Carbon dioxide induced changes in cerebral blood flow and flow velocity: role of cerebrovascular resistance and effective cerebral perfusion pressure.

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German Clinical Trials Register (DRKS00011535).

This article is dedicated in memoriam to our teacher, colleague, and friend Professor Hans Sonntag, who considerably contributed to the concept of this study and died in 2011.
ABSTRACT

**Background:** In addition to cerebrovascular resistance (CVR) zero flow pressure (ZFP), effective cerebral perfusion pressure (CPPe) and the resistance-area-product (RAP) are supplemental determinants of cerebral blood flow (CBF). Until now, the interrelationship of PaCO₂-induced changes in CBF, CVR, CPPe, ZFP, and RAP is not fully understood.

**Materials and methods:** In a controlled crossover trial, we investigated 10 anesthetized patients aiming at PaCO2-levels of 30, 37, 43 and 50 mmHg. CBF was measured with a modified Kety-Schmidt-technique. ZFP and RAP was estimated by linear regression analysis of pressure-flow velocity relationships of the middle cerebral artery. CPPe was calculated as the difference between mean arterial pressure and ZFP, CVR as the ratio CPPe/CBF. Statistical analysis was done by one-way RM-ANOVA.

**Results:** When comparing hypocapnia with hypercapnia CBF showed a significant exponential reduction by 55% and mean VMCA by 41%. CPPe linearly decreased by 17% while ZFP increased from 14 to 29 mmHg. CVR increased by 96% and RAP by 39%; despite these concordant changes in mean CVR and Doppler-derived RAP correlation between these variables was weak (r=0.43).

**Conclusion:** Under general anesthesia hypocapnia-induced reduction in CBF is caused by both, an increase in CVR and a decrease in CPPe, as a consequence of an increase in ZFP.

**Keywords:** carbon dioxide/physiology, cerebrovascular circulation/physiology, resistance, cerebrovascular, ultrasonography, Doppler, transcranial, velocity, blood flow
INTRODUCTION

Arterial partial pressure of carbon dioxide (PaCO₂) is well known to change global cerebral blood flow (CBF) and the flow velocity of the middle cerebral artery (V_{MCA}), which can be explained by an increase or decrease of cerebral vascular resistance (CVR) and partly by cerebral perfusion pressure (CPP). Over a range of PaCO₂ values of 20-80 mmHg for each mmHg increase or decrease in PaCO₂ there is a 2-4% change in CBF or V_{MCA}. However, it is still unclear which of both determinants is more affected by hyperventilation and hypoventilation: CPP, CVR, or both. Both variables are thought to be influenced by arteriolar smooth muscle tone and therefore these variables can be expected to change simultaneously when PaCO₂ varies.

When calculating the CPP, the mean arterial pressure (MAP) is commonly used as effective upstream pressure (EUP) and the intracranial pressure (ICP) as effective downstream pressure (EDP) of the cerebral circulation, because of a Starling resistor phenomenon located at the level of cerebral veins. However, another major component of the effective downstream pressure is the critical closing pressure (CCP), which cannot be directly measured in patients with spontaneous circulation. It has been proven that in vivo pressure-flow relationships are linear for many vascular beds, including the cerebral vessels. Since the introduction of transcranial Doppler ultrasonography (TCD), a number of methods have been developed to assess CCP by extrapolating instantaneous pressure-flow-velocity plots (ABP/V_{MCA}) to zero or by calculation of the intercept of diastolic, mean and/or systolic values of pressures and flow-velocity.

A number of factors other than vascular tone, i.e. central venous pressure (CVP) and ICP, can also influence the EDP in the cerebral circulation. Consequently, the use of the term ‘zero flow pressure’ (ZFP) is considered more appropriate than CCP. It is suggested that, in patients without increased ICP, ZFP is determined by arteriolar tone. The effective cerebral perfusion pressure (CPPe) is defined by the difference between MAP and ZFP. The inverse of the slope of the ABP - V_{MCA} plot is named the ‘resistance area product’ (RAP). In several investigations the RAP has been used as index of CVR.

Recent studies could demonstrate that ZFP varies inversely with changes of PaCO₂. Furthermore, hypocapnia reduced CBF by decreasing CPPE due to the increase in ZFP, whereas the RAP was unaffected. In contrast, McCulloch and colleagues could demonstrate that hyperventilation significantly increases RAP, which seemed to have a greater effect on V_{MCA} than the increase in ZFP.

It is still unclear, which of both components is more affected by hyperventilation and hypoventilation. Describing the pressure-flow (velocity) relationship by a single parameter (CVR) can mislead interpretation and blunt key relationships. As an extreme case, it is possible to have repeated pressure-flow velocity measurements indicating a constant...
estimation of \( CVR = \frac{ABP}{CBF} \) or \( ABP/V_{MCA} \), when in fact there had been changes in both, ZFP and RAP.\(^6\)

Up to now there is no investigation that combines measurements of CBF and \( V_{MCA} \) in order to compare changes of the cerebrovascular \( CO_2 \)-reactivity (\( CO_2-R \)) of global CBF and of \( V_{MCA} \). Similarly, reference calculations of CVR, based on quantitative CBF measurements and calculation of CPPe by determination of ZFP have not yet been compared to changes in RAP. Therefore, we investigated the effects of variation in \( PaCO_2 \) on CBF, \( V_{MCA} \), CPPe, ZFP, RAP and CVR in patients under intravenous anesthesia.

**MATERIAL AND METHODS**

**Design**

The prospective study was designed and performed in a controlled, crossover design at the University of Göttingen Medical Center. The main purpose of this study was to evaluate the cerebrovascular reactivity induced by variations in arterial partial pressure of carbon dioxide in anesthetized patients examined by a combination of two cerebral measurements techniques: transcranial Doppler Sonography and Kety-Schmidt technique. Each patient served as his own control. The study followed the recommendations of the Declarations of Helsinki from 1975-1989 and the European Union Commission and European Medicines Agency (Council Directive 91/507/EEC and 75/318/EEC). Approval was obtained from the local institutional review board (Medical Ethical Committee of the Georg-August-University of Göttingen, Göttingen, Niedersachsen, Germany; No. 07/09/90).

**Endpoints**

The endpoints of the trial were changes in CBF, \( V_{MCA} \), CPPe, ZFP, CVR and RAP.

**Screening, inclusion and informed consent of patients**

Due to logistic reasons we could perform only 1-2 measurements per month. Therefore, standard-screening procedures could not be applied in this crossover trial. Patients were eligible for inclusion if scheduled for elective coronary surgery. Exclusion criteria were being older than 80 years of age, female gender, patient refusal, active neurological disease, and a history of cerebrovascular disease, brain injury, or intracranial surgery. All patients were informed of the purpose of the study and provided written informed consent before being enrolled. None of the eligible patients refused inclusion of the trial. There were no dropouts during the study period.
Sample size calculation

The inter-subject and intra-subject variability of CBF, CPP and CVR has been reported in earlier studies.\textsuperscript{12-15} There is paucity of data of CBF and \(V_{\text{MCA}}\) in humans under general anesthesia, which was necessary for an exact sample size calculation for this crossover trial. We expected a 50\% difference of CBF with an estimated effect size of 0.7-0.8. For a statistical power of 0.8-0.9 the sample size had to be between 7-10 patients. Therefore we projected a sample size of 10 patients.

Anesthesia Procedure

Individual medications were continued until surgery. Intravenous anesthesia was induced and maintained by continuous intravenous administration fentanyl, midazolam and pancuronium. The anesthesia procedure, the details of mechanical ventilation and the methods of catheter insertion have been described in previous reports in detail.\textsuperscript{14,16}

Measurements

CBF was measured using the modified Kety-Schmidt inert gas saturation technique with argon as a tracer gas as described in detail in earlier reports.\textsuperscript{14,16} Blood flow velocity in the proximal (M1) segment of the middle cerebral artery (\(V_{\text{MCA}}\)) was measured by TCD as extensively described in earlier reports.\textsuperscript{5,14} Because TCD measurements of \(V_{\text{MCA}}\) from the transtemporal window fail with above average incidence in elderly female patients we included only male patients in this study.\textsuperscript{17} Arterial blood pressure was measured invasively in the radial artery, isspilateral to the TCD probe.

Measurements were performed aiming at PaCO\(_2\)-levels of A) hyperventilation (30mmHg), B) normoventilation I (37mmHg), C) normoventilation II (43mmHg) and D) hypoventilation (50 mmHg) before surgery. All measurements were performed during hemodynamic and respiratory steady-state conditions. The time interval between the measurements was 20 min. Blood samples were drawn twice, at the beginning and end of each argon wash-in period, to measure hemoglobin concentration and variables of blood gas analysis (ABL; Radiometer, Copenhagen, Denmark). For comparisons with CBF measurements, \(V_{\text{MCA}}\) was averaged over the 10 min period of each argon wash-in maneuver. End-expiratory CO\(_2\) concentrations were continuously recorded to ensure a stable PaCO\(_2\) during argon saturation.

Calculations

The analyses of the ZFP, CPPe and RAP have been performed after the study period. Cerebral ZFP was calculated from data at the beginning and end of each CBF measurement from two simultaneous 10-s recordings (two breathing cycles) of the \(V_{\text{MCA}}\) envelope and arterial pressure curves. Over each 10-s period we first averaged consecutive pairs of diastolic, mean and systolic data of ABP and \(V_{\text{MCA}}\). These data were used in a pres-
sure/flow velocity plot. ZFP was then extrapolated by linear regression analysis of the ABP-$V_{\text{MCA}}$ relationship. A correction of the time delay between ABP and $V_{\text{MCA}}$ signal was not necessary. The ABP axis intercept of the regression line determines the ZFP. The cerebral ZFP was used as a measure of the effective downstream pressure of the cerebral circulation. In the relationship between ABP and $V_{\text{MCA}}$, the RAP is defined as the inverse slope of their linear regression line.

Formulae to calculate CPPe, CVR, and RAP are shown in table 1. The calculation of the carbon dioxide-reactivity was based on the ratio of the individual changes of CBF and PaCO$_2$. To ensure a better comparability with previous studies, we compared CBF and $V_{\text{MCA}}$-values of hyperventilation (A) and hypoventilation (D) with standardized normocapnic CBF and $V_{\text{MCA}}$-values, extrapolated by regression analysis for each patient at a PaCO$_2$-level of 40mmHg. This calculation of CO$_2$-reactivity indicates of the percentage of the changes in CBF and $V_{\text{MCA}}$ per mmHg, which is relatively independent of the particular flow levels and the PaCO$_2$ values.

**Table 1. Formulae to calculate CPPe, CVR, RAP, and CO$_2$-R**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formula</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective cerebral perfusion pressure (CPPe)</td>
<td>$\text{CPPe} = \text{MAP} - \text{ZFP}$</td>
<td>mm Hg</td>
</tr>
<tr>
<td>Cerebral vascular resistance (CVR)</td>
<td>$\text{CVR} = \text{CPPe}/\text{CBF}$</td>
<td>mm Hg/ml/100 g/min</td>
</tr>
<tr>
<td>Resistance area product (RAP)</td>
<td>$\text{RAP} = \text{inverse slope of the relationship of pressure/flow velocity}_{\text{MCA}}$</td>
<td>mm Hg $\times$ s/cm</td>
</tr>
<tr>
<td>Relative carbon dioxide reactivity (CO$_2$-R)</td>
<td>$\text{CO}_2\text{-R} = (\text{parameter } D - A)/\text{parameter extrapolated for normocapnia} / (\text{Pa CO}_2 D - A) \times 100$</td>
<td>%/mm Hg</td>
</tr>
</tbody>
</table>

Abbreviations: MAP, mean arterial pressure; $V_{\text{MCA}}$, cerebral flow velocity of the middle cerebral artery; ZFP, zero flow pressure.

**Statistical Analysis**

The results presented in tables are expressed as mean (standard deviation) unless otherwise stated. Tests for normal distribution of data were done with the D’Agostino-Pearson omnibus K2 method.

The differences between the ventilation steps were assessed using one-way analysis of variance for repeated measures (RM-ANOVA). Multi-comparison analyses between measurements have been done with Holm-Šídák post hoc tests.

The differences between D (hypoventilation) and A (hyperventilation), C (normoventilation II) and A (hyperventilation) as well as B (normoventilation I) and D (hypoventilation) were calculated using t-tests for paired data or Welch-test and non-parametric Wilcoxon signed-rank test, if indicated. To provide an estimate of the effect of changes in PaCO$_2$ levels and their clinical meaningfulness, we calculated mean differences (MD) and their 95% confidence intervals (MD; 95% CI upper bound, lower bound; $P$-value).
order to prevent from type I error, all primary endpoints were tested by one-way ANOVA for repeated measurements followed by Bonferoni multiple comparison tests. All statistical analysis were performed two-sided and a p-value of p<0.05 was considered to be significant. Database sheets were done by MS Excel® for Mac 2011 (Microsoft, Redmond, Washington, USA). Statistical procedures and graphs were made using Prism 6.0 (GraphPad Software, La Jolla, California, USA). Sample size calculation was done with G*Power (University of Düsseldorf, Dept. of Psychology, Düsseldorf, Germany).

RESULTS

The study period was 11 months (February 20th 1991 until January 28th 1992). A total of 10 male patients were included in the study. The mean age of the patients was 56(6) yrs. (median 58, range 48-65 yrs.), mean height 173(6) cm, and mean body weight 77(9) kg. The variability of the four target levels of PaCO2 was small due to the controlled adjustment of mechanical ventilation. Levels of hemoglobin, partial oxygen pressure, blood viscosity and blood temperature were kept constant during the measurements. Cardiac index and stroke volume index changed slightly, which was clinically not relevant. The controlled reduction in PaCO2 caused a small increase of MAP, which could be related to a significant small increase of systemic vascular resistance.

The mean TCD-insonation depth of the MCA was 51(3) mm. TCD signals were of high quality in all patients except one measurement: during the second 10s recording of VMCA and ABP of patient 5 at PaCO2 of 37mmHg showed ABP artifacts. The calculation of the ZFP was thus based on the values at the beginning of the CBF measurement. Hemodynamic and basic metabolic data are presented in table 2. Results of absolute CO2 reactivity of CBF and VMCA are presented in table 3 for the different steps of PaCO2-variation as well in figure 1A and 1B as exponential function.

The effects of ventilatory changes on the cerebral circulation were substantial. Hyperventilation (A) exponentially reduced CBF, in total by 55%, and VMCA by 41%, when compared with hypoventilation (D). Compared to CBF, the exponential decrease of mean VMCA due to changes in PaCO2 was less pronounced (figure 1A, 1B, table 2 and 3A). The decrease in CBF and mean VMCA due to hyperventilation was interrelated to an increase of CVR by 96% (figure 1E, table 2 and 3A) and a decrease of CPPe by 17% (figure 1C, table 2 and 3A). As expected, changing PaCO2 levels from hypoventilation to hyperventilation lead to small increase of the MAP by 7.5%, however post-hoc comparison analysis between measurement (D) and (A) did not reach level of significance (MD 5 (8) mmHg, P=0.25). The CPPe decrease was thus primarily caused by an evident linear increase of ZFP by 15(6) mmHg (figure 1C and 1D, table 2, and table 3A). Hyperventilation (A) increased RAP only by 39%, when compared with hypoventilation (D) (figure
### Table 2. Hemodynamic and metabolic variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dimension</th>
<th>PaCO₂ level</th>
<th>ANOVA</th>
<th>MC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>mm Hg</td>
<td>29</td>
<td>37</td>
<td>44</td>
</tr>
<tr>
<td>CBF&lt;sup&gt;a&lt;/sup&gt;</td>
<td>mL/100 g/min</td>
<td>31</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td>V&lt;sub&gt;MCA&lt;/sub&gt; mean&lt;sup&gt;a&lt;/sup&gt;</td>
<td>cm/s</td>
<td>31</td>
<td>34</td>
<td>40</td>
</tr>
<tr>
<td>ZFP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>mm Hg</td>
<td>29</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>CPPe&lt;sup&gt;a&lt;/sup&gt;</td>
<td>mm Hg</td>
<td>47</td>
<td>52</td>
<td>54</td>
</tr>
<tr>
<td>CVR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>mm Hg/mL/100 g/min</td>
<td>1.53</td>
<td>1.52</td>
<td>1.28</td>
</tr>
<tr>
<td>RAP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>mm Hg × s/cm</td>
<td>1.60</td>
<td>1.69</td>
<td>1.49</td>
</tr>
<tr>
<td>CI</td>
<td>L/min/m²</td>
<td>2.1</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>SVI</td>
<td>mL/beat/m²</td>
<td>38</td>
<td>39</td>
<td>41</td>
</tr>
<tr>
<td>MAP</td>
<td>mm Hg</td>
<td>76</td>
<td>74</td>
<td>71</td>
</tr>
<tr>
<td>SVRI</td>
<td>mL/beat/m²</td>
<td>2,741</td>
<td>2,668</td>
<td>2,298</td>
</tr>
<tr>
<td>Hb</td>
<td>mg/dL</td>
<td>12.1</td>
<td>12.0</td>
<td>12.3</td>
</tr>
<tr>
<td>PaO₂</td>
<td>mm Hg</td>
<td>118</td>
<td>119</td>
<td>119</td>
</tr>
<tr>
<td>Visc</td>
<td>mPa × s</td>
<td>4.3</td>
<td>4.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Temp</td>
<td>C</td>
<td>35.3</td>
<td>35.2</td>
<td>35.3</td>
</tr>
</tbody>
</table>

Abbreviations: CBF, cerebral blood flow; CI, cardiac index; CPPe, effective cerebral perfusion pressure; CVR, cerebrovascular resistance; Hb, hemoglobin concentration; MAP, mean arterial pressure; ns, non significant; PaCO₂, arterial partial pressure of CO₂; PaO₂, arterial partial pressure of O₂; RAP, resistance area product; s, significant; SVI, stroke volume index; SVRI, systemic vascular resistance; Temp, blood temperature; Visc, blood viscosity; V<sub>MCA</sub>, blood flow velocity of the middle cerebral artery; ZFP, zero flow pressure. Effect of the PaCO₂ level (RM-ANOVA = analysis of variance for repeated measures P < 0.05). MC = multiple group comparison (Holm–Sidak, P < 0.05): Significant difference between means between: 1 = A versus B, 2 = A versus C, 3 = A versus D, 4 = B versus C, 5 = B versus D, 6 = C versus D. *End points.
### Table 3. Effect of changes in PaCO₂ levels on primary end points

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dimension</th>
<th>MD (s.d.)</th>
<th>CI limits*</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(A) Comparison between D (PaCO₂ = 50 mm Hg) versus A (PaCO₂ = 29 mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaCO₂ §*</td>
<td>mm Hg</td>
<td>23 (2)</td>
<td>20 to 25</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CBF $§$§#*</td>
<td>mL/100 g/min</td>
<td>42 (18)</td>
<td>22 to 62</td>
<td>0.0003</td>
</tr>
<tr>
<td>$V_{MCA}$ mean $§$§#*</td>
<td>cm/s</td>
<td>21 (8)</td>
<td>13 to 30</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>ZFP §*</td>
<td>mm Hg</td>
<td>-15 (6)</td>
<td>-22 to -8</td>
<td>0.0002</td>
</tr>
<tr>
<td>CPPe §*</td>
<td>mm Hg</td>
<td>10 (8)</td>
<td>1 to 18</td>
<td>0.0210</td>
</tr>
<tr>
<td>CVR §*</td>
<td>mm Hg/mL/100 g/min</td>
<td>-0.70 (0.33)</td>
<td>-1.06 to -0.34</td>
<td>0.0007</td>
</tr>
<tr>
<td>RAP §*</td>
<td>mm Hg × s/cm</td>
<td>-0.42 (0.29)</td>
<td>-0.74 to -0.10</td>
<td>0.0097</td>
</tr>
<tr>
<td><strong>(B) Comparison between C (PaCO₂ = 44 mm Hg) versus A (PaCO₂ = 29 mm Hg)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>PaCO₂ §*</td>
<td>mm Hg</td>
<td>15 (2)</td>
<td>13 to 16</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CBF $§$§#*</td>
<td>mL/100 g/min</td>
<td>14 (12)</td>
<td>1 to 27</td>
<td>0.0330</td>
</tr>
<tr>
<td>$V_{MCA}$ mean $§$§#*</td>
<td>cm/s</td>
<td>9 (6)</td>
<td>2 to 16</td>
<td>0.0116</td>
</tr>
<tr>
<td>ZFP §*</td>
<td>mm Hg</td>
<td>-11 (6)</td>
<td>-17 to -5</td>
<td>0.0010</td>
</tr>
<tr>
<td>CPPe §</td>
<td>mm Hg</td>
<td>7 (7)</td>
<td>-0.3 to 14</td>
<td>0.0650</td>
</tr>
<tr>
<td>CVR §</td>
<td>mm Hg/mL/100 g/min</td>
<td>-0.24 (0.33)</td>
<td>-0.60 to 0.11</td>
<td>0.2689</td>
</tr>
<tr>
<td>RAP §</td>
<td>mm Hg × s/cm</td>
<td>-0.11 (0.25)</td>
<td>-0.38 to 0.16</td>
<td>0.7940</td>
</tr>
<tr>
<td><strong>(C) Comparison between D (PaCO₂ = 50 mm Hg) versus B (PaCO₂ = 37 mm Hg)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>PaCO₂ §*</td>
<td>mm Hg</td>
<td>15 (2)</td>
<td>13 to 17</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CBF $§$§#*</td>
<td>mL/100 g/min</td>
<td>38 (17)</td>
<td>20 to 56</td>
<td>0.0004</td>
</tr>
<tr>
<td>$V_{MCA}$ mean $§$§#*</td>
<td>cm/s</td>
<td>19 (5)</td>
<td>13 to 25</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>ZFP §*</td>
<td>mm Hg</td>
<td>-10 (6)</td>
<td>-17 to -3</td>
<td>0.0047</td>
</tr>
<tr>
<td>CPPe §</td>
<td>mm Hg</td>
<td>4 (7)</td>
<td>-3 to 12</td>
<td>0.5271</td>
</tr>
<tr>
<td>CVR §</td>
<td>mm Hg/mL/100 g/min</td>
<td>-0.69 (0.22)</td>
<td>-0.85 to -0.53</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>RAP §</td>
<td>mm Hg × s/cm</td>
<td>-0.52 (0.34)</td>
<td>-0.76 to -0.28</td>
<td>0.0062</td>
</tr>
</tbody>
</table>

Abbreviations: CBF, cerebral blood flow; CI, confidence interval; CPPe, effective cerebral perfusion pressure; CVR, cerebrovascular resistance; MD, mean difference; PaCO₂, arterial partial pressure of CO₂; RAP, resistance area product; s.d., standard deviation; $V_{MCA}$ = blood flow velocity of the middle cerebral artery; ZFP, zero flow pressure. The $P$ values, which refer to the difference between two measurement points, were calculated using two-sided t-test for paired data (§). Because the variances of some outcome variables differed between two measurement points, these parameters were additionally examined by Welch test (#) and nonparametric Wilcoxon signed-rank test ($§$), which showed that the differences persist ($P < 0.05$). To prevent from type I error, statistical analysis of end points was adjusted by one-way ANOVA for repeated measurements with Bonferroni multicomparsion procedure (*).
Figure 1: Cerebral hemodynamic variables under different levels of PaCO₂

Values of CBF (figure 1A), mean VMCA (figure 1B), CPPe (figure 1C), ZFP (figure 1D), CVR (figure 1E) and RAP (figure 1F) under four different levels of PaCO₂. Data are mean (SD) for each measurement. The P values were calculated using analysis of variance for repeated measures (p<0.05).

CBF = cerebral blood flow, CI = confidence interval, CPPe = effective cerebral perfusion pressure, CVR = cerebrovascular resistance, MD = mean differences, PaCO₂ = arterial partial pressure of CO₂, RAP = resistance area product, SD = standard deviation, VMCA = blood flow velocity of the middle cerebral artery, ZFP = zero flow pressure.
The changes in mean CVR and RAP were thus concordant, nevertheless the correlation analysis of individual data showed only a weak linear connection (Pearson r = 0.43, CI 0.14 to 0.66, P = 0.005).

The curves of CBF, V_{MCA}, CVR and RAP showed an exponential slope (figure 1). Thus, percent changes between the ventilation-steps might be quantitatively different. For statistical analysis we consequently compared C (normoventilation II) versus A (hyperventilation) and B (normoventilation I) versus D (hypoventilation) of all endpoints.

When comparing level C (normoventilation II) with A (hyperventilation) CBF was reduced by 28% and mean V_{MCA} by 21% (table 3B). The decrease of CPPe by 12% was caused by an increase of ZFP by 74%, which exceeded the small increase in MAP. CVR increased by 0.24(0.30) mmHg/mL/100 g/min and RAP by 0.11 (0.25) mmHg*s/cm (table 3B), which were not significant.

When comparing level D (hypoventilation) with B (normoventilation I) the CBF was decreased by 50% and mean V_{MCA} by 36% (table 3C). Here the relative increase of ZFP was high (93%) but absolute changes were small which lead only to a small reduction in CPPe by 7%. The RAP showed a less pronounced increase (by 46%) than the CVR, which increased by 91% (table 3C).

Cerebrovascular CO₂-reactivity of CBF was 1.88 (0.89) mL/100 g/min/mmHg, corresponding to a relative change of 4.13 (1.27) %/mmHg. The cerebrovascular CO₂-reactivity of mean V_{MCA} was 0.95(0.36) cm/s/mmHg, corresponding to a relative change of 2.47 (0.48) %/mmHg, which was significantly lower than the relative CO₂-reactivity of CBF (MD -1.67; 95% CI: -2.46, -0.87; p=0.001).

**DISCUSSION**

We investigated the effects of variations in PaCO₂ on cerebral blood flow, blood flow velocity, effective cerebral perfusion pressure, cerebrovascular resistance, zero flow pressure and resistance area product in cardiovascular patients undergoing fentanyl-midazolam anesthesia. The three most prominent results of our study are:

(i) hypocapnia-induced reduction in CBF under general anesthesia was the effect of both, an increase in CVR and a decrease in CPPe, as a consequence of an increase in ZFP;

(ii) the increases of mean CVR and RAP associated with hypocapnia were concordant. Nevertheless, correlation analysis showed only a weak linear relationship;

(iii) variation of PaCO₂ within a range of 30-50 mmHg induced changes in CBF and V_{MCA}, which both followed an exponential function. However, V_{MCA} systematically underestimated changes in global cerebral blood flow resulting in a discrepancy between cerebrovascular CO₂ reactivity measured by CBF and V_{MCA}.
Examining the functional capacity of the cerebrovascular autoregulatory system by altering blood pressure is difficult and possibly hazardous to be performed as a routine clinical procedure. Therefore, Bloor and colleagues introduced CO₂-reactivity measurements to evaluate cerebral vascular reactivity.²⁴

In our study we could demonstrate that the cerebrovascular CO₂-reactivity (within a PaCO₂ range of 30-50 mmHg) of CBF as well as VMCA followed an exponential shape. Other authors described the cerebral CO₂-reactivity response curve of CBF in humans as a linear or exponential function.¹¹,¹⁵,²⁵,²⁶ Correspondingly, CO₂-response curves of the VMCA measured by TCD have been described as a linear, logarithmic, exponential or as a sigmoid function.²⁶,²⁷

Today, VMCA and its indices are routinely used to assess components of cerebral circulation and cerebrovascular resistance. A good correlation between changes in VMCA and changes in CBF has been found during carotid endarterectomy.²⁸ During cardiopulmonary bypass and in patients with cerebrovascular diseases, correlations between CBF and VMCA values are rather weak with wide between-patient variation.¹⁴,²⁹

In a number of previous studies relative changes in CBF were reflected by changes in VMCA in a proportional manner, suggesting that MCA diameter remains constant.³⁰ Nevertheless, other investigations could demonstrate that illness and interventions as orthostasis, migraine attacks, CO₂-rebreathing or vasoactive medicine could change the MCA diameters by 5-12%.³¹-³⁴

Reports of cerebrovascular CO₂-reactivity of CBF and VMCA in humans give inconsistent results. Additionally, comparisons between cerebrovascular CO₂-reactivity of CBF and VMCA in humans under general anesthesia are very rare. In our study we could demonstrate that the cerebrovascular CO₂-reactivity (within a range of 30-50mmHg) of CBF as well as VMCA showed an exponential function. However, the relative cerebrovascular CO₂-reactivity measured by TCD was lower when compared to measurements of CBF by the Kety-Schmidt technique, especially under hypercapnia. The most probable explanation is that changes of PaCO₂ do not only cause changes in vascular diameter at the arteriolar level but may also cause minor changes in MCA main trunk diameter resulting in a slight systematic difference between relative changes in flow and flow velocity. A similar phenomenon had been described during hypothermic CPB.¹⁴

Our study is the first report that combines measurement of global CBF with the determination of ZFP and CPPe. The CVR is commonly defined by the law of Darcy as the quotient between the driving blood pressure and CBF (“classical model" CVR=(MAP-ICP)/CBF). This model has limitations, because it assumes that flow or flow velocity only reaches zero when the driving pressure is zero, which is unlikely, at least in the absence of intracranial hypertension.⁶ Patients without cerebrovascular disease are expected to have a normal ICP between 7-15 mmHg in supine position.³⁵ Hyperventilation reduces cerebral blood volume and consequently decreases ICP.³⁶ But the reduction of ICP in-
duced by hyperventilation in patients without cerebrovascular disease is expected to be small. Thus, the main determinant of the driving pressure would be the MAP. Our patients showed only a small increase of MAP and SVRI, which was in accordance with previous findings. Nevertheless, measures of MAP do not directly reflect the effective CPP because the CPP may be modified by factors other than MAP and ICP.

Studies of other organs have shown, that the EDP can be determined by a Starling resistor located at arteriolar level. These studies verified the theory of Permutt and Riley showing that two forces, the extramural pressure (ICP in the case of the brain) and arteriolar wall tension determine CCP. Arteriolar wall tension arises from a combination of the stretched elastic components of the vessel wall and active contraction of vascular smooth muscle. Thus, the driving pressure for the flow through arterioles is, under many conditions, not the difference between arterial (inflow) pressure and venous (outflow) pressure, but rather the difference between arterial pressure and CPP.

The CCP of the cerebral circulation cannot be directly measured in patients with spontaneous circulation. It has been confirmed that in vivo pressure-flow relationships are straight lines for many vascular beds and for the cerebral vessels as well. Since the introduction of TCD, a number of methods have been developed to assess CCP non-invasively by extrapolating instantaneous pressure-flow velocity plots (ABP/VMCA) to zero from continuous recordings or by extrapolation of the ZFP from a linear function given by diastolic, mean and/or systolic values of pressures and flow-velocity.

In a former investigation we suggested the hypothesis of two Starling resistors in a series connection, one (proximal) at the precapillary level of cerebral resistance vessels (CCP_{art}) and a second (distal) at the level of collapsible cerebral veins (CCP_{ven}). The effective downstream pressure of the cerebral circulation may be determined by either CCP_{art}, CCP_{ven} (i.e. ICP), or jugular venous pressure, depending on which one is highest. Thus, a number of factors other than vascular tone, i.e. CVP and ICP, can also influence EDP in the cerebral circulation. Consequently, the use of the term ‘zero flow pressure’ (ZFP) is considered more appropriate than CCP.

Using the difference between MAP and ZFP as the driving pressure of the cerebral circulation offers advantages in understanding pathophysiology, because changes of the effective downstream pressure will be reflected by this kind of calculation (“alternative model” CVR=(MAP-ZFP)/CBF). The reduction of CPPe due to hyperventilation in our patients was clinically relevant, which is in accordance with previous findings.

When using TCD, an estimate of vascular resistance can be derived from the ratio between blood pressure and blood flow velocity, which equals the product of the peripheral resistance and the cross-sectional area of the vessel at the site of insonation, the resistance area product (RAP). Up to now, there is no investigation that compared measurements of global CBF and V_{MCA} in order to compare changes in CVR with RAP. In our study the increase of mean CVR and RAP due to changes of PaCO_{2} was concordant.
Nevertheless, correlation analysis of individual data showed only a weak linear relationship. There are only a few trials about variations of PaCO₂ and its effect on CBF and CVR as well as on VMCA and RAP in patients or volunteers without cerebral disease. They all showed an inverse reaction of CBF and CVR. Recent TCD-studies could demonstrate that the RAP varies inversely with changes in PaCO₂. Thus, the RAP might have potential to reflect CO₂-induced changes of vascular resistance. Some investigations showed that hypocapnia increases ZFP, whereas the RAP seems to be unaffected. Conversely, McCulloch and colleagues found that RAP increased significantly with hypocapnia, and the increase in RAP appeared to have a greater effect on VMCA than the increase in ZFP. In the relationship between flow velocity and arterial blood pressure, the RAP has been defined as the inverse slope of their linear regression line. RAP can thus be expressed as RAP=(MAP-ZFP)/VMCA. The CVR is defined “classically” as CVR=(MAP-ICP)/CBF or in our study “alternatively” as CVR=(MAP-ZPF)/CBF. In our patients, the CO₂-reactivity-curve of the VMCA showed a smaller increase than CBF when comparing levels from hyperventilation to hypoventilation, which affects the slope of the pressure-flow (velocity) relationship and consequently also limits the validity of RAP as an index of CVR. Recently, Panerai et al. could demonstrate that the RAP is related to myogenic properties of the cerebrovascular system, while ZFP reflects metabolism and cerebrovascular reactivity to CO₂. Our data, however, could not explain the regulatory mechanism between these two factors.

Some methodological aspects of our study have to be considered. First, the a priori sample size calculation was based on estimation of the effect size, because of a lack of data regarding CBF and VMCA under general anesthesia in humans. A post-hoc calculation showed a statistical power of 99% for the CBF and VMCA (n=10, α error probability = 0.05; effect size CBF of 2.3 and effect size VMCA 2.6).

Furthermore, the type of anesthesia may have potential influence on the results of our study. Induction of anesthesia with fentanyl and midazolam leads to a moderate, but proportional reduction of CBF and cerebral metabolism. The cerebrovascular CO₂ reactivity in our patients favorably compared with data in conscious patients. Although we thus have no reason to assume, that intravenous anesthesia with fentanyl and midazolam per se may have affected the CO₂-induced changes in CBF and VMCA in our patients, the results of this study cannot a priori be extrapolated to other types of anesthesia.

Similarly, the external validity of our data could be limited by the fact that our patients were suffering from coronary artery disease and concomitant asymptomatic cerebrovascular disease cannot completely be excluded despite normal cerebrovascular CO₂-reactivity. Therefore, the conclusions from our study results should be limited to this patient population.
CONCLUSIONS

Hypocapnia-induced reduction in CBF under general anesthesia is caused by both, an increase in CVR and a decrease in CPPe, as a consequence of an increase in ZFP. The increases in CVR and RAP associated with hypocapnia were concordant. Nevertheless, correlation analysis showed only a weak linear relationship. Using the RAP as an index of CVR has thus limitations. The cerebrovascular CO₂ reactivity of V_{MCA} was lower when compared to CBF. The most probable explanation is that changes of PaCO₂ do not only cause changes in vascular diameter at the arteriolar level but might also cause minor changes in MCA main trunk diameter, resulting in a slight systematic difference between relative changes in flow and flow velocity.

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