MCA V\text{mean} and the arterial lactate-to-pyruvate ratio correlate during rhythmic handgrip

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Rasmussen, Peter, Peter Plomgaard, Rikke Krogh-Madsen, Yu-Sok Kim, Johannes J. van Lieshout, Niels H. Secher, and Børn Quistorff. MCA V\text{mean} and the arterial lactate-to-pyruvate ratio correlate during rhythmic handgrip. J Appl Physiol 101: 1406–1411, 2006. First published June 22, 2006; doi:10.1152/japplphysiol.00423.2006.—Regulation of cerebral blood flow during physiological activation including exercise remains unknown but may be related to the arterial lactate-to-pyruvate (L/P) ratio. We evaluated whether an exercise-induced increase in middle cerebral artery mean velocity (MCA V\text{mean}) relates to the arterial L/P ratio at two plasma lactate levels. MCA V\text{mean} was determined by ultrasound Doppler sonography at rest, during 10 min of rhythmic handgrip exercise at ∼65% of maximal voluntary contraction force, and during 20 min of recovery in seven healthy male volunteers during control and a ∼15 mmol/l hyperglycemic clamp. Cerebral arteriovenous differences for metabolites were obtained by brachial artery 1 and retrograde jugular venous catheterization. Control resting arterial lactate was 0.78 ± 0.09 mmol/l (mean ± SE) and pyruvate 55.7 ± 12.0 μmol/l (L/P ratio 16.4 ± 1.0) with a corresponding MCA V\text{mean} of 46.7 ± 4.5 cm/s. During rhythmic handgrip the increase in MCA V\text{mean} to 51.2 ± 4.6 cm/s was related to the increased L/P ratio (23.8 ± 2.5; P = 0.79; P < 0.01). Hyperglycemia increased arterial lactate and pyruvate to 1.9 ± 0.2 mmol/l and 115 ± 4 μmol/l, respectively, but it did not significantly influence the L/P ratio or MCA V\text{mean} at rest or during exercise. Conversely, MCA V\text{mean} did not correlate significantly, neither to the arterial lactate nor to the pyruvate concentrations. These results support that the arterial plasma L/P ratio modulates cerebral blood flow during cerebral activation independently from the plasma glucose concentration.

cerebral blood flow; cerebral activation; exercise; hyperglycemia; transcranial ultrasound Doppler

GENERALLY PHYSIOLOGICAL ACTIVATION increases tissue energy metabolism and blood flow (11, 12, 19). Although global cerebral blood flow (CBF) is maintained relatively stable, the regional flow increases during exercise in activated areas of the brain, e.g., as accessed by the transcranial ultrasound Doppler (TCD) sonography determined mean blood flow velocity in basal arteries (V\text{mean}) (21), by functional magnetic resonance imaging or by positron emission tomography (25). A variety of factors contribute to flow regulation such as mean arterial pressure (MAP), cardiac output (CO) (17), local vasodilator compounds including ATP and adenosine (15), the arterial carbon dioxide (P\text{ACO}_2) (23, 29) and oxygen (P\text{AO}_2) (23) tensions, the arterial glucose (31) and lactate content (18), and the arterial plasma lactate-to-pyruvate ratio (L/P ratio) (18, 27, 32). The separate contribution of each of these mechanisms to blood flow control during physiological activation of the brain, however, is uncertain. Thus increased regional blood flow appears uncoupled from metabolic rate because oxygen and glucose provision increase more than consumption (6, 11, 12).

In an upright position MAP is important for cerebral perfusion pressure because the cerebral circulation is not supported by a siphon (7, 13, 14). However, during exercise alterations in MAP do not seem to play a significant role because, although MAP does increase during static exercise, middle cerebral artery (MCA) V\text{mean} is elevated only for some 3 s (28) and then decreases to the resting level for the remaining exercise bout. MCA V\text{mean} also remains unchanged during postexercise muscle ischemia, despite MAP remaining elevated by ∼40% (20). Furthermore, the increase in MCA V\text{mean} is confined to the contralateral hemisphere of the exercising arm (20). The increase in MCA V\text{mean} upon exercise depends on the concomitant increase in CO, as illustrated by the inability of patients with atrial fibrillation to increase MCA V\text{mean} unless CO increases as well (16). The cerebral circulation is reactive to changes in P\text{ACO}_2, at rest (23) and during exercise (29) and P\text{ACO}_2 increases during light to moderate dynamic exercise along with MCA V\text{mean} whereas both variables decrease during intense exercise (21, 26). However, during rhythmic handgrip there is a similar increase in MCA V\text{mean} although P\text{ACO}_2 does not change significantly, indicating that the MCA V\text{mean} response to exercise does not depend on P\text{ACO}_2 (20).

Increased blood flow and reduced cerebral autoregulatory capacity develop in response to elevated plasma glucose (22). For example, hyperglycemia increases forearm blood flow independently of sympathetic activity (31). Also, the L/P ratio takes part in regulation of blood flow in activated tissue such as brain and muscle (18, 19, 27, 32). To the extent allowed by the respective monocarboxylate carriers, plasma lactate and pyruvate equilibrate with the cytosolic concentrations of the two metabolites. These are, in turn, believed to be in near equilibrium with the cytosolic free NADH and NAD\text{+} concentrations via the reaction catalyzed by lactate dehydrogenase. During activation of the brain an increased rate of glycolysis may lead to increased L/P ratio of the cytosol, which could provide a signal for flow increase, either locally or transmitted downstream via the blood or lymph to other tissues. Similarly,
increased lactate production in activated muscle may contribute to an increase in brain blood flow through an increased L/P ratio of venous blood draining from the muscle and transmitted to the brain (27). It has been suggested that the mechanistic coupling between the L/P ratio and flow is via changes in ratio of cytosolic free NAD+ and NADH concentrations affecting the nitric oxide synthase system in the endothelial cells (18, 19, 27, 32).

We manipulated arterial L/P ratio with rhythmic handgrip exercise and evaluated its relationship to MCA Vmean. The relationship between MCA Vmean and the L/P ratio was further studied at different levels of arterial lactate. This was obtained by clamping arterial glucose at euglycemic (~5 mmol/l) and hyperglycemic (15 mmol/l) levels, while choosing an exercise protocol in which changes in PaCO2 are limited (2).

**METHODS**

Seven healthy men at an age of 22–29 yr participated in this study (body weight 80.9 (12.2 SD) kg, height 185.2 (10.8 SD) cm). The subjects underwent a clinical examination and it was verified that renal, hepatic, and thyroid function, hemoglobin, white blood cells counts, plasma electrolytes, and resting plasma glucose and insulin were normal. The subjects provided informed consent to the study as approved by the Ethical Committee of Copenhagen and Frederiksborg (KF 01-257245).

**Experimental design.** Before the experiment the subjects were familiarized with the protocol and individual maximal voluntary handgrip contraction was determined. On the following day the subjects reported to the laboratory after an overnight fast. Under local anesthesia (2%, lidocaine), a catheter was placed retrograde with Seldinger technique in the right internal jugular vein (1.6 mm, 14 gauge; ES-04706, Arrow International). Placement of the catheter was guided by an ultrasound image, and the catheter was subsequently advanced to the bulb of the vein. Arterial blood was drawn from a catheter in the brachial artery (1.1 mm, 20 gauge) of the nondominant arm. Cerebral perfusion was evaluated by TCD (Transcan, EME, Uberlingen, Germany) in the contralateral MCA. Depending on the position with the best signal-to-noise ratio, the proximal part of the MCA was insonated at a depth of 40–60 mm from the temporal bone and the probe was secured with a headband. Using TCD for evaluation of cerebral perfusion has several advantages compared with image-diagnostic methods such as positron emission tomography or infusion of tracer substances. Being noninvasive and robust against movement artifacts, TCD is used to gauge brain activation paradigms such as whole body exercise that cannot be evaluated inside a scanner. MAP was measured with a transducer (Edwards Life Sciences, Irving, CA) placed at the level of the heart. Blood pressure data were interfaced with a Dialogue-2000 (IBC-Danica), sampled at 200 Hz (DI-720, Datasq) and stored. Offline the arterial pressure waveform was analyzed for changes in left ventricular stroke volume (SV) and heart rate (HR) by using Modelflow (Beatscope software, BMI-TNO, The Netherlands). The SV was computed by simulation of a nonlinear, time-varying model of the aortic input impedance (3). The CO was the product of SV and HR, and the modeled flow tracks changes in SV and CO with a ~2% accuracy (34).

After this preparation the subjects rested in a semisupine position with the back and head supported at an angle of ~45° for 1½ h. Following sampling of blood and cerebral and systemic circulatory variables, the subjects performed 10 min of rhythmic handgrip exercise at 65% of the maximal voluntary contraction in that 2 s of contraction was followed by 4 s of recovery. During handgrip exercise rating of perceived exertion (RPE; Borg 6–20 scale) (4) was noted every minute. Blood and circulatory variables were sampled after 5 and 9 min of handgrip exercise and after 1, 3, 7, and 20 min of recovery. After 30 min of recovery, the arterial plasma glucose was clamped at ~15 mmol/l, and after 3 h of hyperglycemic clamp the handgrip exercise protocol was repeated.

**Hyperglycemic clamp.** The hyperglycemic clamp was established according to DeFronzo et al. (8). Glucose (1.0 mol/l) was infused intravenously to maintain a blood glucose level of 15 mmol/l with the rate of infusion adjusted by a computer-controlled infusion pump according to the arterial blood glucose level. To maintain potassium at the baseline value, isotonic saline with potassium (51 mmol/l) was infused continuously. In addition, the catheters were kept open by flushing a total of ~1,000 ml of isotonic saline during the study.

**Blood samples.** Samples for measurement of glucose and potassium concentrations were taken every 10 min, and steady-state hyperglycemia were reached after ~1 h. Blood was sampled in preheparinized syringes and arterial and venous hemoglobin saturation and O2 content as well as pH and PaCO2 were obtained immediately on an ABL 725 apparatus (Radiometer, Copenhagen, Denmark). CO2 content was calculated according to Douglas et al. (9). EDTA plasma was analyzed enzymatically for glucose, lactate, and pyruvate on an automatic analyzer (Cobas Faro, Roche, Basel, Switzerland).

**Statistical analysis.** A statistical package was used for the analysis (SigmaStat, Build 3.0, SPSS, Chicago, IL) and a P value < 0.05 was considered statistically significant. Two-way ANOVA for repeated measures was used to identify differences across glucose levels and time. Post hoc pairwise multiple comparisons vs. rest were performed with the Holm-Sidak method. Correlation strength was assessed with Pearson’s test, and data are presented as means ± SE except RPE scores, presented as median and range.

**RESULTS**

**Cardiovascular variables.** HR was 66 ± 2 beats/min, CO 5.5 ± 0.3 l/min, and MAP 95 ± 3 mmHg and increased during exercise (P < 0.001; Table 1) associated with no significant changes in PaCO2 or arterial pH. During hyperglycemia PaCO2 (P < 0.001) and pH (P < 0.01) were lower, and HR (P < 0.01) and CO (P < 0.001) were higher, while MAP remained unaffected. Both during exercise and in the recovery all variables showed similar responses as established during and after normoglycemic exercise.

**Perceived exertion.** During control exercise, RPE increased from 11 (7–13) after 1 min of exercise and to 17 (14–18) after 9 min (P < 0.001). Hyperglycemia had no effect on the RPE score.

**Cerebral oxygen, glucose, lactate, and pyruvate extraction.** The cerebral arteriovenous (a-v) difference for oxygen tended to declined (but not significantly) during exercise but increased in the early recovery (P < 0.01; Table 2). Conversely, the cerebral a-v difference for both lactate (−0.06 ± 0.01 to 0.01 ± 0.02 mmol/l; P < 0.01) and pyruvate (−9.9 ± 2.2 to 5.8 ± 2.5 μmol/l; P < 0.001) increased with handgrip exercise and returned to baseline 20 min after exercise. There was no significant change in the glucose a-v difference.

Throughout hyperglycemia the cerebral a-v difference for oxygen was lower (P < 0.01), whereas the a-v differences for lactate (P < 0.01) and pyruvate (P < 0.05) were higher but displayed the same response to handgrip as during control exercise. Hyperglycemia had no significant effect on the cerebral a-v difference for glucose (Table 2).

**Cerebral metabolic ratios.** No significant changes in the cerebral metabolic ratios of oxygen-to-carbohydrate or carbon dioxide-to-carbohydrate were observed during the handgrip exercise (Table 2). Hyperglycemia had no significant effect on the cerebral metabolic ratios.
MCA $V_{\text{mean}}$ in relation to arterial and venous L/P ratio.

Resting arterial lactate was 0.8 ± 0.1 mmol/l, pyruvate was 56 ± 12 μmol/l, and the L/P ratio 16.4 ± 1.0 with a corresponding MCA $V_{\text{mean}}$ of 46.7 ± 4.5 cm/s. During rhythmic handgrip arterial plasma lactate increased and reached a zenith at 1.5 ± 0.2 mmol/l after 9 min ($P < 0.001$ vs. preexercise) and decreased during the recovery to the baseline value (0.8 ± 0.1 mmol/l) after 20 min (Fig. 1). Conversely, the arterial pyruvate concentration did not increase during the first 5 min of exercise, but it did increase after exercise with a peak after 3 min of recovery (84 ± 15 μmol/l; $P < 0.001$ vs. preexercise). The arterial pyruvate concentration returned to baseline (58 ± 11 μmol/l) after 20 min of recovery.

The L/P ratio increased with handgrip exercise to 23.8 ± 2.5 ($P < 0.001$) after 5 min together with MCA $V_{\text{mean}}$ (to 51.2 ± 4.6 cm/s; $P < 0.01$) and the L/P ratio remained elevated until 3 min of recovery. Thus MCA $V_{\text{mean}}$ and the L/P ratio correlated ($r = 0.89; P < 0.01$; Fig. 2). The L/P ratio did not change between the arterial and the venous blood coming from the brain.

During hyperglycemia arterial lactate (1.9 ± 0.2 mmol/l; $P < 0.01$) and pyruvate (115 ± 4 μmol/l; $P < 0.01$) were higher compared with control exercise, but the response to exercise was similar to that established during control exercise. Neither the L/P ratio nor MCA $V_{\text{mean}}$ were, however, significantly elevated at baseline compared with control exercise nor was the response to exercise different from that observed during control exercise (Fig. 1). Although there was no significant correlation between MCA $V_{\text{mean}}$ and the L/P ratio during hyperglycemia ($r = 0.70; P = 0.06$; Fig. 2), the combined normo- and hyperglycemic data did correlate ($r = 0.70; P < 0.01$).

### DISCUSSION

CBF is under influence of numerous factors during exercise, e.g., MAP, CO, and $P_{\text{aco}}$, but no single variable accounts for the increase in regional CBF in response to cerebral activation. The arterial L/P ratio has been proposed as a regulating parameter (18, 27) that could account for the increased regional flow to activated regions of the brain. The present study extends this literature by showing that, in response to exercise, blood flow in the territory of a large cerebral artery changes together with plasma L/P changes. An additional finding is that this relationship is unaffected by the changes in lactate and pyruvate levels induced by hyperglycemia.

### Table 2. Cerebral arterio venous differences and metabolic ratios

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Rest</th>
<th>5 min</th>
<th>9 min</th>
<th>11 min</th>
<th>13 min</th>
<th>17 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>N</td>
<td>3.3±0.1</td>
<td>3.0±0.3</td>
<td>2.9±0.2</td>
<td>3.6±0.2</td>
<td>3.5±0.4</td>
<td>3.4±0.3</td>
</tr>
<tr>
<td>HY**</td>
<td>2.5±0.2</td>
<td>2.6±0.2</td>
<td>2.6±0.2</td>
<td>2.9±0.2</td>
<td>2.9±0.2</td>
<td>2.9±0.2</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>N</td>
<td>0.06±0.08</td>
<td>0.05±0.04</td>
<td>0.05±0.03</td>
<td>0.57±0.04</td>
<td>0.64±0.06</td>
<td>0.54±0.04</td>
</tr>
<tr>
<td>HY</td>
<td>0.62±0.20</td>
<td>0.13±0.33</td>
<td>0.43±0.15</td>
<td>0.53±0.22</td>
<td>0.09±0.17</td>
<td>0.44±0.10</td>
<td>0.49±0.14</td>
</tr>
<tr>
<td>Pyruvate, μmol/l</td>
<td>N</td>
<td>0.00±0.01</td>
<td>0.04±0.01</td>
<td>0.00±0.02</td>
<td>0.01±0.02</td>
<td>0.00±0.02</td>
<td>-0.03±0.01</td>
</tr>
<tr>
<td>HY**</td>
<td>0.03±0.01</td>
<td>0.05±0.02</td>
<td>0.09±0.04</td>
<td>0.05±0.03</td>
<td>0.04±0.05</td>
<td>0.03±0.03</td>
<td>0.04±0.02</td>
</tr>
<tr>
<td>$O_2$</td>
<td>N</td>
<td>1.4±1.5</td>
<td>6.6±12</td>
<td>6.8±0.8</td>
<td>6.2±0.4</td>
<td>5.6±0.5</td>
<td>7.0±0.5</td>
</tr>
<tr>
<td>Glu+/1/5Lac+1/5Pyr</td>
<td>N</td>
<td>6.4±0.7</td>
<td>7.4±0.2</td>
<td>6.8±1.0</td>
<td>6.2±0.4</td>
<td>5.6±0.5</td>
<td>7.0±0.5</td>
</tr>
<tr>
<td>$CO_2$</td>
<td>N</td>
<td>6.4±0.7</td>
<td>7.4±0.2</td>
<td>6.8±1.0</td>
<td>6.2±0.4</td>
<td>5.6±0.5</td>
<td>7.0±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 7$ subjects. $O_2$, oxygen; Glu, glucose; Lac, lactate; Pyr, pyruvate; $CO_2$, carbon dioxide. Time relative to exercise start. *, † and ‡P < 0.05, 0.01, and 0.001, respectively, vs. normoglycemia or rest. Symbols at HY indicate differences between N and HY trials.
Exchange of lactate and pyruvate between plasma and the intracellular compartment represents a means of organ-organ interaction on the metabolic level. A well-known example of such interorgan metabolic coupling is the Cori cycle, in which the plasma-carried substrates lactate, pyruvate, and alanine institute a close metabolic coupling between the energy metabolism of the muscle and the pathways of gluconeogenesis, lipogenesis, and ureagenesis in the liver. Similarly, major organ-organ interactions via lactate are demonstrated between muscle and brain (6). It is less clear how organ-organ coupling at the level of the cytosolic redox state plays a regulatory role, albeit almost all major pathways of the cell may be affected by changes in the redox state. It is, therefore, an attractive idea, first suggested by Ido et al. (19), that the increase in plasma L/P ratio occurring in working skeletal muscle may induce appropriate flow regulation in other organs. NADH is a candidate as a sensor of cellular energy need and also a possible mediator of tissue blood flow (19). In all major tissues, including muscle, liver, heart, erythrocytes, and brain, the cytosolic enzyme lactate dehydrogenase is abundantly expressed and is believed to be in a near equilibrium state (18). Thus the L/P ratio of the cytosol of a given cell can be expected to reflect the cytosolic NADH-to-NAD⁺ ratio multiplied by the LDH equilibrium constant, assuming one single cytosolic compartment of the individual cell.

To what extent the extracellular (plasma) L/P ratio is a reflection of the intracellular L/P ratio is unknown but would...
be expected to depend on the activity and kinetics of the relevant monocarboxylate carriers (33). Conversely, for the plasma L/P ratio to elicit a flow regulatory effect, lactate and pyruvate have to enter the intracellular compartment of the endothelium, the glia cells, and/or the neurons to modulate the cytosolic NADH-to-NAD⁺ ratio and thereby affect the vasocostriction systems. Table 2 demonstrates a positive arteriovenous difference for both pyruvate and lactate, indicating their uptake in the brain, and these events were correlated with increased MCA V̇mean. Figure 1 further demonstrates how the arterial L/P ratio increases during handgrip and Fig. 2 how the change correlates with MCA V̇mean.

However, CO is coupled to the exercise, as is the rise of lactate and the L/P ratio, and the blood flow to motor cortex. The coupling between regional CBF and exercise has not been elucidated, and it might be the NADH-to-NAD⁺ ratio or the NADH concentration, or both, as related to the L/P ratio, that determine the magnitude of blood flow. The present data only present a correlation and a role of systemic L/P changes in localized effect in the brain is speculative. On the other hand, this study expands the findings of previous reports by observing that increased lactate with unchanged L/P ratio does not cause a flow increase in the brain. This finding is significant because it indicates that an increase in lactate alone does not qualify as signal for increased CBF.

In order for the plasma L/P ratio to qualify as a signal for flow increase of the brain, the CBF and the L/P ratio must not only correlate. The plasma L/P ratio change must also precede the flow changes. During intense activation of the brain, the flow increases within seconds (10), but the temporal resolution of the measurements of the present study was insufficient to verify that notion. With the exercise protocol of the present study arterial lactate and pyruvate are not expected to change until 20–30 s after initiation of handgrip (1). Thus, during the first part of the activation, the observed increase in cerebral perfusion is not likely to be driven by muscle-derived L/P ratio changes but rather by regional cerebral metabolism. Thereafter it could be modulated by the arterial L/P ratio. The time resolution of the measurements does not allow conclusions as to whether the increase in CBF occurred before the L/P ratio increase, and our findings therefore do not provide direct evidence of a causal relationship between increased plasma L/P ratio and MCA V̇mean. It should be noted that the jugular venous L/P ratio does not differ from the arterial L/P ratio, indicating that the brain does not contribute to L/P changes in the blood in the present experiment.

Diabetes mellitus is associated with increased blood plasma glucose and is connected with increased blood flow and reduced cerebral autoregulatory capacity (22). These perturbations of blood flow could be due to a permanent change in the L/P ratio. In humans and animals hyperglycemia increases arterial lactate concentration (2), L/P ratio (24), and blood flow (31). The present study is compatible with the idea that the flow perturbations associated with diabetes mellitus are related to a permanently increased L/P ratio. However, hyperglycemia failed to mimic this aspect of diabetes mellitus as the L/P ratio was not elevated.

MCA is robust to changes in PaCO₂ (5, 30), and PaCO₂ did not change with exercise; we regard MCA V̇mean as a valid measure of CBF. We consider it therefore unlikely that the changes in flow were due to a CO₂-elicited vasodilatation or constriction.

The present study supports the notion of a correlation between cerebral perfusion and arterial L/P ratio.

**GRANTS**

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