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Age, aerobic fitness, and cerebral perfusion during exercise: role of carbon dioxide

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Flück D, Braz ID, Keiser S, Hüpippn F, Haider T, Hilty MP, Fisher JP, Lundby C. Age, aerobic fitness, and cerebral perfusion during exercise: role of carbon dioxide. Am J Physiol Heart Circ Physiol 307: H515–H523, 2014. First published June 20, 2014; doi:10.1152/ajpheart.00177.2014.—Middle cerebral artery mean velocity (MCAvmean) is attenuated with increasing age both at rest and during exercise. The aim of this study was to determine the influence of the age-dependent reduction in arterial PCO2 (PaCO2) and physical fitness herein. We administered supplemental CO2 (CO2 trial) or no additional gas (control trial) to the inspired air in a blinded and randomized manner, and assessed middle cerebral artery mean flow velocity during graded exercise in 1) 21 young [Y; age 24 ± 3 yr (±SD)] volunteers of whom 11 were trained (YT) and 10 considered untrained (YUT), and 2) 17 old [O; 66 ± 4 yr] volunteers of whom 8 and 9 were considered trained (OT) and untrained (OIT), respectively. A resting hypercapnic reactivity test was also performed. MCAvmean and PaCO2 were lower in O [44.9 ± 1.0 mmHg (±SE)] compared with Y (59.3 ± 2.3 cm/s and 34 ± 1 mmHg, P < 0.01) at rest, independent of aerobic fitness level. The age-related decreases in MCAvmean and PaCO2 persisted during exercise. Supplementation CO2 reduced the age-associated decline in MCAvmean by 50%, suggesting that PaCO2 is a major component in the decline. On the other hand, relative hypercapnic reactivity was neither influenced by age (P = 0.46) nor aerobic fitness (P = 0.36). Although supplemental CO2 attenuated exercise-induced reduction in cerebral oxygenation (near-infrared spectroscopy), this did not influence exercise performance. In conclusion, PaCO2 contributes to the age-associated decline in MCAvmean at rest and during exercise; however exercise capacity did not diminish this age effect.

Cerebral blood flow (CBF) is meticulously regulated to ensure an adequate perfusion of the brain. With exercise middle cerebral artery mean velocity (MCAvmean, a surrogate measure of CBF) is increased until ~60% of maximal oxygen uptake (VO2max) but thereafter declines toward resting levels (10, 20, 28). In young healthy individuals this drop in cerebral perfusion is likely the consequence of a hyperventilation facilitated reduction in PaCO2 and hence augmented cerebral vasoconstriction (39). Accordingly, administration of CO2 to the inspired air during exercise abolishes the decrease in MCAvmean (44, 45), and during vigorous exercise MCAvmean is regulated by PaCO2 and only to a lesser extent influenced by cerebral metabolism, mean arterial pressure (MAP), cardiac output, or sympathetic nerve activity (35).

Compared with young healthy individuals a reduced CBF (24) and MCAvmean has consistently been reported in the aged population both at rest (2, 7, 9, 15, 25, 27, 49) and during exercise (9, 10, 28, 32). Although a reduced MCAvmean response in aged humans is observed with exercise, its pattern follows that of young individuals, i.e., an initial increase which is then followed by a decline as the exercise intensity becomes intense (9, 10, 28, 32). The regulating mechanisms for the reduction in CBF with age remain uncertain and global brain atrophy (11), decreased neuronal activity (29), increased arterial stiffness (12, 50), and reduced cerebrovascular reactivity (12, 22) have all been proposed as important factors. In addition, the concomitantly occurring and age-dependent decrease in PaCO2 at rest (9) and during exercise (9, 28) may be involved in the age-associated reduction in cerebral perfusion. This however has not been tested experimentally, and is one aim of the present study. An age-related reduction in CO2 reactivity could also diminish the CBF response to exercise. The effect of aging on CO2 reactivity is not clear, however, as hypercapnic reactivity has been demonstrated to be either unchanged (15, 24, 42), reduced (4, 22, 48), or even elevated (49) with increasing age. One reason for the discrepancies could be related to the use of humans over a narrow age range, and with varying degrees of physical fitness. Thus a further aim related to this study was to quantify CO2 reactivity and to investigate its association to the decrease in MCAvmean with age.

A high aerobic fitness level appears to attenuate the age-related decline in resting CBF (39) and MCAvmean (1–3, 46), although this has not been universally observed (4, 49). Whether fitness is a factor affecting CBF during exercise

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remains unclear. Moreover, aerobic fitness may affect hypercapnic cerebrovascular reactivity (4, 32, 49), although these findings are also contradictory as increased (3, 32), unchanged (49) and decreased (46) hypercapnic reactivity has been reported in aerobically trained individuals. Again potential influencing factors for the widespread results could include differences in age and fitness levels across studies.

Accordingly the purpose of the present study was fourfold: 1) to determine the effect of age on hypercapnic reactivity compared with healthy young individuals, 2) to establish the importance of the age-related drop in PaCO2 on MCAvmean by administering supplemental CO2 to the inspiration during exercise, 3) to examine the effects of physical fitness on hypercapnic reactivity and the age-related decrease in MCAvmean by comparing aged and young individuals that are aerobically trained or untrained, and 4) to investigate whether maintaining MCAvmean and thereby cerebral oxygenation would lead to an improvement in exercise capacity in young and older individuals. We hypothesized that supplementing the inspired air during exercise, and that this difference would also be accompanied by a higher hypercapnic reactivity. Finally we expected that improvements in MCAvmean elicited by CO2 supplementation would not lead to an improved exercise capacity.

**METHODS**

All experimental protocols and procedures conformed to the Declaration of Helsinki and were approved by the ethical committee of the Swiss Federal Institute of Technology Zürich (EK 2013-N-17). Prior to participation, a detailed verbal and written explanation of the study was provided, and written informed consent to take part in this study was obtained from each participant. Subjects were screened by means of a general health questionnaire to identify any history or symptoms of cardiovascular (e.g., hypertension), pulmonary, metabolic, or neurological disease or use of medications. We recruited 21 young (YT) and 24 older (OT) males to participate in the study. Consequently 11 young trained (VO2max: 65.6 ± 1.0 ml·kg⁻¹·min⁻¹; YT), 10 young untrained (49.6 ± 1.8 ml·kg⁻¹·min⁻¹; YT), 8 older trained (40.5 ± 2.5 ml·kg⁻¹·min⁻¹; OT), and 9 older untrained (29.6 ± 1.3 ml·kg⁻¹·min⁻¹; OT) individuals participated in the study (Table 1).

During a preliminary visit to the laboratory, subjects were familiarized with the exercise tests and the study set-up. On the second visit to the laboratory a hypercapnic reactivity test plus two maximal incremental exercise tests were performed, one without and one with additional CO2 to inspiration. Between subjects the tests were conducted at different times of the day; however, within-subject comparison data were assessed at the same time of the day. Subjects were requested to abstain from strenuous physical activity for 24 h, and alcohol and caffeine for 12 h prior to experimental sessions. Monitoring procedures associated with medical safety during exercise were performed in real-time by a physician and included a 12-lead ECG, oscillometric noninvasive blood pressure measurements with a 3 min monitoring interval and a ST elevation check of I-AVF, V1-V6, in a 1-min interval. Termination criteria for the exercise test were systolic blood pressure above 220 mmHg or a 10-mmHg drop below baseline, ST anomalies, arrhythmias, and symptoms such as dizziness, syncope, or cyanosis. Posttest monitoring was for 15 min if asymptomatic and if BP and ECG reached baseline values. No test participants were excluded on the basis the above investigations.

**Experimental measures.** MCAvmean was assessed using transcranial Doppler ultrasonography (Doppler Box, DWL, Sipplingen, Germany) with a 2 MHz probe placed over the right temporal window, prepared with ultrasound gel. The probe was held in place with a snug-fitting headgear. Mean arterial pressure (MAP) was recorded continuously and noninvasively via finger photoplethysmography (Nexfin, BMYE B.V., Amsterdam, Netherlands) and heart rate (HR) was assessed by a monitor belt (Cosmed Quark b2, Rome, Italy). MCAvmean and MAP were sampled at 1,000 Hz and stored for offline analysis (LabChart 7 Pro v7.3.5 and Powerlab, ADInstruments, Bella Vista, NSW, Australia).

| Table 1. Age, weight, height, BMI, MAP, absolute and relative VO2max, PaCO2, MCAvmean and absolute, relative and CVC CO2 reactivity at rest in young and older, trained and untrained individuals |
|---|---|---|---|---|---|---|---|
| | Young | | Old | | | |
| | Trained | Untrained | Trained | Untrained | | |
| Age, yr | 22 ± 0 | 25 ± 1 | 65 ± 0 | 67 ± 1 | <0.01 | 0.06 | 0.78 |
| Weight, kg | 71.0 ± 2.3 | 78.8 ± 2.7 | 72.1 ± 2.5 | 82.3 ± 3.9 | <0.01 | 0.06 | 0.69 |
| Height, m | 1.82 ± 0.02 | 1.81 ± 0.02 | 1.72 ± 0.01* | 1.79 ± 0.02 | <0.01 | 0.07 | 0.05 |
| BMI, kg/m² | 21.4 ± 0.5 | 23.9 ± 0.8 | 24.2 ± 0.9 | 25.4 ± 1.1 | 0.02 | 0.04 | 0.44 |
| MAP, mmHg | 91.3 ± 1.8 | 92.3 ± 1.8 | 92.0 ± 3.4 | 94.2 ± 2.8 | 0.67 | 0.60 | 0.85 |
| Wmax, W | 388 ± 16 | 318 ± 12 | 238 ± 10 | 216 ± 17 | <0.01 | <0.01 | 0.13 |
| Absolute VO2max, l/min | 4.66 ± 0.14 | 3.88 ± 0.12 | 2.90 ± 0.16 | 2.42 ± 0.12 | <0.01 | <0.01 | 0.32 |
| Relative VO2max, ml·min⁻¹·kg⁻¹ | 65.6 ± 1.0 | 49.6 ± 1.8 | 40.5 ± 2.5 | 29.6 ± 1.3 | <0.01 | <0.01 | 0.15 |
| PaCO2, mmHg | 34.8 ± 0.5 | 32.8 ± 0.9 | 30.4 ± 0.8 | 29.4 ± 0.9 | <0.01 | 0.07 | 0.50 |
| MCAvmean, cm/s | 56.1 ± 2.7 | 62.9 ± 3.3 | 49.7 ± 4.9 | 40.6 ± 2.8* | <0.01 | 0.76 | 0.04 |
| Absolute CO2 reactivity, cm·s⁻¹·mmHg⁻¹ | 1.99 ± 0.37 | 1.57 ± 0.26 | 1.95 ± 0.36 | 1.82 ± 0.21 | 0.73 | 0.38 | 0.64 |
| Relative CO2 reactivity, %/mmHg | 3.42 ± 0.68 | 2.80 ± 0.29 | 3.64 ± 0.36 | 3.32 ± 0.54 | 0.49 | 0.38 | 0.78 |
| CVC CO2 reactivity, cm·s⁻¹·mmHg⁻² | 0.016 ± 0.003 | 0.013 ± 0.002 | 0.010 ± 0.003 | 0.009 ± 0.001 | 0.06 | 0.47 | 0.71 |

Values are means ± SE. BMI, body mass index; MAP, mean arterial pressure; VO2max, maximal oxygen uptake; PaCO2, estimated arterial partial pressure of CO2; MCAvmean, middle cerebral artery mean velocity; CVC, cerebrovascular conductance. *P < 0.05 vs. young. Young trained and untrained and older trained and untrained are compared at rest.
By wearing a mask covering nose and mouth (Hans Rudolph) respiratory variables were measured breath-by-breath using a spirometer. A capillary blood sample was obtained from the right ear lobe at BL, and during exercise at 100 W, at 75% maximal workload (Wmax), and at exhaustion. Capillary PCO2 (PcapCO2), pH, and HCO3− were measured using a blood gas analyzer (ABL800, Radiometer, Copenhagen, Denmark). Cerebral and muscle tissue oxygenation (cerebral and muscle SjO2) were continuously assessed on the left forehead and right vastus lateralis muscle, respectively, by near-infrared spectroscopy (NIRS; Invos-5100c, Covidien, Mansfield, MA).

**Hypercapnic reactivity test.** Hypercapnic reactivity test was performed by adding CO2 to the inspired air (Altitrainer, SMTEC, Nyon, Switzerland) with subjects in a supine position. After a 10-min resting phase, PaCO2 (estimated from end-tidal PCO2) was recorded for 3 min following which the CO2 reactivity test protocol was undertaken. The protocol consisted of three steps of 120 s: step 1 (PaCO2 = +1.5 mmHg above resting values), step 2 (PaCO2 = +6.5 mmHg above resting values), and recovery (PaCO2 = +1.5 mmHg above resting values). Maintaining PaCO2 at 1.5 mmHg above resting values facilitates PaCO2 control (21) and reduces breath-to-breath variability in cerebral blood flow velocity (19).

**Exercise test.** During the preliminary visit to the laboratory each subject performed a maximal incremental exercise test on a cycle ergometer (Monark E 839, Varberg, Sweden) to determine their Wmax and VO2max, using a protocol starting with a warm-up period of 5 min at 100 W (YT), 150 W (YT), 20 W (OUT), or 50 W (OT). Thereafter the workload was increased every minute by 30 W (YT, YT, YT, and OT) or 20 W (OT) until exhaustion.

On the second visit to the laboratory subjects completed two incremental exercise tests in a blinded and randomized manner without (control trial) and with supplemental inspired CO2 (CO2 trial). The trials were separated by at least 90 min to limit any potential carryover effect (41). The workload selected during the exercise protocol was based on the Wmax achieved during the maximal incremental exercise test performed on the first visit. Both the control trial and the CO2 trial followed the same individualized protocol consisting of two absolute workloads (60 W, 100 W) and four relative workloads (25% Wmax, 50% Wmax, 75% Wmax, and 100% Wmax). The first four workloads were set to last 3 min each, the fifth workload for 2 min, and the final workload (100% Wmax) was performed until exhaustion. The duration that the subjects were able to sustain 100% Wmax was estimated from end-tidal PCO2 (PETCO2) by the equation (37):

\[
\text{estimated PaCO2} = 2.367 + 0.884 \times \text{PETCO2}
\]

During the exercise tests PaCO2 was estimated from PETCO2 by the equation (23):

\[
\text{estimated PaCO2} = 5.5 + 0.9 \times \text{PETCO2} - 0.0021 \times V_T
\]

where tidal volume (VT) is in milliliters.

Resting MCAvmean, MAP, and PaCO2 were averaged over the last minute of the resting phase prior to the hypercapnic reactivity test. Baseline, absolute, and relative exercise intensity values represent an average over 1 min whereas the “last 20 s” time point represents an average of the last 20 s of each exercise trial. This was chosen to reduce the chance of missing the changes in the measured parameters at maximal exercise shortly before exhaustion. Cerebrovascular con-

**RESULTS**

All 38 volunteers completed the study. Independent of the participant’s physical fitness level there was a main age effect between the YT+UT and the OT+UT (Table 1). Briefly, resting MAP was similar in the two groups. PaCO2 and MCAvmean were higher in YT+UT compared with OT+UT. Furthermore, an interaction for MCAvmean between age and training was found and post hoc analysis revealed an age difference within the untrained groups.

**Maximal workload and oxygen uptake with exercise.** Wmax achieved by YT+UT was 35% higher (354 ± 13 vs. 227 ± 11 W, P < 0.01) than in OT+UT. Within the age groups, trained participants reached 18% and 9% (YT+UT and OT+UT) higher Wmax compared with the untrained participants (388 ± 10 vs. 318 ± 13 W, P < 0.01, YT vs. YT; 238 ± 10 vs. 216 ± 19 W, P < 0.01, OT vs. OT). Absolute and relative VO2max are presented in Table 1. VO2, carbon dioxide production (VCO2), and respiratory exchange ratio (RER) are presented in Table 2.

**Hypercapnic reactivity test.** PaCO2 was increased (P < 0.01) from step 1 to step 2 in YT+UT (38.1 ± 0.5 to 42.2 ± 0.6 mmHg) and OT+UT (34.5 ± 0.7 to 38.5 ± 0.7 mmHg). Concomitantly MAP was augmented by 3.3 ± 0.8 and 5.3 ± 0.8 mmHg in YT+UT and OT+UT, respectively. Hypercapnic reactivity data (Table 1) are presented in absolute and relative MCAvmean as well as absolute CVC changes to 1 mmHg increase in PaCO2. Neither of these differed between the age groups and trained vs. untrained study volunteers. CVC CO2 reactivity, however, tended (P = 0.06) to be slightly lower with age.

PaCO2, MCAvmean, MAP, and CVC in the control vs. the CO2 exercise trial. Figure 1 illustrates PaCO2, MCAvmean, MAP, and CVC data from the control and CO2 trial for YT+UT (YT and YT pooled) and OT+UT (OT and OT pooled). In the control trial, PaCO2 was elevated with increasing exercise intensity until ~75% Wmax and thereafter decreased in both age groups. In accordance with the lower PaCO2 at BL, PaCO2 during exercise (control trial) was also lower (P < 0.01) in OT+UT compared with YT+UT. CO2 was administrated to inspiration during exercise in the CO2 trial to keep PaCO2 above 40 mmHg (YT+UT, 42.6 ± 0.4 mmHg; OT+UT, 45.2 ± 0.3 mmHg). Analysis of values at absolute exercise intensities revealed an interaction of age, condition, and intensity (P < 0.01, Fig. 1A). Post hoc analysis demonstrated lower PaCO2 values in the control trial of the OT+UT compared with OT+UT CO2 trial and YT+UT control and CO2 trial at 60 and 100 W. A main effect of age (P < 0.01) and an interaction between intensity and condition (P < 0.01) was observed within the relative exercise intensities data. At 75%, 100% Wmax and last
20 s, PaCO₂ was elevated in the CO₂ trial compared with the control trial in both age groups.

Figure 1B presents MCAvmean values during the control and CO₂ trial for both age groups. Throughout exercise MCAvmean in the OT vs. UT was lower compared with YT+UT. MCAvmean was higher at 100% Wmax and the last 20 s in the CO₂ trial compared with the control trial in YT+UT and OT+UT.

MAP responses to exercise are presented in Fig. 1C. Training status did not show an effect on MAP response during absolute or relative exercise intensities in both age groups (P > 0.58). CVC for the control and CO₂ trial and both age groups is presented in Fig. 1D. At absolute and relative exercise intensities a main effect of age was apparent. In agreement with MCAvmean, CVC was also increased at 100% Wmax and the last 20 s in the CO₂ trial compared with the control trial in both age groups.

Ventilation and exercise performance in the control vs. the CO₂ exercise trial. Ventilation (V̇E) was elevated with increasing exercise intensity (P < 0.01); however, it was not different between YT+UT and OT+UT at BL, 60 W, and 100 W (14.7 ± 0.6 vs. 14.6 ± 0.7, 34.7 ± 0.8 vs. 34.7 ± 1.6, and 47.1 ± 0.9 vs. 51.4 ± 2.0 l/min, P = 0.22). Analysis of V̇E during relative exercise intensities revealed an interaction between age and intensity (P < 0.01), and at 75% Wmax (96.8 ± 3.1 vs. 73.8 ± 2.6 l/min), 100% Wmax (134.5 ± 3.4 vs. 87.8 ± 2.8 l/min), and last 20 s (140.0 ± 3.6 vs. 90.6 ± 3.1 l/min) V̇E was elevated in YT+UT vs. OT+UT. A main effect of condition was only apparent when absolute intensities [e.g., 100 W in OT+UT: 48.9 ± 2.4 vs. 53.9 ± 3.2 l/min, control vs. CO₂ trial (P < 0.01)] were evaluated, whereas there was no main effect of condition when relative exercise intensities were assessed (P = 0.52).

The duration at 100% Wmax was lower in the OT+UT compared with the YT+UT (P < 0.01), independent of their fitness level (P = 0.49). Additionally both age groups sustained a shorter duration at 100% Wmax during the CO₂ trial (YT, 106 ± 11 s; YUT, 83 ± 11 s; OT, 50 ± 11 s; OUT, 48 ± 11 s) compared with the control trial (YT, 116 ± 12 s; YUT, 85 ± 11 s; OT, 61 ± 10 s; OOUT, 87 ± 16 s; P = 0.01).

Trained vs. untrained study volunteers. Figure 2 illustrates the same data as in Fig. 1 except that age groups are divided into a trained (YT and OT) and untrained group (YUT and OUT) according to their V̇O₂max (Table 1). A main effect of training status was not evident for MCAvmean, MAP, CVC, and V̇E at absolute or relative exercise intensities. PaCO₂ during relative exercise intensities, however, demonstrated a main effect of training status. An interaction between intensity and training status was observed, along with an interaction between condition and training status. PaCO₂ values during the control trial in both age groups were lower in the untrained vs. trained volunteers. Training status did not exert an effect on PaCO₂ when compared at absolute exercise intensities.

Relative changes in PaCO₂, MCAvmean and CVC during control and CO₂ trial. Percent changes from baseline are similar to that observed for the absolute values (Fig. 1) except that the magnitude of the age-related difference in the MCAvmean and PaCO₂ response to exercise was diminished. The supplemental CO₂ given to inspiration resulted in a greater increase in CO₂ in the OT+UT compared with the YT+UT during exercise at the same absolute intensity.

Capillary blood samples. Due to an insufficient volume of blood being obtained from some of the participants these data are derived from n = 13 (8 YT+UT and 5 OT+UT). PcapCO₂ values are in agreement with the estimated PaCO₂ values from the spirometer (r = 0.76, P < 0.01). Baseline pH did not differ between YT+UT (7.40 ± 0.01) and OT+UT (7.41 ± 0.01, P = 0.80). At exhaustion pH in the YT+UT was 7.27 ± 0.02 and 7.27 ± 0.02 for control and CO₂ trial, respectively, and in OT+UT pH was 7.34 ± 0.01 and 7.33 ± 0.01. A main effect of

Table 2. Oxygen uptake, carbon dioxide production, and respiratory exchange ratio during exercise in the control trial in young and older, trained and untrained individuals

<table>
<thead>
<tr>
<th></th>
<th>Young (n = 11)</th>
<th>Old (n = 8)</th>
<th>P Values</th>
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</thead>
<tbody>
<tr>
<td>V̇O₂, ml/min</td>
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<tr>
<td>60 W</td>
<td>1,587 ± 75</td>
<td>1,563 ± 36</td>
<td>1,424 ± 94</td>
<td>1,319 ± 80</td>
<td>&lt; 0.01</td>
<td>0.51</td>
</tr>
<tr>
<td>100 W</td>
<td>2,059 ± 76</td>
<td>2,097 ± 75</td>
<td>1,896 ± 81</td>
<td>1,757 ± 99</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>25% Wmax</td>
<td>1,994 ± 95</td>
<td>1,830 ± 65</td>
<td>1,442 ± 116</td>
<td>1,196 ± 87</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>50% Wmax</td>
<td>3,112 ± 129</td>
<td>2,839 ± 127</td>
<td>2,156 ± 134</td>
<td>1,785 ± 111*</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>75% Wmax</td>
<td>4,080 ± 174</td>
<td>3,482 ± 134</td>
<td>2,656 ± 138*</td>
<td>2,279 ± 131*</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>100% Wmax</td>
<td>4,459 ± 141</td>
<td>3,692 ± 138</td>
<td>2,732 ± 235*</td>
<td>2,429 ± 156*</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
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<tr>
<td>V̇CO₂, ml/min</td>
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<tr>
<td>60 W</td>
<td>1,197 ± 55</td>
<td>1,215 ± 55</td>
<td>1,177 ± 85</td>
<td>1,099 ± 68</td>
<td>0.12</td>
<td>0.81</td>
</tr>
<tr>
<td>100 W</td>
<td>1,685 ± 70</td>
<td>1,844 ± 80</td>
<td>1,655 ± 75</td>
<td>1,588 ± 99</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>25% Wmax</td>
<td>1,614 ± 70</td>
<td>1,553 ± 61</td>
<td>1,172 ± 114</td>
<td>971 ± 70</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>50% Wmax</td>
<td>2,667 ± 82</td>
<td>2,654 ± 120</td>
<td>1,925 ± 124*</td>
<td>1,615 ± 81*</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>75% Wmax</td>
<td>3,849 ± 143</td>
<td>3,604 ± 130</td>
<td>2,591 ± 134*</td>
<td>2,181 ± 101*</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>100% Wmax</td>
<td>5,231 ± 212</td>
<td>4,296 ± 190</td>
<td>2,897 ± 245*</td>
<td>2,550 ± 125*</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
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</table>

Values are means ± SE. V̇O₂, oxygen uptake; V̇CO₂, carbon dioxide production; RER, respiratory exchange ratio. Wmax, maximal workload. P values represent ANOVA results. *P < 0.05 vs. young, †P < 0.05 vs. trained.
age ($P < 0.01$) was observed in pH, but there was no main effect of either condition ($P = 0.14$) or training status ($P = 0.44$). No main effects of condition ($P = 0.99$) or training ($P = 0.23$) were found for HCO$_3^-$, but there was an interaction between age and intensity ($P < 0.01$). At exhaustion HCO$_3^-$ was lower in YT vs. OT in the control trial and CO$_2$ trial (18.2 ± 0.9 vs. 20.7 ± 0.3 mmol/l and 18.3 ± 0.6 vs. 20.5 ± 0.5 mmol/l, $P < 0.01$).

Cerebral and vastus lateralis muscle oxygenation. Cerebral StO$_2$ was increased in response to supplemental CO$_2$ whereas muscle StO$_2$ was unaffected (Fig. 3). Changes in cerebral and muscle oxygenation followed a similar pattern in both age groups; however, greater decreases in muscle oxygenation were observed in the young group when relative exercise intensities were compared.

**DISCUSSION**

In support of the study aims the major findings include: 1) confirmation of the age-associated decrease in PaCO$_2$ and
MCA\textsubscript{V-mean} at rest and during exercise; 2) hypercapnic cerebrovascular reactivity is not altered with increased age; 3) the experimentally induced increase in PaCO\textsubscript{2} abolished \textasciitilde50% of the age-related reduction in MCA\textsubscript{V-mean} during exercise; 4) aerobic fitness in young and older humans does not influence MCA\textsubscript{V-mean} at rest or during exercise; and 5) improvements in cerebral oxygenation by CO\textsubscript{2} administration do not lead to improved exercise performance in either young or older study participants.

**Influence of age on resting PaCO\textsubscript{2} and MCA\textsubscript{V-mean}**. The reduction in resting MCA\textsubscript{V-mean} with age observed in the present study is in agreement with studies assessing CBF using transcranial Doppler ultrasonography (7, 9, 15, 32, 49), the Kety-Schmidt technique (25), and arterial spin labeling MRI (27). Possible mechanisms for the age-related decrease in CBF include decreased neuronal activity (29), increased arterial stiffness (50), reduced cerebrovascular reactivity (22), and global brain atrophy (11), although the latter has been argued not to contribute to the age-related decline in CBF (6). Reduced PaCO\textsubscript{2} has also been associated to the age-related decrease in MCA\textsubscript{V-mean} (9) and in agreement herewith we observed that PaCO\textsubscript{2} and MCA\textsubscript{V-mean} are both lower in the older study participants. Others, however, have not demonstrated such a relationship (15, 28, 32, 49), although three of four of these studies reported that PaCO\textsubscript{2} was 1 to 2.8 mmHg lower in the older subjects. A possible explanation for PaCO\textsubscript{2} to be lower with age in some humans could be secondary to age-related metabolic acidosis (13). However, in a follow up study the same authors reported elevated blood acidity in the elderly, but no age-related difference in PaCO\textsubscript{2} was reported (14). Furthermore, in the present study resting capillary blood pH was not reduced with age, and does hence not support an
MCAvmean, PaCO2, and CVC values were observed throughout test the importance of PaCO2 for the age-associated decrease in MCAvmean, other factors are also involved. It has been suggested that differences in PaCO2 may account for 30% of the age-related decline in MCAvmean (1), and thus according to the present study this may be somewhat an underestimate. As MCAvmean and PaCO2 in young and old participants present an age-related difference already at rest, the difference observed during exercise could derive from here. The percentage change in MCAvmean during exercise was similar in young and old and in agreement with a previous study (28), although at 50% VO2peak a lower MCAvmean response was observed in the elderly. Similarly, the percent changes from baseline in PaCO2 were comparable in young and old participants. Thus exercise does not appear to exacerbate the age-related difference in PaCO2 and MCAvmean manifested at rest.

As PaCO2 accounts for ~50% of the age related decrease in MCAvmean, other factors must also contribute to the decline. As mentioned decreased neuronal activity (29), increased arterial stiffness (50) and reduced cerebrovascular reactivity (4, 22, 48) are potential contributors. Cerebrovascular reactivity, which in the current study was assessed as hypercapnic reactivity, demonstrated no difference between the two groups when expressed as absolute and relative changes in MCAvmean in support of previous studies (15, 24). Unfortunately none of these studies determined CVC reactivity. In the present study a trend (P = 0.06) toward a reduced hypercapnic reactivity was evident. In contrast an increase in hypercapnic reactivity, calculated as relative and CVC changes, in elderly has been reported (49). Since no apparent explanation seems at hand for the discrepancies, the inconsistencies in hypercapnic reactivity results might arise from differences in the CBF measurement techniques, CO2 stimulus, and age range of the study participants. In future studies blood pressure changes and therefore CVC reactivity should also be taken into consideration.

Despite having a lower MCAvmean this may not necessarily also lead to a lower brain oxygenation in the elderly, as a reduced oxygen delivery to the brain may be compensated for by an augmented oxygen extraction and thereby maintain cerebral oxygenation (34). Fisher et al. (9) however demonstrated similar cerebral oxygenations despite differences in cerebral perfusion between young and older individuals. In the present study brain delta oxygenation also did not differ between age groups; one limitation, however, is that we assessed tissue oxygenation index and not absolute oxy- and deoxyhemoglobin values or direct arterio-venous differences.

The influence of aerobic fitness on MCAvmean and hypercapnic reactivity. Physical activity has been suggested to maintain MCAvmean and cerebrovascular reactivity (2, 32). In the present study aerobic fitness neither in the young nor in the old group influenced resting MCAvmean or hypercapnic reactivity. Recent studies (4, 49) also reported a lack of an association between fitness and MCAvmean in young vs. older individuals. In contrast, life-long physical activity/training has shown an attenuated age-dependent decline in resting MCAvmean (2, 3) and CBF (2, 3, 46). When the same relative exercise intensities were compared the trained study participants displayed a higher PaCO2 in the control trial, and this was independent of age. The reason could be related to the fact that trained

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Fig. 3. Relative changes (delta) to baseline (BL) for cerebral (brain StO2; A) and muscle (leg StO2; B) tissue oxygenation in response to exercise performed with (circles) or without (triangles) administered CO2 to inspiration in young (black symbols) and old (gray symbols) participants. Values are means ± SE. P values represent ANOVA results.

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age-related acidosis, at least in the subjects participating in the present study.

PaCO2 and MCAvmean during exercise in old and young humans. In the present study PaCO2 increased gradually in the young and older groups until an exercise intensity of ~75% Wmax was reached, and thereafter declined in agreement with previous work (10, 20). Although MCAvmean, when expressed as absolute and relative changes in MCAvmean, demonstrated no difference between the two groups which in the current study was assessed as hypercapnic reactivity, showed a pattern similar, the decline, however, was less apparent. Cerebrovascular conductance (CVC; MCAvmean/MAP) exhibited a diminished elevation with increasing exercise intensity and was markedly decreased at maximal exercise (Fig. 1). This could be indicative of MAP being the dominating force increasing CBF during the early light phase of exercise (39) and the subsequent switch to PaCO2 becoming the dominant regulator of CBF at higher exercise intensities.

Age did not influence the response pattern of MCAvmean, PaCO2, or CVC; however, in the older group, lower MCAvmean, PaCO2, and CVC values were observed throughout compared with the younger participants (Fig. 1). Possible mechanisms underlying this age effect remain unclear. To test the importance of PaCO2 for the age-associated decrease in MCAvmean, young and old volunteers performed an exercise trial with CO2 administered to the inspired air. This approach has been used in different settings (8, 36, 44) and proven effective in maintaining or increasing PaCO2 and MCAvmean (36, 44). When increasing PaCO2 in the aged pop-
participants were exercising at a higher absolute workload and hence also at a higher VO₂ and in most instances therefore also higher PaCO₂. The elevated PaCO₂ in trained subjects did not lead to a concomitant higher MCAvmean although trained study volunteers, especially the trained older, had a higher MCAvmean during exercise, although not reaching statistical significance (P = 0.16).

Several studies have investigated the influence of aerobic fitness on hypercapnic reactivity, and in agreement with our results Zhu et. al (49) demonstrated no effect of aerobic fitness on hypercapnic reactivity. As indicated with respect to the effects of age, the effects of aerobic exercise capacity on hypercapnic reactivity are inconsistent and range from increased (3) to decreased (46) reactivities in trained individuals.

The influence of cerebral oxygenation on exercise limitations. The gradual decline in CBF with exercise intensity above a certain threshold also reduces cerebral oxygenation. This has been speculated to lead to centrally mediated fatigue (17, 33). A unique feature of CO₂ supplementation is that whereas MCAvmean and cerebral oxygenation are increased, oxygenation of the exercising skeletal muscles remains unaffected by the intervention (Fig. 3), and hence the isolated influence of altered brain oxygenation can be assessed. In the present study a reduced decline in cerebral oxygenation did not result in a higher exercise capacity. Our results are in line with recent work (44, 45) and hence support that brain oxygenation is not a parameter of importance for fatigue development during maximal exercise. Indeed, supplemental CO₂ administration to the inspired air tended to reduce exercise performance as the duration of the 100% Wmax step was shorter during the CO₂ trial. One potential reason for the negative influence of CO₂ administration to exercise performance is a hyperventilation-induced acidification (45, 47). Previous studies have not assessed blood gas variables at maximal exercise when CO₂ was administered. Capillary pH was unaffected by the supplemental CO₂ gas. CO₂ induced hyperventilation could direct blood flow away from the exercising muscles and support the additional work and metabolic demand of the respiratory muscles. However, ventilation did not differ between the control and CO₂ trial.

Limitations. We assessed MCAvmean by TCD as a surrogate for CBF. MCAvmean is a measure of blood flow velocity and not a flow in absolute terms. Nevertheless the two are highly correlated (5). The majority of studies have illustrated that the diameter of the MCA does not change in response to hypercapnia (16, 38). Thus assessment of blood velocity using TCD has been accepted as a reliable measure of CBF and is widely used to study age and exercise-related effects (9, 10, 28, 32). In the present study we focused on PaCO₂ as it is one of the major CBF regulators; however, whether it is PaCO₂ or in fact pH, or both, that are responsible for the hypercapnia-induced vasodilation remains uncertain. Studies manipulating arterial pH, extravascular pH, and PaCO₂ proposed changes in extravascular pH to be responsible for the hypercapnia-induced vasodilation (18, 26). In the present study baseline pH values did not differ between age groups, suggesting and agreeing with the above that capillary pH may not be the main factor responsible for the decrease in MCAvmean in older individuals. It can, however, not be ruled out that extravascular pH may have been different in the two populations and thereby at least partly involved.

Trained study volunteers were included independent of their regimen of training conducted. This could have become a problem since it is still debated whether resistance training leads to increased arterial stiffness (31) or not (40). However, since none of the subjects were predominantly engaged in resistance training this is likely not to have influenced the study outcome. Since only male volunteers were examined this also implies that the obtained data may not necessarily be valid for female although differences are not apparent (30). The PaCO₂ values reported in the present study are derived from PETCO₂; however, there are studies reporting good correlations between the two. Also in the present study the actually obtained capillary blood gases correlated well with the PaCO₂ estimated from the obtained PETCO₂.

Conclusion. In conclusion, reduced CBF was associated with a lower PaCO₂ at rest and during exercise in older men. Supplemental CO₂ diminished the age-related reduction in CBF by ~50% during exercise. An elevated aerobic capacity did not lead to increased cerebrovascular health either in our young or in our older study participants. Finally, despite the maintained oxygenation during the CO₂ trial exercise performance did not improve, indicating decreased cerebral oxygenation not to be a limiting factor.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


