The isoenzyme pattern of LDH does not play a physiological role; except perhaps during fast transitions in energy metabolism
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**Introduction:** A paper describing increased brain lactate concentration with age recently caught our attention [1]. The proposed explanation for the increased brain lactate with age was a shift in the brain lactate dehydrogenase (LDH) isoenzyme pattern, which also occurred with age. While the observations are highly interesting, we found the explanation unlikely under steady state conditions, since LDH is regarded a near-equilibrium reaction and since the equilibrium constant of all the LDH isoenzymes, of course, are the same [2]. We therefore decided to evaluate the question further, including a brief historical review, since a quick examination of common textbooks of biochemistry on the LDH isoenzyme question suggests that it is in fact common place to confer significant physiological importance to the different kinetic properties of the isoforms of LDH [3-9].

**Historical:** The concept of isoenzymes evolved in the late 1950’s and early 1960’s. In case of LDH it became clear that five isoenzymes numbered 1-5 could be distinguished according to electrophoretic mobility [10] and that there was a tissue specific distribution of these isoenzymes [10, 11]. The various isoenzymes appeared to be tetramers of two different subunits, M and H. Isoenzyme 5 is composed of M-subunits only and is the predominant form in skeletal muscle and isoenzyme 1 is composed of H-subunits only and is found primarily in heart tissue. Isoenzyme 2-4 contains both subunits. Later studies showed that two proteins M and H are the products of the genes Ldh-A and Ldh-B, respectively, and the LDH enzyme presents with the following five isoenzymes: M₄, MH₃, M₂H₂, M₃H and H₄.

In the 1960’s it was furthermore demonstrated that the LDH isoenzymes showed different kinetic properties with respect to substrate affinity and inhibition, where the M-dominated forms have a 3.5 – 7 times higher Kᵥm value for pyruvate than H-dominated. (Table 1). The H₄ isoenzyme is furthermore sensitive to substrate inhibition by pyruvate at concentrations above ~0.2 mM, whereas the M₄ isoenzyme appear little affected by pyruvate concentrations as high as 5 mM [12-14]. Also, the H₄ isoenzyme exhibits substrate inhibition by lactate above 20-40 mM, whereas the M₄ isoenzyme is much less inhibited by high lactate concentrations [14].

**Discussion**

The structural basis for the differences in kinetic properties was recently reported [15]. The large majority of measurements of the mentioned kinetic parameters have been carried out at 20 or 25 ºC. In this context it should be noted that Kᵥm-values for pyruvate increase with temperature [13, 16] and are doubled at 37 ºC compared to 25 ºC, and that substrate inhibition by pyruvate may be less pronounced at 37 ºC than at 20-25 ºC, especially for the heart enzyme [13, 17] although there is disagreement regarding pyruvate inhibition [16]. Thus, there may be only small differences, if any, in kinetic constants among the LDH isoforms at physiological temperature.

Historically, the tissue characteristic LDH isoenzymes distribution led to hypotheses of possible functional implications. This concept was pioneered by Kaplans group in particular [18, 19], where it was stated: “The susceptibility of heart lactic dehydrogenase (H subunits) to inhibition by pyruvate is compatible with adaption of the heart to aerobic metabolism of pyruvate and NADH. Skeletal muscle enzyme (M subunits), on the other hand, resists inhibition by pyruvate, and enables the tissue to derive energy from anaerobic pathways when oxygen is limited and pyruvate accumulates” [18]. This somewhat uncritical interpretation of the biochemical function of LDH isoenzymes seems to have been transmitted through most textbooks since, as mentioned above (e.g. 3-9).

**Analysis of the physiological role of LDH isoenzymes:** Below we discuss the possible physiological roles, if any, of the LDH isoenzymes with brain and skeletal muscle as examples.

The H-dominated isoenzymes have a lower Kᵥm value for pyruvate and lactate than M-dominated forms and are inhibited by pyruvate concentrations in the physiological range (Table 1). As mentioned above this is often interpreted to cause LDH isoenzymes composed primarily of M-subunits to preferentially catalyze the reduction of pyruvate → lactate and conversely, that isoenzymes composed primarily of H-subunits predominantly should catalyze oxidation of lactate to...
pyruvate [1,3-9] However, in spite of changed kinetic
constants the equilibrium constant, \( K_{eq} \), is the same for
all isoenzymes since it is the same chemical reaction
being catalyzed. This is stated in the Haldane Equation,
relating \( K_{eq} \) with the kinetic constants of the forward
and reverse reaction:

\[
K_{eq} = \frac{(V_{max} \cdot K_{mp})}{(V_{max} \cdot K_{ms})}
\]

Furthermore, the total LDH activity is high in most
tissues compared to metabolic flux (see Table 1) and the
reaction therefore likely to be close to equilibrium under
steady state conditions.

Consequently, it would seem unlikely that the LDH
isoenzyme pattern in itself could influence the
tissue lactate concentration. This conclusion was, in
fact, also reached in a recent study performing
mathematical modeling of the LDH reaction,
concluding that the decisive parameter was the total
LDH activity and not the isoenzyme pattern [20].

The situation may, however, be quite different during
metabolic transitions in energy metabolism where in
particular the glycolytic flux can undergo large rapid
changes. We propose that under such conditions there
may be significant physiological effect of the
isoenzyme pattern of a given tissue.

In brain under normal physiological conditions it seems
that the intracellular lactate concentration is significantly higher (2-4 mM) than in the arterial blood
[21]. This implies that the LDH reaction is in fact, not a
dead-end reaction as is normally assumed, since there
will be a net concentration gradient mediated flux in the
reaction. In the brain this is indicated by a small but
statistically significant net output of lactate at rest [22,
23]. Theoretically, this could affect steady state lactate
concentration as a result of a changed LDH isoenzyme
pattern. However, with a high total activity of LDH this
is likely to be a minor effect [20]. A different situation
might apply during the initial phase of brain activation
(e.g. as occurring when initiating intense mental or
physical exercise), where there may be a burst of
anaerobic metabolism in the brain [24, 25] and therefore
presumably a sharp increase in the pyruvate
concentration. In that case the kinetics of the
intracellular lactate concentration could be affected by
the LDH isoenzyme pattern. But again, this applies only
during the non-steady state situation of the brain
activation.

Such non-equilibrium effects on the LDH reaction
should be even more visible in skeletal muscle upon the
transition from rest to work. Here the glycolytic flux
can easily change by a factor of 50 [26] and an active
involvement of the LDH reaction is suggested by
observed training effects on the kinetics of the LDH
reaction [27]. Under these conditions the LDH reaction
is likely to be temporarily far from equilibrium and the
kinetic effects of the pyruvate concentration on the
LDH flux could be large.

It is under such conditions that the prevalent high LDH
activity becomes metabolically important by providing
an efficient way of buffering the pyruvate concentration
excursions and indeed supplying the necessary NADH
to uphold the glycolytic flux and the resulting ATP
production. In that context, it is interesting to consider
what purpose the rather low \( K_i \) for pyruvate of the H-
form might serve, since it would tend to slow down the
“buffering” by the LDH reaction of the pyruvate
concentration. One suggestion could be that it would
keep the pyruvate high for a longer period of time in the
initial phase of glycolytic flux acceleration would
therefore favor a subsequent acceleration of the
pyruvate dehydrogenase (PDH) pathway. But this
suggestion does not seem to be supported by the low \( K_m \)
of PDH for pyruvate, which is about an order of
magnitude lower than even normal resting pyruvate
concentrations [28].

Apart from PDH, there are however, several other
reactions connecting pyruvate to the intermediary
metabolism. For the alanine amino-transferase (ALAT)
reaction, a high pyruvate concentration would favor
glutamate conversion to \( \alpha \)-ketoglutarate and
consequently an increase in the TCA intermediary pool,
which would seem useful under conditions of an
eminent TCA acceleration and could therefore be
considered a feed forward mechanism. The same effect
would result by way of the pyruvate carboxylase
reaction.

Therefore, it is not unlikely that the isoenzyme pattern
of LDH does after all play an important physiological
role under non-steady state conditions, and a
quantitative evaluation through mathematical modeling
of the above suggestions are needed.

In the context of the present evaluation of the LDH
reaction it is noteworthy that the beta cells of the
pancreatic islets does not express Ldh-A or the lactate
and pyruvate transporter Metl [29]. This is likely to be
linked with the special function of the glycolytic flux of
these cells as a sensor mechanism of the glucose
concentration. i.e., without LDH activity, and therefore
no lactate buffering on the pyruvate concentration, the
magnitude of the glycolytic flux may be directly
transmitted to TCA flux and ATP formation and
subsequent insulin release. If LDH was present in high
amounts or if pyruvate or lactate could be taken up in
the beta cells, a bout of intense physical exercise, which results in very significant increase in systemic lactate, would transmit a pyruvate signal to the beta cell mitochondria and therefore potentially cause an irrelevant insulin secretion.

**Conclusion:** All in all we find that the LDH isoenzyme pattern most probably is without effect on the intracellular lactate concentration and the explanation advanced by [1], is probably incorrect since their experiments on aging effects reflect steady state conditions. However, during fast metabolic transitions involving significant changes in the pyruvate concentration, primarily resulting from major changes in glycolytic rate, the LDH isoenzyme pattern may well play an important role in the compounded metabolic response to altered energy metabolism. However, this latter part of the conclusion depends strongly on resolving the issue as to whether the reported K_m differences for pyruvate between LDH isoenzymes are actually present at 37º C [13,16,17].

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**REFERENCES**

### Table 1. Kinetic properties of the LDH isoenzymes of different species.

K\textsubscript{m} values are in mM and V\textsubscript{max} given as U/g wet wt.

#### $K_m$ Pyruvate as substrate:

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>$K_m$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td></td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td>0.17-0.27</td>
<td>27</td>
</tr>
<tr>
<td>Bovine</td>
<td></td>
<td>0.14</td>
<td>30</td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
<td>0.09</td>
<td>30</td>
</tr>
</tbody>
</table>

#### $K_m$ Lactate as substrate:

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>$K_m$</th>
<th>$K_m$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td></td>
<td>9.34</td>
<td>23.0</td>
<td>14</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td>15-17</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Bovine</td>
<td></td>
<td>9</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
<td>7</td>
<td>40</td>
<td>30</td>
</tr>
</tbody>
</table>

#### $V_{max}$ Pyruvate as substrate:

$V_{max}$ values in U/g wet wt.

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>$V_{max}$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human*</td>
<td>Brain</td>
<td>46</td>
<td>31</td>
</tr>
<tr>
<td>Human*</td>
<td>m. vastus</td>
<td>175</td>
<td>31</td>
</tr>
<tr>
<td>Rabbit*</td>
<td>m. soleus</td>
<td>167</td>
<td>31</td>
</tr>
<tr>
<td>Rabbit*</td>
<td>m. tibialis anterior</td>
<td>1458</td>
<td>31</td>
</tr>
<tr>
<td>Dog*</td>
<td>Heart</td>
<td>125</td>
<td>31</td>
</tr>
<tr>
<td>Rat*</td>
<td>m. plantaris</td>
<td>740-889</td>
<td>27</td>
</tr>
<tr>
<td>Rat*</td>
<td>Liver</td>
<td>83</td>
<td>31</td>
</tr>
</tbody>
</table>

*Assuming 3.5 g wet wt/g dry wt.