Cerebral oxygenation and metabolism during exercise following three months of endurance training in healthy overweight males

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Running title: Training and brain metabolism

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Abstract

Endurance training improves muscular and cardiovascular fitness, but the effect on cerebral oxygenation and metabolism remains unknown. We hypothesized that three months of endurance training would reduce cerebral carbohydrate uptake with maintained cerebral oxygenation during submaximal exercise. Healthy overweight males were included in a randomized, controlled study (training: n = 10; control: n = 7). Arterial and internal jugular venous catheterization was used to determine concentration differences for oxygen, glucose and lactate across the brain and the oxygen to carbohydrate index [molar uptake of oxygen / (glucose + ½lactate); OCI], changes in mitochondrial oxygen tension ($\Delta$P$_{\text{MitoO}_2}$) and the cerebral metabolic rate of oxygen (CMRO$_2$) were calculated. For all subjects, resting OCI was higher at the three-month follow-up (6.3 ± 1.3 compared to 4.7 ± 0.9 at baseline, mean ± SD; P < 0.05) and coincided with a lower plasma epinephrine concentration (P < 0.05). Cerebral adaptations to endurance training manifested when exercising at 70% of maximal oxygen uptake (~211 W). Before training, both OCI (3.9 ± 0.9) and $\Delta$P$_{\text{MitoO}_2}$ (-22 mmHg) decreased (P < 0.05), whereas CMRO$_2$ increased by 79 ± 53 micromol 100 g$^{-1}$ min$^{-1}$ (P < 0.05). At the three-month follow-up, OCI (4.9 ± 1.0) and $\Delta$P$_{\text{MitoO}_2}$ (-7 ± 13 mmHg) did not decrease significantly from rest and were than compared to before training (P < 0.05) and CMRO$_2$ did not increase. This study demonstrates that endurance training attenuates the cerebral metabolic response to submaximal exercise as reflected in a lower carbohydrate uptake and maintained cerebral oxygenation.

Key words: brain carbohydrate uptake, catecholamines, cerebral mitochondrial oxygen tension, oxygen to carbohydrate index
Introduction

Endurance training improves physical performance as reflected in enhanced muscle metabolism and cardio-vascular fitness. However, cerebral metabolism might also adapt to endurance training. Cerebral energy metabolism is covered almost exclusively by oxidation of glucose since the molar ratio between the brain oxygen (O$_2$) uptake to that of glucose (the O$_2$ - glucose index; OGI) is close to 6 (23). However, glucose is not the only substrate that supports brain metabolism since lactate is recognized as energy substrate for neurons (30). Therefore, the total amount of carbohydrate taken up by the brain relative to that of O$_2$ is considered when calculating changes in brain metabolism in the O$_2$ - carbohydrate index [OCI = O$_2$ / (glucose + ½ lactate)]. A ratio of ~5.7 is an often reported resting value although OCI may be as low as ~4 (32) and above 6 (23). During intense activation of the brain as exemplified by maximal whole body exercise, there is a consistent decrease in OCI to a lowest reported value of 1.7 since the cerebral carbohydrate uptake increases more than that of O$_2$ (39).

OCI decreases not only during exercise. In a positron emission tomography (PET) based evaluation of brain metabolism, OGI decreased in the visual cortex from a resting value of 4.1 to 2.8 in response to intense visual stimulation. Following arterial and internal jugular venous catheterization, there is some recovery in OCI, e.g. from ~4 to ~5 over an hour (32) suggesting that OCI is vulnerable to the discomfort associated with catheterization and the anxiety provoked when subjects are confined in a scanner. In support, short-term stress hormones appear responsible for the decrease in OCI. When infusion of epinephrine establish an arterial plasma concentration comparable to that elicited during exercise at 70% of maximal oxygen uptake (VO$_{2\text{max}}$) (15), OCI decreases (32). In contrast, a similar infusion of norepinephrine is without an effect on OCI supporting that a β$_2$-adrenergic receptor mechanism plays an important role in regulation of cerebral carbohydrate uptake (8; 17).
Besides carbohydrate, the brain relies on a continuous and uninterrupted supply of O2. During light to moderate submaximal exercise, cerebral oxygenation is elevated, whereas it decreases during maximal exercise (31; 36) and a decrease in cerebral oxygenation may limit exercise performance. Calculation of the cerebral mitochondrial O2 tension (P_{MitoO2}) integrates a global measure of cerebral oxygenation where a reduction in P_{MitoO2} of more than 5-6 mmHg is associated with elevated cerebral lactate production, a low OCI and development of fatigue (24; 31). Also, the global cerebral metabolic rate of O2 (CMRO2) remains stable during moderate exercise, but CMRO2 increases during strenuous exercise (28; 31).

Exercise is associated with activation of the sympathetic nervous system as reflected in an exponential rise in plasma catecholamines with work rate (16). In skeletal muscles, catecholamines enhances glucose and lactate turnover (16) and since brain tissue expresses adrenergic receptors (19), circulating catecholamines that crosses the blood brain barrier (35; 40), may influence cerebral carbohydrate metabolism. Based on the finding that OCI decreases in response to epinephrine administration (32) and with the assumption that the sympathetic response to exercise is attenuated following training (16), we hypothesized that endurance training would reduce cerebral carbohydrate uptake with maintained cerebral oxygenation during submaximal exercise. In addition, we considered that OCI decreases to a lower value during maximal exercise since plasma catecholamine concentrations increase to a greater level in trained subjects (16), and that termination of exercise is associated with a similar decrease in P_{MitoO2}. 
Materials and Methods

Seventeen sedentary healthy males participated in the study after written informed consent as approved by the local ethical committee (H-KF-2006-6443) in accordance with the principles established in The Declaration of Helsinki. The subjects were included in the study based on the following criteria: no use of medication, normal levels of fasting plasma glucose (≤ 5.6 mM), and arterial pressure (< 130/85 mmHg, systolic/diastolic, respectively) with no known predisposition to type 2 diabetes (no first-order relatives diagnosed with type 2 diabetes). In order to obtain a large effect of endurance training, the subjects were selected to be physically inactive as assessed by no involvement in regular physical training (judged by interview and a questionnaire) and to demonstrate a maximal oxygen uptake (VO2max) lower than 45 ml O2 per kg per minute. For purposes unrelated to the present report, we were also interested in the effect of training on fat metabolism and the subjects needed to be overweight (body mass index 25-30 kg per m²) with a body fat content of ~20%-25%. Upon inclusion in the study, the subjects were randomly assigned to either endurance training or to a control group. Accordingly, 10 subjects were assigned to endurance training (30 ± 5 years, 93 ± 9 kg, and 183 ± 7 cm) and 8 subjects were assigned to the control group (32 ± 6 years, 96 ± 7 kg, and 183 ± 4 cm). One subject in the control group decided to leave the study and his data were excluded from the analysis.

Pretesting

Graded exercise on a cycle ergometer (Ergometrix 800S, Ergo-line GmbH, Germany) was used to assess VO2max. In order to familiarize the subjects to ergometer cycling, the subjects performed two bouts of maximal exercise prior to the first determination of VO2max. Cycling began at 75 W and after 4 min, the workload was increased 25 W each minute until exhaustion. Pulmonary ventilation, VO2 and exhalation of carbon dioxide (VCO2) were registered every 10 s by
an on-line system (Oxycon Pro, Jaeger, Würzburg, Germany). Criteria for reaching VO₂max were a leveling off in VO₂ with increasing workload and a pulmonary ventilation resulting in a respiratory exchange ratio > 1.14. Heart rate (HR) was monitored by telemetry (WearLink® 31 transmitter, Polar Electro, Kempele, Finland) while body composition, fat mass, lean body mass, and total body mass were assessed by Dual Energy X-ray Absorptiometry (DEXA) scanning (Prodigy Bone Densiometer System, GE Lunar Corporation, Madison, WI and Lunar Prodigy Advance and enCORETM 2006 software ver.10.50.080).

**Endurance training**

The control subjects were asked to continue their (sedentary) lifestyle throughout the three months of study. They reported to the laboratory for determination of VO₂max before and after the intervention period lasting three months. The training group performed endurance type exercise seven days a week and each session lasted ~60 min or until the target energy expenditure of ~2500 kJ was reached. Since the procedures at baseline and at the three-month follow-up were performed on a cycle ergometer, the mode of exercise was primarily cycling, but the subjects were allowed to use other modes of exercise such as running or ergometer rowing. The exercise intensity was on average ~70% of maximal HR equivalent to ~65% of VO₂max. All training sessions were supervised for the first 2-3 weeks and the subjects wore the HR monitor during all training sessions to verify that the required intensity was achieved and also to estimate the average energy expenditure. The training intensity was adjusted to any changes in VO₂max.

**Procedures**

On the days of the study, the subjects had no restrictions in diet but abstained from strenuous physical activity on the previous day. Upon arrival at the laboratory, the subjects were
placed in a hospital bed and tilted slightly head-down. Under local anesthesia (lidocain, 2%) and
guided by ultrasound, a catheter (1.6 mm; ES-04706, Arrow International, PA) was inserted
retrograde in the right internal jugular vein and advanced to its bulb at the base of the skull. These
blood samples were considered to represent blood leaving the brain with a small contribution from
cerebrospinal fluid drained from the sinus sagitalis. A second catheter (1.1 mm) was inserted in the
left brachial artery. After catheterization, the subjects were placed supine and they recovered for 1 h
before exercise to offset changes in OCI caused by “arousal” and the nociceptive stimuli associated
with catheterization (6).

Experimental protocol

To evaluate brain metabolism during exercise, the subjects performed ergometer
cycling (Ergomedic 874E, Monarch, Stockholm, Sweden) before and after the intervention. Before
endurance training or the control period, the subjects cycled for 5 min at a light intensity whereafter
the workload was increased to 70% \( \text{VO}_{2}\text{max} \). That intensity was maintained for 15 min and blood
samples were obtained simultaneously from the brachial artery and the internal jugular vein after 5,
10 and 15 min. The subjects then rested for 30 min until the next exercise bout that included
incremental cycling. Subjects cycled for 4 min at 60%, 70%, 80%, 90% and 100% of \( \text{VO}_{2}\text{max} \) and
each 4-min bout was separated by 6 min of recovery and blood samples were obtained at the end of
each workload. This exercise protocol was repeated after the intervention, except that subjects
cycled for 30 min, with the first 15 min of cycling adjusted to the pre-training \( \text{VO}_{2}\text{max} \) (same
absolute intensity) and the last 15 min at an intensity corresponding to 70% \( \text{VO}_{2}\text{max} \) adjusted to the
post-endurance training \( \text{VO}_{2}\text{max} \) (same relative intensity). Otherwise the subjects performed the
same exercise protocol before and after the intervention.
**Measurements**

Mean blood velocity of the proximal segment of the left middle cerebral artery (MCA Vmean) was monitored by transcranial Doppler sonography through the posterior temporal ultrasound window at a depth of 48-60 mm (Multidop X, DWL, Sipplingen, Germany). Once the optimal signal to noise ratio was obtained, the probe (2-MHz and 20 mm in diameter) was mounted on a headband and acoustic coupling was secured by adhesive ultrasonic gel (Tensive, Parker Laboratories, Orange, NJ). MCA Vmean was calculated from the integral of the maximal frequency Doppler shifts over one heartbeat and thirty second averages was calculated to avoid variation.

Mean arterial pressure (MAP) was measured through a transducer (Edwards Life Sciences, Irvine, CA) placed at the level of the heart and connected to a monitor (Dialogue-2000 IBC-Danica Electronic, Denmark) with sampling at 100 Hz (Di-720, Dataq, Akron, OH) for off-line analysis of HR and cardiac output (CO), as assessed from the pressure curve using Modelflow (4) and the software was an online real-time version of Beatscope® (FMS, Amsterdam, The Netherlands). The method uses a non-linear three-element model of the aortic input impedance and simulates aortic flow waveforms from an arterial pressure signal. Aortic impedance and arterial compliance depend on the elastic properties of the aorta and calculations are based on the height, weight, age and gender of the subject. Integrating the aortic flow waveform per beat provides left ventricular stroke volume and thereby CO. HR, CO, MAP, and MCA Vmean were recorded throughout the experiment and averaged over the last 5 min during the 70% VO₂max exercise bout and over the last minute at each workload during the incremental exercise. At the end of each exercise load, the subjects expressed their rating of perceived effort (RPE) in a presentation of the Borg scale, which provides an estimate of the exercise intensity (5).

Blood samples were purged of any atmospheric content and immediately analyzed using an ABL 725 (Radiometer, Copenhagen, Denmark). Although pyruvate is a viable
carbohydrate source in fuelling cerebral activity, pyruvate was not included in the analysis based on the assumption that its uptake by the brain is at least an order of magnitude smaller than that of lactate (25). OCI and OGI were calculated and both ratios were considered to be independent of changes in cerebral blood flow (6). The fractional extraction of O₂, glucose and lactate was calculated as the arterial to jugular venous concentration difference (a-v diff) divided by the arterial concentration.

Calculations

Capillary O₂ saturation (S\textsubscript{cap}O₂) was calculated as

\[
S\textsubscript{cap}O_2 = \frac{S_aO_2 + S_vO_2}{2}.
\]

(12; 24). Based on the assumption that capillary recruitment does not manifest within the brain, the capillary O₂ tension (P\textsubscript{cap}O₂) is

\[
P\textsubscript{cap}O_2 = P_{50a}^{Hb} \sqrt{\frac{S\textsubscript{cap}}{1 - S\textsubscript{cap}}}
\]

where S\textsubscript{a}O\textsubscript{2} is the arterial O\textsubscript{2} saturation, S\textsubscript{v}O\textsubscript{2} the internal jugular venous O\textsubscript{2} saturation, P\textsubscript{50a}\textsuperscript{Hb} PO\textsubscript{2} when hemoglobin is half saturated and h\textsubscript{a} the Hill coefficient for arterial blood. The P\textsubscript{50a}\textsuperscript{Hb} was estimated on the ABL 725 (Radiometer) and h\textsubscript{a} was calculated.
PMitoO₂ depend on the balance between the O₂ supply, O₂ extraction, and O₂ conductance from the capillary to the mitochondria (L). PMitoO₂ is determined from PcapO₂, CMRO₂, and oxygen diffusibility (L).

\[
P_{\text{MitoO₂}} = P_{\text{capO₂}} - \frac{\text{CMRO}_2}{L}
\]

where CMRO₂ is derived from the cerebral arterial-venous difference for O₂ with a resting global CBF estimated to 46 ml 100 g⁻¹ min⁻¹ (18) adjusted in proportion to changes in MCA Vmean and assuming a constant vessel diameter (33). In a matching subject cohort L was calculated to be 4.4 µmol 100 g⁻¹ min⁻¹ mmHg⁻¹ (24). All variables at rest and during submaximal and maximal workloads represent averages over 30 sec obtained at the time of the blood sampling.

The analysis of plasma catecholamine concentration was performed by ¹²⁵I – Radioimmuno assay (Labor Diagnostica Nord, Nordhorn, Germany) according to the manufacturer’s guidelines. As soon as the blood samples were obtained, blood was centrifuged in EDTA tubes (3500 rpm for 15 min at 4 °C) and plasma was sampled and immediately stored at −80 °C for later analysis.
Statistics

A three-way analysis of variance (ANOVA) with repeated measures was performed with time (before vs. after), treatment (training vs. control) and mode (rest vs. various exercise intensities) as factors. In case of significant main effects, pair-wise multiple comparison procedures were performed by the Holm-Sidak method. Since we had plasma samples from only seven subjects in the training group and five in the control group, the subjects with no catecholamine analysis were excluded from that statistical analysis. Thus, twelve subjects were included in the comparison performed by a two-way ANOVA with repeated measures. Data are presented as means ± SD and in the figures as means ± SEM. P < 0.05 was considered statistically significant using SAS version 9.1 (SAS Institute Inc. Cary, N.C.).
Results

Exercise tolerance and hemodynamics

Endurance training caused a ~25% increase in VO$_2$max (from 3.4 ± 0.4 to 4.1 ± 0.3 l min$^{-1}$, P < 0.01), whereas VO$_2$max did not significantly change in the control subjects (from 3.4 ± 0.2 to 3.4 ± 0.1 l min$^{-1}$). The maximal workload accomplished by the trained subjects increased by ~20% (from 302 ± 45 to 365 ± 39 W, P < 0.05; Table 1) increasing the workload corresponding to 70% of VO$_2$max from 211 ± 31 to 256 ± 28 W (P < 0.05). Accordingly, RPE was lower during exercise at 211 W in the three-month follow-up [13 (10-15) vs. 16 (14-19), respectively; median (range); P < 0.05]. CO and HR increased from rest to submaximal exercise (P < 0.05; Table 1) with a further increase during maximal exercise. Both CO and HR were lower at 211 W following three months of endurance training (P < 0.05).

Arterial oxygen, glucose and lactate concentrations

The arterial O$_2$ content was elevated during exercise in both groups of subjects (Table 2). In subjects randomized to training, the arterial glucose concentration decreased during submaximal exercise both before and after the intervention (P < 0.05). Arterial lactate increased to the same extent during exercise in both groups of subjects, but endurance training attenuated the lactate production at 211 W (P < 0.05).

Cerebral metabolism

At the three-month follow-up, resting levels of a-v diff for O$_2$, glucose and lactate were similar to the baseline levels in both groups of subjects. This was also the case for the exercise-induced changes in the a-v diff for O$_2$, glucose and lactate (Table 2). Endurance training
reduced the a-v diff for O₂, glucose and lactate when exercising at 211 W. There was no significant effect of training in response to exercise at the same relative workload and at maximal intensity. In both groups of subjects the a-v diff for carbohydrate (glucose + ½ lactate) was reduced at rest at the three-month follow-up (P < 0.05). The reduced a-v diff for carbohydrate following training was also evident at 211 W.

The resting OCI remained unaffected following three months of training. Before training, exercise at 211 W caused a decrease in OCI (P < 0.05; Fig. 1), but at the three-month follow-up, OCI did not decrease at that workload and was higher as compared to before training (P < 0.05). When exercising at 70% of maximal workload adjusted to the improvement in exercise capacity (256 W), there was a decrease in OCI (P < 0.05). Also, during maximal exercise, OCI decreased to the same value both before and after three months of training (P < 0.05).

The control subjects had a higher resting OCI at the three-month follow-up (P < 0.05; Fig. 1). During exercise at 70% of maximal workload (~204 W) and during maximal exercise (~290 W), OCI decreased both before and after the intervention (P < 0.05; Fig. 1) and the decrease in OCI during submaximal exercise was comparable (25 ± 17% vs. 32 ± 23% before and after, respectively). During exercise, the arterial plasma concentrations of epinephrine and norepinephrine increased (P < 0.05) to the same extent before and after the intervention in both groups of subjects (Fig. 2).

When subjects from both groups were evaluated together, resting OCI and OGI were higher at the three-month follow-up (4.7 ± 0.9 and 4.6 ± 1.0 before vs. 6.3 ± 1.3 and 5.7 ± 1.1 after, respectively; P < 0.05, Fig. 3). Furthermore, the resting arterial plasma concentration of epinephrine was lower at the three-month follow-up (0.28 ± 0.11 nM) compared to baseline (0.34 ± 0.11 nM; n = 7 from the training group and n = 5 from the control group, P < 0.05) with no significant change in the resting arterial plasma norepinephrine concentration.
**Cerebral oxygenation**

In both groups of subjects, the cerebral capillary $O_2$ saturation and partial pressure ($S_{\text{cap}O_2}$ and $P_{\text{cap}O_2}$) decreased during submaximal and maximal exercise (Table 3). Before training, $\text{CMRO}_2$ increased and $P_{\text{Mito}O_2}$ decreased at 211 W ($P < 0.05$; Fig. 4), but there was no further increase in $\text{CMRO}_2$ or decrease in $P_{\text{Mito}O_2}$ during maximal exercise. At the three-month follow-up, $\text{CMRO}_2$ did not increase and there was no decrease in $P_{\text{Mito}O_2}$ at 211 W. However, at 256 W, the increase in $\text{CMRO}_2$ and the decrease in $P_{\text{Mito}O_2}$ were evident with no further change during maximal exercise. For the control group, $\text{CMRO}_2$ increased and $P_{\text{Mito}O_2}$ decreased to the same extent during submaximal and maximal exercise before and after the control period.
Discussion

The novel finding of this study is that brain metabolism adapts to endurance training when humans perform submaximal exercise. The OCI and $P_{\text{MitoO}_2}$ did not decrease and CMRO$_2$ did not increase at the workload that before training corresponded to 70% VO$_{2\text{max}}$ (211 W), whereas the decrease in OCI and $P_{\text{MitoO}_2}$ and the increase in CMRO$_2$ was maintained when the workload was adjusted to the increased exercise capacity (256 W). This was the case although three months of endurance training did not significantly affect the plasma catecholamine response to exercise. Another finding of this study is that subjects, independently of training, adapted to participating in an experiment involving catheterization with reduced anxiety. This was illustrated by a lower cerebral carbohydrate uptake and, thus, a higher OCI and OGI at the three-months follow-up compared to baseline. The higher OCI and OGI coincided with lower plasma epinephrine levels.

A typical response to strenuous exercise is a decrease in OCI caused by an increased cerebral carbohydrate uptake (glucose + $\frac{1}{2}$lactate) relative to that of O$_2$ (6), but the mechanism responsible for the decrease in OCI are not established. The decrease in OCI takes place independently of O$_2$ availability (39) and also in the absence of a significant increase in arterial glucose and lactate concentration during prolonged exercise (20). The decrease in OCI may be explained by intermittent glycogen synthesis and breakdown (34), but that hypothesis implies accumulation of lactate or efflux from the brain where OCI decreases mainly due to an increased cerebral lactate uptake. Furthermore, all lactate taken up by the brain is oxidized during resting conditions with the majority (~87%) also oxidized during intense exercise (38) and in the rat, cerebral glycogen turnover is slow during cerebral activation (10). The decrease in OCI may also be associated with the mental effort required to sustain exercise at a given intensity (7). At least mental stress and the associated release of stress hormones affect OCI and epinephrine may activate
cerebral glycolysis resulting in an increased energy turnover by astrocytes as reported for skeletal muscles and the liver (37).

Three months of endurance training influenced cerebral carbohydrate uptake during submaximal exercise. At the same absolute workload (211 W), OCI did not decrease and remained higher than the corresponding value before training, but when the workload was adjusted to the change in exercise capacity (256 W), OCI decreased to the same extent as before training (Fig. 1). This was in contrast to the observation in the control subjects for whom, although OCI was higher after the intervention, a decrease in OCI was observed both before and after the intervention at 70% of VO$_{2\text{max}}$. The higher OCI following training reflected an attenuated cerebral carbohydrate uptake (Table 2), while the lactate uptake remained proportional to its arterial concentration. Following training, the arterial lactate concentration was lower at a given workload, indicative of improved oxidative capacity in skeletal muscles and possibly enhanced lactate elimination (11). On that basis, the reduced cerebral lactate uptake in the training group was expected. The reduced cerebral glucose and O$_2$ uptake during submaximal exercise may reflect an adaptation to endurance training since the uptake increased even though the arterial concentrations were unchanged. The lower O$_2$ uptake may reflect a lower cerebral oxidative stress following training that is supported by the maintained P$_{\text{MitoO}_2}$, resulting in a reduced requirement for glucose in support of brain metabolism. Alternatively, it may be that the brain is capable of obtaining an increased metabolic capacity following endurance training, an adaptation comparable to skeletal muscles.

The higher OCI, the maintained P$_{\text{MitoO}_2}$ and the lack of increase in CMRO$_2$ at the same absolute workload (211 W) illustrate a reduced mental effort required to sustain exercise as reflected by a lower RPE following training (Table 2). The reduction in OCI may be linked to the development of central fatigue since OCI decreases when exercise becomes demanding and RPE exceeds 15 (7; 8), whereas OCI remains unaffected during light to moderate exercise corresponding
to 30-60% VO$_2$max or RPE below 15 (1). Similarly, a decrease in P$_{\text{MitO}_2}$ of more than 5-6 mmHg is associated with the development of central fatigue (21). In this study, there was an association between OCI and the change in P$_{\text{MitO}_2}$ (P = 0.05) and it may be that exercising at any given submaximal intensity before training is associated with a greater level of neural activation to sustain the required intensity and, thus, a greater central neural drive to the muscles. Conversely, feedback from the working muscles may affect the central nervous system in a way that affects performance. Attenuated feedback to the brain from opioid sensitive muscle afferents increases the mental effort of performing a maximal cycling time trial resulting in an overshoot of central neural drive (3). Also, inhibition in recruitment of active muscles is reduced when performing the same time trial with epidural anesthesia (2). Based on these results, we speculate that neural feedback from the exercising muscles in the untrained state is influenced by accumulation of metabolites in the working muscles, that such feedback affects the central motor drive and, consequently, decreases OCI. Endurance training may reduce the amount brain activation needed for a specific type of performance (27). Furthermore, verbal encouragement also influences performance (26) and highlights the potential influence of motivation and sensory input on performance and fatigue. Since the subjects were aware of the catheterization procedure and the exercise protocol at their second visit to the laboratory, they may have been more “relaxed” and hence needed less central activation both at “rest” and during submaximal bouts of exercise.

In contrast to our expectations, there was no effect of endurance training on OCI, ΔP$_{\text{MitO}_2}$ and plasma catecholamines during maximal exercise. Performing an unfamiliar task, such as arm cranking, reduces OCI less than leg exercise (9) and when maximal exercise is performed in a semi-supine position, OCI is reduced to a lesser extent compared to what is established during maximal ergometer cycling (7). The reduction in OCI observed in this study is, therefore, what could be expected when taking the low fitness level of the subjects into consideration. The lack of a
further decrease in OCI following training may reflect that the subjects did not increase their fitness level to a degree comparable to, e.g., well-trained rowers in whom OCI decreases to 1.7 during maximal ergometer rowing (39).

The higher resting OCI and OGI at the three-month follow-up occurred independently of training since it manifested mainly in the control group (Fig. 3). This may be interpreted as an attenuated stress response to the discomfort associated with the catheterization procedure. Epinephrine accelerates cerebral carbohydrate uptake (32) and the lower resting cerebral carbohydrate uptake at the three-month follow-up coincided with a lower plasma epinephrine level (Fig. 3). A correlation between plasma catecholamines and primary motor cortex activation has been established (29). However, other hormones related to the stress response such as glucocorticoids and vasopressin may influence brain metabolism and their response to the catheterization procedure should be considered when evaluating the cerebral metabolic response.

Limitations

We used transcranial Doppler as a measure of changes in global cerebral blood flow even though it tracks changes in blood velocity in the middle cerebral artery. However, the use of transcranial Doppler as a measure of flow is justified since increases in MCA Vmean during exercise are in parallel with the inflow of the internal carotid artery (13), with the “initial slope index” of the $^{133}$Xenon clearance-determined cerebral blood flow (14), and with regional cerebral blood flow measurements by PET (22). Also, the cerebral capillary and mitochondrial oxygenation is based on calculations from arterial and internal jugular blood samples rather than based on real measurements. There are many assumptions and possible errors in the estimation of the cerebral mitochondrial oxygen tension (12; 24). We acknowledge that the absolute values may not be
accurate, but the changes in $P_{\text{MitO}_2}$ are likely not an effect of the formalism and are taken to reflect real changes.

**Perspectives and Significance**

Brain metabolism has not previously been evaluated following endurance training in humans and we demonstrate that during submaximal, but not maximal exercise, the brain adapts to three months of endurance training with reduced carbohydrate uptake, maintained oxygenation and unchanged oxygen consumption possibly due to less central neural drive or attenuated feedback from the working muscles. Also, we observed a higher resting OCI and OGI when subjects reported to the laboratory and were catheterized for the second time. This likely reflects reduced anxiety since the plasma epinephrine level was lower on that occasion and suggests that subjects adapt to participating in experiments with reduced anxiety.

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Disclosure

The authors declare no conflicts of interest.
References


Figure legends.

**Figure 1.** The oxygen to glucose index (OGI; \( \frac{O_2}{\text{glucose}} \)) and the oxygen to carbohydrate index (OCI; \( \frac{O_2}{\text{glucose} + \frac{1}{2}\text{lactate}} \)) at rest and during exercise before (open circles), and after (filled circles) three months of endurance training (n = 10) or a three-month control period (n = 7). 211 W = 70% of the maximal workload established before training. 256 W = 70% of the maximal workload established after training. 204 W = 70% of the maximal workload established before and after the control period. * P < 0.05 vs. rest and † P < 0.05 vs. before. Values are mean ± SEM.

**Figure 2.** Arterial concentrations of epinephrine and norepinephrine at rest and during exercise before (open circles) and after (filled circles) three months of endurance training (n = 7) or a three-month control period (n = 5). 211 W = 70% of the maximal workload established before training. 256 W = 70% of the maximal workload established after training. 204 W = 70% of the maximal workload established before and after the control period. * P < 0.05 vs. rest. Values are mean ± SEM.

**Figure 3.** The oxygen to glucose index (OGI; \( \frac{O_2}{\text{glucose}} \)), the oxygen to carbohydrate index (OCI; \( \frac{O_2}{\text{glucose} + \frac{1}{2}\text{lactate}} \)) and the arterial epinephrine and norepinephrine concentration at rest before and after three months of endurance training and a control period. Data are pooled (n = 17 for OGI and OCI and n = 12 for epinephrine and norepinephrine). * P < 0.05 vs. before. Individual values are indicated with gray circles and group mean is black circles.

**Figure 4.** The cerebral metabolic rate of O\(_2\) (CMRO\(_2\)) and changes in the cerebral mitochondrial O\(_2\) tension (\(P_{\text{Mito}O_2}\)) at rest and during exercise before (open circles), and after (filled circles) three months of endurance training (n = 10) and a three-month control period (n = 7). 211 W = 70% of
the maximal workload established before training. 256 W = 70% of the maximal workload established after training. 204 W = 70% of the maximal workload established before and after the control period. * P < 0.05 vs. rest and † P < 0.05 vs. before. Values are mean ± SEM.
Table legends.

Table 1. Workload, rating of perceived exertion (RPE), heart rate (HR), cardiac output (CO), mean arterial pressure (MAP) and middle cerebral artery mean flow velocity (MCA Vmean) at rest and during submaximal and maximal exercise before and after three months of endurance training. Training group (n = 10); control group (n = 7). * P < 0.05 vs. rest, † P < 0.05 vs. before and # P < 0.05 vs. same absolute workload after training (211 W). Values are mean ± SD except RPE where values are median (range).

Table 2. Arterial concentrations, arterial to internal jugular venous concentration differences (a-v diff) and the fractional extraction of O₂, glucose and lactate at rest and during submaximal and maximal exercise before and after three months of endurance training. Training group (n=10); control group (n=7). * P < 0.05 vs. rest, † P < 0.05 vs. before, ‡P < 0.05 vs. same absolute workload before training, and # P < 0.05 vs. same absolute workload after training (211 W). Values are mean ± SD.

Table 3. Arterial pH, arterial hemoglobin concentration (Hb), arterial partial pressure of carbon dioxide (PₐCO₂), arterial and internal jugular venous partial pressures of oxygen (PₐO₂ and PᵥO₂), arterial and internal jugular venous O₂ saturation (SᵥO₂ and SₐO₂) and brain capillary oxygen saturation (ScapO₂) and partial pressure (PcapO₂) at rest and during submaximal and maximal exercise before and after three months of endurance training. Training group (n=10); control group (n=7). * P < 0.05 vs. rest. **P < 0.01 vs. rest. † P < 0.05 vs. before and # P < 0.05 vs. same absolute workload after training (211 W). Values are mean ± SD.
Table 1. Workload, rating of perceived exertion (RPE), heart rate (HR), cardiac output (CO), mean arterial pressure (MAP) and middle cerebral artery blood flow velocity (MCA Vmean) at rest and during submaximal and maximal exercise before and after three months of endurance training or a similar control period.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Same absolute workload</th>
<th>Same relative workload</th>
<th>Maximal workload</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Training (211 W)</td>
<td>Training (256 W)</td>
<td>Control (~204 W)</td>
<td>Training</td>
</tr>
<tr>
<td><strong>Workload (W)</strong></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>211 ± 31</td>
<td>211 ± 31</td>
<td>203 ± 17</td>
<td>205 ± 13</td>
</tr>
<tr>
<td><strong>RPE</strong></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>16 (14-19)</td>
<td>13 (10-15) †</td>
<td>16 (13-18)</td>
<td>15 (13-17)</td>
</tr>
<tr>
<td><strong>HR (beats per min)</strong></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>67 ± 8</td>
<td>66 ± 7</td>
<td>170 ± 11*</td>
<td>169 ± 14*</td>
</tr>
<tr>
<td></td>
<td>58 ± 7</td>
<td>65 ± 8</td>
<td>154 ± 14*†</td>
<td>171 ± 11*#</td>
</tr>
<tr>
<td><strong>CO (l per min)</strong></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>6.8 ± 0.6</td>
<td>6.4 ± 0.9</td>
<td>17.7 ± 1.2*</td>
<td>18.2 ± 2.0*</td>
</tr>
<tr>
<td></td>
<td>6.9 ± 1.0</td>
<td>6.9 ± 0.8</td>
<td>16.8 ± 1.7*†</td>
<td>17.7 ± 2.2*</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>90 ± 4</td>
<td>89 ± 4</td>
<td>107 ± 11*</td>
<td>109 ± 10*</td>
</tr>
<tr>
<td></td>
<td>89 ± 4</td>
<td>88 ± 7</td>
<td>98 ± 5*</td>
<td>102 ± 10*</td>
</tr>
<tr>
<td><strong>MCA Vmean (cm per sec)</strong></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>55 ± 6</td>
<td>52 ± 9</td>
<td>59 ± 6</td>
<td>52 ± 7</td>
</tr>
<tr>
<td></td>
<td>56 ± 4</td>
<td>52 ± 8</td>
<td>55 ± 8</td>
<td>55 ± 9</td>
</tr>
<tr>
<td></td>
<td>60 ± 2</td>
<td>50 ± 7</td>
<td>50 ± 7</td>
<td>50 ± 7</td>
</tr>
</tbody>
</table>
Table 2. Arterial concentrations, arterial to internal jugular venous concentration differences (a-v diff) and the fractional extraction of O2, glucose and lactate during rest, submaximal and maximal exercise before and after three months of endurance training or a similar control period.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Same absolute workload</th>
<th>Same relative workload</th>
<th>Maximal workload</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Training</td>
<td>Control</td>
<td>Training</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>(211 W)</td>
<td>(256 W)</td>
<td>(~204 W)</td>
<td></td>
</tr>
<tr>
<td>Arterial O2 (mM)</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>8.49 ± 0.53</td>
<td>8.49 ± 0.57</td>
<td>8.49 ± 0.53</td>
<td>9.31 ± 0.60*</td>
</tr>
<tr>
<td>Arterial Glucose (mM)</td>
<td>6.16 ± 0.64</td>
<td>6.22 ± 0.61</td>
<td>6.22 ± 0.61</td>
<td>9.39 ± 0.63*</td>
</tr>
<tr>
<td>Arterial Lactate (mM)</td>
<td>1.05 ± 0.36</td>
<td>1.29 ± 0.48</td>
<td>1.04 ± 0.48</td>
<td>9.61 ± 3.13*</td>
</tr>
<tr>
<td>A-V diff O2 (mM)</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>2.89 ± 0.59</td>
<td>2.72 ± 0.34</td>
<td>2.89 ± 0.59</td>
<td>2.49 ± 0.60*</td>
</tr>
<tr>
<td>A-V diff Glucose (mM)</td>
<td>0.65 ± 0.17</td>
<td>0.53 ± 0.16</td>
<td>0.65 ± 0.17</td>
<td>0.58 ± 0.15*</td>
</tr>
<tr>
<td>A-V diff Lactate (mM)</td>
<td>-0.04 ± 0.07</td>
<td>-0.01 ± 0.12</td>
<td>-0.04 ± 0.07</td>
<td>-0.04 ± 0.11</td>
</tr>
<tr>
<td>A-V diff Glucose + ½Lactate (mM)</td>
<td>0.63 ± 0.18</td>
<td>0.54 ± 0.18†</td>
<td>0.63 ± 0.18</td>
<td>0.56 ± 0.17</td>
</tr>
<tr>
<td>E_oxygen (%)</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>34 ± 6</td>
<td>32 ± 3</td>
<td>34 ± 6</td>
<td>29 ± 6</td>
</tr>
<tr>
<td>E_glucose (%)</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>11 ± 3</td>
<td>9 ± 3</td>
<td>11 ± 3</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>E_lactate (%)</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>-5 ± 11</td>
<td>-1 ± 7</td>
<td>-5 ± 11</td>
<td>-7 ± 5</td>
</tr>
</tbody>
</table>
Table 3. Arterial pH, arterial hemoglobin (Hb) concentration, arterial partial pressure of carbon dioxide (PaCO₂), arterial and internal jugular venous partial pressures of oxygen (PaO₂ and PvO₂), arterial and internal jugular venous O₂ saturation (SaO₂ and SvO₂) and brain capillary oxygen saturation (ScapO₂) and partial pressure (PcapO₂) at rest and during submaximal and maximal exercise before and after three months of endurance training or a similar control period.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Same absolute workload</th>
<th>Same relative workload</th>
<th>Maximal workload</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Training</td>
<td>Control</td>
<td>Training (211 W)</td>
<td>Control (~204 W)</td>
</tr>
<tr>
<td>Arterial pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>7.41 ± 0.01</td>
<td>7.41 ± 0.01</td>
<td>7.34 ± 0.01*</td>
<td>7.30 ± 0.04*</td>
</tr>
<tr>
<td>After</td>
<td>7.41 ± 0.01</td>
<td>7.41 ± 0.01</td>
<td>7.39 ± 0.02†</td>
<td>7.37 ± 0.04*</td>
</tr>
<tr>
<td>Arterial Hb (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>8.6 ± 0.5</td>
<td>8.8 ± 0.4</td>
<td>9.7 ± 0.7*</td>
<td>9.7 ± 0.7*</td>
</tr>
<tr>
<td>After</td>
<td>8.6 ± 0.6</td>
<td>8.5 ± 0.6</td>
<td>9.7 ± 0.6*</td>
<td>9.4 ± 0.7</td>
</tr>
<tr>
<td>PₐCO₂ (kPa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>5.13 ± 0.29</td>
<td>5.27 ± 0.32</td>
<td>4.53 ± 0.33*</td>
<td>4.74 ± 0.31*</td>
</tr>
<tr>
<td>After</td>
<td>5.35 ± 0.32</td>
<td>5.21 ± 0.27</td>
<td>4.95 ± 0.34</td>
<td>4.71 ± 0.24*</td>
</tr>
<tr>
<td>PₐO₂ (kPa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>14.2 ± 2.2</td>
<td>11.8 ± 0.7</td>
<td>13.4 ± 1.4</td>
<td>13.0 ± 1.3</td>
</tr>
<tr>
<td>After</td>
<td>13.0 ± 1.5</td>
<td>13.0 ± 2.3</td>
<td>12.4 ± 0.7</td>
<td>12.6 ± 0.7</td>
</tr>
<tr>
<td>SvO₂ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>98.4 ± 0.9</td>
<td>98.4 ± 0.4</td>
<td>97.7 ± 0.9</td>
<td>97.9 ± 0.8</td>
</tr>
<tr>
<td>After</td>
<td>98.3 ± 0.8</td>
<td>98.3 ± 0.9</td>
<td>98.0 ± 0.6</td>
<td>98.0 ± 0.9</td>
</tr>
<tr>
<td>SvO₂ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>64.9 ± 5.2</td>
<td>72.0 ± 10.4</td>
<td>55.9 ± 8.5*</td>
<td>63.8 ± 10.1</td>
</tr>
<tr>
<td>After</td>
<td>66.2 ± 3.0</td>
<td>65.0 ± 6.8</td>
<td>62.9 ± 6.2</td>
<td>61.3 ± 4.0</td>
</tr>
<tr>
<td>ScapO₂ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>82 ± 3</td>
<td>85 ± 5</td>
<td>77 ± 4*</td>
<td>81 ± 5*</td>
</tr>
<tr>
<td>After</td>
<td>82 ± 2</td>
<td>82 ± 3</td>
<td>80 ± 3*†</td>
<td>76 ± 2*</td>
</tr>
<tr>
<td>PcapO₂ (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>44 ± 3</td>
<td>50 ± 9</td>
<td>40 ± 3*</td>
<td>44 ± 6*</td>
</tr>
<tr>
<td>After</td>
<td>45 ± 2</td>
<td>44 ± 3</td>
<td>43 ± 3*†</td>
<td>39 ± 2*</td>
</tr>
</tbody>
</table>
Figure 4

Training

Control

CMRO$_2$ [µmol 100g$^{-1}$ min$^{-1}$]

 lowers

△PMiO$_2$ [mmHg]

Rest 21 W 24 W MAX

Rest 24 W MAX

* Before

- After