LETTERS TO THE EDITOR

Effects of Training of Short Duration on the Levels of Zinc and Carbonic Anhydrase Isoenzymes in Human Erythrocytes

Keywords: Training—Exercise—Zinc—Carbonic Anhydrase Isoenzymes—pH—Erythrocytes

Carbonic anhydrase (EC 4.2.1.1, CA) is a zinc metalloenzyme which reversibly catalyzes the hydration of carbon dioxide to bicarbonate and hydrogen ions; that is, CA plays an important role in gas transport and acid/base equilibrium. In erythrocytes, CA isoenzymes have been designated as B type (CA-B) and C type (CA-C) according to immunological and genetic criteria. Our recent studies showed that although the levels of CA-B and CA-B-dependent activity in human erythrocytes are decreased immediately after acute physical exercise, no significant changes occur in the levels of CA-C or CA-C-dependent activity, and that the active CA-B enzymes are converted in part to inactive enzymes during heavy physical exercise of short duration, possibly by decreased zinc binding. It is well known that sustained physical training regimens induce a relatively mild, apparently functional anemia. This pseudoanemic condition has been termed “sports anemia.” In the present study, experiments on the effect of training on the levels of zinc and CA isoenzyme activities in human erythrocytes were performed. The possible significance of zinc and CA isoenzymes under heavy physical training of short duration is discussed in connection with the changes in hematocrit and hemoglobin (Hb) levels in the venous blood.

Subjects: The subjects comprised 5 untrained male medical students (21–23 years of age). After fasting for about 12 hours, they took a rest in a supine position for 30 min in the morning and then worked on a bicycle ergometer (Monark, Sweden) with a load of 200 W for 30 min. The training protocol consisted of a 30-min ride on the bicycle ergometer at the same load as the initial ride, 6 times/week for 2 weeks. Blood samples were collected from the antecubital vein in a zinc-free heparinized plastic syringe just before and immediately after the exercise session, prior to and following the training program. Dry α-cellulose and micro-crystalline cellulose (Sigma Chemical Company, U.S.A.) for removing white cells and platelets from the whole blood were used to prepare hemolysates for erythrocyte parameter assays according to the method of Beutler et al. The Hb content of the hemolysate and whole blood was measured by the cyanmethemoglobin method.
Venous blood gas analysis: The venous blood gas was analyzed with a pH/blood gas analyzer model 513 (Instrumentation Lab., U.S.A.), and the value was corrected automatically for body temperature.

Determination of zinc concentration: The zinc concentration in the hemolysates was determined using a Shimadzu model AA 640-13 atomic absorption spectrophotometer (Japan) after an ashing process.

Immunological measurement of CA isoenzymes: The levels of CA-B and CA-C isoenzymes were measured according to a single radial immunodiffusion technique\textsuperscript{1,5} employing a slight modification of the method described by Funakoshi and Deutsch\textsuperscript{2}.

Assay of CA isoenzyme activity: The esterase activity of CA was assayed according to a slight modification of the procedure of Armstrong \textit{et al.}\textsuperscript{1,6} Each CA isoenzyme activity was determined according to the modified method of Kondo \textit{et al.}\textsuperscript{1,7}, based on that originally described by Axen \textit{et al.}\textsuperscript{1,8}.

### Table 1. Effects of 2 weeks training on the levels of venous blood parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before training</th>
<th>After training</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Just before exercise</td>
<td>Immediately after exercise</td>
</tr>
<tr>
<td>Hemoglobin (g/100 ml)</td>
<td>14.7 (\pm 0.39)</td>
<td>15.7 (\pm 0.36)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45.4 (\pm 1.08)</td>
<td>48.5 (\pm 1.21)</td>
</tr>
<tr>
<td>pH</td>
<td>7.293 (\pm 0.0041)</td>
<td>7.212 (\pm 0.0247)</td>
</tr>
<tr>
<td>PCO(_2) (mmHg)</td>
<td>56.4 (\pm 1.94)</td>
<td>44.1 (\pm 1.69)</td>
</tr>
<tr>
<td>PO(_2) (mmHg)</td>
<td>26.9 (\pm 1.34)</td>
<td>46.9 (\pm 4.84)</td>
</tr>
<tr>
<td>SO(_2) (%)</td>
<td>34.1 (\pm 4.09)</td>
<td>63.0 (\pm 5.86)</td>
</tr>
<tr>
<td>Base excess (mEq/l)</td>
<td>0.420 (\pm 0.9447)</td>
<td>-9.52 (\pm 1.481)</td>
</tr>
<tr>
<td>HCO(_3^-) (mM/l)</td>
<td>26.5 (\pm 1.10)</td>
<td>17.3 (\pm 1.12)</td>
</tr>
<tr>
<td>TCO(_2) (mM/l)</td>
<td>28.2 (\pm 1.15)</td>
<td>18.6 (\pm 1.15)</td>
</tr>
</tbody>
</table>

The training protocol consisted of a 30-min ride on a bicycle ergometer with a load of 200 W, 6 times/week for 2 weeks.

Number of cases=5.

Values are expressed as mean\(\pm\)S.E.

Significant differences from the respective values before training: \(a\) \textit{p}<0.05, \(b\) \textit{p}<0.02, \(c\) \textit{p}<0.01.

\(\triangle\)=Changes in levels of venous blood parameters after 30 min physical exercise.
Statistical methods: Differences between pre- and post-training values were tested for significance using a paired t-test.

Table 1 shows the levels of the venous blood parameters just before and immediately after exercise both before and after training. After training, the hematocrit level just before exercise and the Hb concentration immediately after exercise decreased (p<0.05 and p<0.01, respectively) and the pH value immediately after exercise rose (p<0.02). In addition, there was an increasing or decreasing tendency in Hb, pH, and PO₂ values just before exercise or hematocrit and PO₂ values immediately after exercise (p<0.1). Table 2 summarizes the effects of training on the erythrocyte zinc and CA isoenzyme concentrations. There was an increase in the levels of zinc, CA-B, total CA activity, and CA-B-dependent activity just before exercise (p<0.01, p<0.05, p<0.05, and p<0.02, respectively) and a decrease in the change of zinc concentration after exercise (p<0.05). An increasing or decreasing tendency was also observed in the specific activity of CA-B just before exercise, the total CA activity immediately after exercise or the change in CA-B level after exercise as a result of training (p<0.1).

Table 2. Effects of 2 weeks training on the levels of zinc and carbonic anhydrase isoenzymes in erythrocytes.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before training</th>
<th></th>
<th>After training</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Just before</td>
<td>Immediately after</td>
<td>Just before</td>
<td>Immediately after</td>
</tr>
<tr>
<td></td>
<td>exercise</td>
<td>exercise</td>
<td>exercise</td>
<td>exercise</td>
</tr>
<tr>
<td>Total zinc (µg/gHb)</td>
<td>50.2 ± 1.91</td>
<td>43.4 ± 2.65</td>
<td>56.1 ± 2.47</td>
<td>44.5 ± 2.02</td>
</tr>
<tr>
<td></td>
<td>−6.98 ± 1.859</td>
<td>△</td>
<td>−11.6 ± 1.47a</td>
<td>△</td>
</tr>
<tr>
<td>Immunological level (mg/gHb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA-B</td>
<td>14.1 ± 0.48</td>
<td>11.9 ± 0.36</td>
<td>16.1 ± 0.75a</td>
<td>12.3 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>−2.22 ± 0.254</td>
<td>△</td>
<td>−3.86 ± 0.797</td>
<td>△</td>
</tr>
<tr>
<td>CA-C</td>
<td>1.74 ± 0.095</td>
<td>1.76 ± 0.096</td>
<td>1.84 ± 0.041</td>
<td>1.76 ± 0.036</td>
</tr>
<tr>
<td></td>
<td>0.014 ± 0.0236</td>
<td>△</td>
<td>-0.076 ± 0.0599</td>
<td>△</td>
</tr>
<tr>
<td>Esterase activity (units/gHb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total activity</td>
<td>17.5 ± 1.96</td>
<td>13.8 ± 1.43</td>
<td>23.8 ± 0.83a</td>
<td>17.4 ± 1.37</td>
</tr>
<tr>
<td></td>
<td>−3.68 ± 1.434</td>
<td>△</td>
<td>−6.34 ± 1.238</td>
<td>△</td>
</tr>
<tr>
<td>CA-B-dependent activity</td>
<td>10.7 ± 1.24</td>
<td>6.98 ± 1.036</td>
<td>15.5 ± 0.656</td>
<td>9.24 ± 1.331</td>
</tr>
<tr>
<td></td>
<td>−3.76 ± 1.325</td>
<td>△</td>
<td>−6.26 ± 1.149</td>
<td>△</td>
</tr>
<tr>
<td>CA-C-dependent activity</td>
<td>6.76 ± 0.731</td>
<td>6.83 ± 0.685</td>
<td>8.30 ± 0.487</td>
<td>8.20 ± 0.447</td>
</tr>
<tr>
<td></td>
<td>0.082 ± 0.1314</td>
<td>△</td>
<td>−0.104 ± 0.1139</td>
<td>△</td>
</tr>
<tr>
<td>Specific activity (units/mg isoenzyme)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA-B</td>
<td>0.754 ± 0.0686</td>
<td>0.582 ± 0.0697</td>
<td>0.970 ± 0.0587</td>
<td>0.746 ± 0.0689</td>
</tr>
<tr>
<td></td>
<td>−0.172 ± 0.0875</td>
<td>△</td>
<td>−0.224 ± 0.0892</td>
<td>△</td>
</tr>
<tr>
<td>CA-C</td>
<td>3.88 ± 0.389</td>
<td>3.91 ± 0.388</td>
<td>4.52 ± 0.299</td>
<td>4.60 ± 0.281</td>
</tr>
<tr>
<td></td>
<td>0.028 ± 0.0296</td>
<td>△</td>
<td>0.078 ± 0.1423</td>
<td>△</td>
</tr>
</tbody>
</table>

Number of cases = 5.
Values are expressed as mean ± S.E.
Significant differences from the respective values before training: a p<0.05, b p<0.02, c p<0.01.
△ = changes in levels of zinc and carbonic anhydrase isoenzymes in erythrocytes after 30 min physical exercise.
In agreement with the findings of our previous studies\(^5,6\), there was a significant
decrease in the levels of CA-B, total CA activity, and CA-B-dependent activity
immediately after exercise both before and after the training program (p<0.01,
p<0.01, and p<0.05, respectively). The zinc concentration also showed a decrease
immediately after exercise under the two conditions (p<0.05). Several authors\(^17,19,20\)
have previously reported that, although the levels of CA-B in human
erthrocytes may vary considerably under certain physiological or pathological
conditions, no significant changes occur in the levels of CA-C. Under acute
physical exercise, however, the physiological meaning of the decreases in levels of
CA-B and CA-B-dependent activity remains to be elucidated\(^5,6\). The 2-week
training caused an increase in the levels of zinc, CA-B, total CA activity, CA-B-
dependent activity, and specific activity of CA-B in erythrocytes and pH and PO\(_2\)
in the venous blood, respectively. It may be that an adaptive increase in the levels
of CA-B and CA-B-dependent activity of the erythrocytes occurs with increased
alkalosis during physical training; however, the HCO\(_3^-\) level remained unchanged
after training. The Hb and hematocrit levels decreased after training. Lindemann\(^8\)
has suggested that such “sports anemia” is caused by mechanical damage to ery-
throcytes. In addition, Yoshimura\(^12\) has indicated that destruction of erythrocytes
may be regarded as one of the adaptive reactions which promote growth or hypertro-
fy of muscles and regeneration of new and strong erythrocytes in strenuous
physical training. In the present study, however, it seemed unlikely that a hemo-
lytic effect could explain the decrease in Hb, hematocrit or CA-B levels, in view of
the unchanged CA-C levels in the erythrocytes after training. The readings
obtained for the concentrations of zinc and enzymes were undoubtedly higher than
those expected from the change in Hb or hematocrit. Oelshlegel \(\text{et al.}\)^21 reported
that zinc binding to erythrocyte 2,3-diphosphoglycerate (2,3-DPG) can cause an
increase in erythrocyte oxygen affinity. On the other hand, Hořejší and Komár-
ková\(^22\) indicated that CA shifts the oxygen dissociation curve to the right and
causes a decrease in erythrocyte oxygen affinity. Our previous study\(^5\) revealed
negative correlations between the changes in 2,3-DPG level on the one hand, and
those in CA-B level and total CA activity on the other after acute physical exercise.
It is known that training also causes an increase in arterial and venous 2,3-DPG
levels\(^23\). We are at present continuing work along the above lines to clarify the
association between CA, zinc, 2,3-DPG, and Hb in erythrocytes under physical
training.

This study was presented in part to the “Fifth International Symposium on the

**References**


*Department of Hygiene and Preventive Medicine, Asahikawa Medical College,*
*4-5-3-11 Nishikagura, Asahikawa, 078-11 Japan*

*Department of Public Health,*
*Asahikawa Medical College,*
*Asahikawa, 078-11 Japan*

*Biochemistry Laboratory, Cancer Institute, Hokkaido University*
*School of Medicine, West 7, North 15, Kita-ku, Sapporo 060, Japan*

(Received August 2, 1982)