode ruimte ventilatie (*Eigen waarneming*).
5. Het Gamma assortiment is onontbeerlijk bij het in elkaar knutselen van een proefopstelling (*Eigen waarneming*).
6. De waarheid is de som van gedeelde percepties.
7. Medische specialisten zouden tijdens hun opleiding meer over management moeten leren als ze later geacht worden dit te beheersen.
8. Je best doen is niet altijd genoeg.
9. Ik zou het vermogen om ergens vreugde uit te halen willen voorschrijven als middel tegen depressie.
10. Boerendochters hebben minder kans op allergie en atopie ("hygiene hypothese", o.a. Braun-Fahrlander et al, *Clin Exp Allergy* 1999).

