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Adaptations of Lactate Metabolism in Iron-Deficient Rats (41633)

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Abstract. Effects of dietary iron deficiency on lactate metabolism were studied in weanling female rats. Following an iron-deficient diet for 5 weeks, mean hemoglobin concentration was lowered to 6.4 g/dl relative to 12.2 in the control group. Mean plasma iron levels were 58 and 162 μg/dl, respectively. Significantly elevated resting lactate levels were observed in whole blood and plasma from iron-deficient anemic (relative to control) rats. Total activity of lactate dehydrogenase (LDH) was elevated in soleus and gastrocnemius muscles in response to iron deficiency from 269 ± 51 to 364 ± 60 (Mean ± SD) and from 265 ± 65 to 372 ± 61 $IU \cdot 10^{-3} \cdot g^{-1}$, respectively. The LDH activity in heart was lowered from 700 ± 61 to 593 ± 45 $IU \cdot 10^{-3} \cdot g^{-1}$. The M_3H and M_2H_2 isozymes in soleus were increased from 12.7 ± 2.8 to 20.4 ± 5.8% and from 19.4 ± 6.1 to 28.2 ± 3.6%, respectively. Similar increase was observed in M_2H_2 and MH_3 in gastrocnemius from 9.8 ± 0.9 to 14.8 ± 2.0% and from 17.4 ± 2.0 to 20.5 ± 2.3%, respectively. The H_4 isozyme was significantly reduced in soleus, gastrocnemius, and plantaris muscles from 27.7 ± 4.7 to 12.4 ± 4.4, from 15.8 ± 1.9 to 7.2 ± 2.9, and from 10.5 ± 2.9 to 3.9 ± 2.1%, respectively. It was suggested that iron-deficiency anemia induces an elevation of lactate production following an increase in total LDH activity and change in LDH isozyme patterns.

Severe iron deficiency causes a reduction in iron-containing oxidative enzyme activities as well as hemoglobin (Hb), myoglobin, and cytochromes (1–6). Thus, maximal potential for aerobic energy production is impaired. Severe iron-deficiency anemia also causes lactic acidosis in man and animals (7–10). Resting venous blood lactate is elevated in anemic human subjects (7). Finch *et al.* (8) reported that iron-deficient rats had significantly greater resting lactate concentration in vena cava blood than control rats had, even after the Hb concentration of both groups had been adjusted by exchange transfusion to approximately 10 g/dl. Impaired oxygen utilization capacity and elevated lactate production in tissues were suggested to explain their findings (8). However, it is unclear whether such lactate elevation involves some metabolic adaptation in tissues and/or blood that may be the cause of the elevated lactate concentrations at rest. Therefore, this study was designed to investigate the concentration of lactate, the activity of lactate dehydrogenase (LDH), and its isozyme patterns in various tissues of iron-deficient rats.

Methods. Thirteen newly weaned, female Sprague–Dawley rats were randomly divided into iron-deficient ($n = 8$) and control groups ($n = 5$). Rats were fed a basal solid diet containing <4 ppm Fe; control diets contained 250 ppm Fe as $FeSO_4$ (6). Food and deionized water were supplied *ad libitum* for 5 weeks.

Following a 12-hour starvation with water present, resting animals were anesthetized with ether. Approximately 2 ml of blood was withdrawn using a heparinized syringe from the external jugular vein. Some of the blood was immediately pipetted into 6% trichloroacetic acid (TCA) solution for lactate determination (11). Hemoglobin was measured by the cyanmethemoglobin method. The time course of blood lactate levels after sampling was tested in another study. The blood was kept in ice and the tube was capped tightly. Lactate levels increased gradually *in vitro,* then plateaued after 25–30 min. Since separation of plasma was done approximately 30 min after the withdrawal, plasma lactate was higher than

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TABLE I. HEMATOLOGICAL STATUS OF IRON-DEFICIENT AND NORMAL RATS

	n	Hemoglobin (g/dl)	Plasma iron (μg/dl)	Plasma protein (g/dl)	Blood lactate (mmole/liter)	Plasma lactate (mmole/liter)
Iron deficient	8	6.4 ± 0.4	58 ± 20	5.0 ± 0.5	5.14 ± 1.94	6.99 ± 2.18
Control	5	12.2 ± 0.6***	162 ± 33***	5.6 ± 0.5	1.53 ± 0.30**	2.48 ± 0.45***

Note. Values are expressed as mean ± SD. $^{**}P < 0.01$ and $^{***}P < 0.001$ by unpaired *t* tests.

whole-blood lactate which was deproteinized immediately. The remaining blood was centrifuged for the determination of plasma lactate, protein (refractometer), and iron (electrothermal atomic absorption spectrophotometry). The rats were then killed by ether over-inhalation. Tissues (soleus, gastrocnemius, plantaris, heart, liver, and brain) were immediately removed and frozen in liquid nitrogen prior to the determination of LDH activity and LDH isozyme pattern.

Whole blood was mixed with distilled water to hemolyze the cells for LDH activity determination; the mixture was centrifuged, and the supernatant was sampled. Tissues were homogenized in 0.25 *M* sucrose/0.10 *M* KH_2PO_4 (pH 7.4). The total LDH activity was measured spectrophotometrically at 340 nm immediately after the sample was mixed with NAD^+ and lactate substrate solution (Sigma Chemical Co., kit No. 225-UV). The LDH isozymes were separated by polyacrylamide electrophoresis in a gel of the following composition: 6.35% acrylamide/0.15% bisacrylamide (w/v); 0.10 *M* 2-amino-2-methyl-2,3-propanediol/0.01 *M* boric acid buffer (pH 10.2); 0.05% TEMED (v/v); 0.0028% ammonium persulfate (w/v). Electrophoresis was

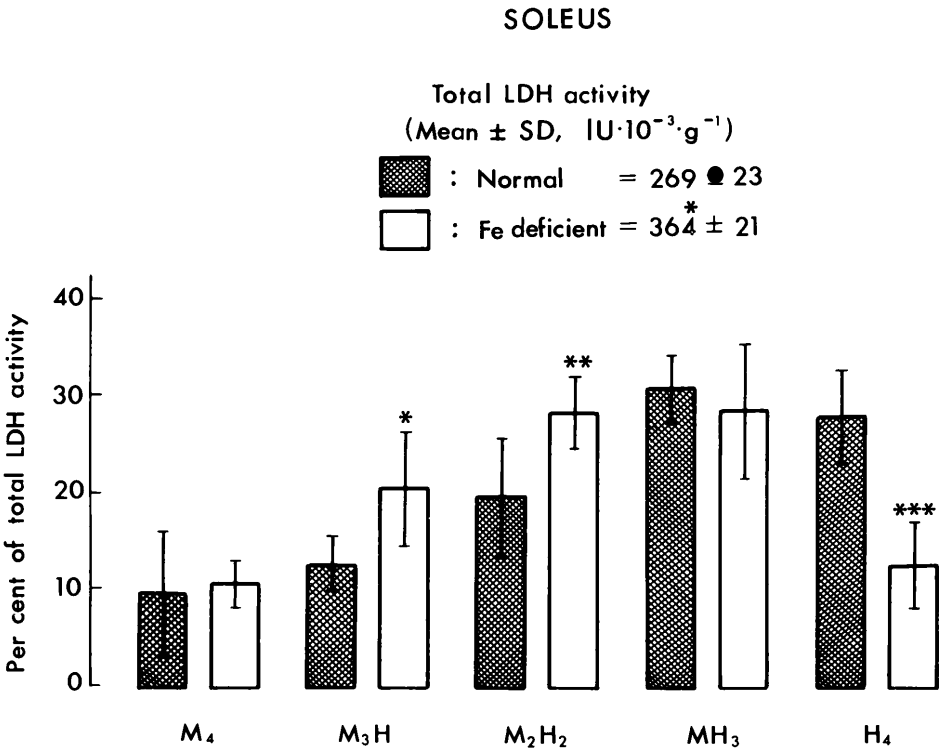

FIG. 1. LDH isozyme distribution in iron-deficient and normal rat soleus muscles. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ by unpaired *t* tests.

TABLE II. EFFECTS OF DIETARY IRON DEFICIENCY ON LDH ACTIVITY AND LDH ISOZYME PATTERNS IN RATS[a]

Tissue	Total activity[b]		H_4[c]		MH_3		M_2H_2		M_3H		M_4	
	+Fe[d]	−Fe[e]	+Fe	−Fe	+Fe	−Fe	+Fe	−Fe	+Fe	−Fe	+Fe	−Fe
Whole blood	6.1 ± 1.8	6.5 ± 2.6	—	—	—	—	—	—	—	—	—	—
Plasma	1.3 ± 0.6	1.5 ± 1.3	—	—	—	—	—	—	—	—	—	—
Soleus	269 ± 51	364 ± 60*	27.7 ± 4.7	12.4 ± 4.4***	30.6 ± 3.5	28.3 ± 6.8	19.4 ± 6.1	28.2 ± 3.6**	12.7 ± 2.8	20.4 ± 5.8*	9.6 ± 6.2	10.4 ± 2.4
Gastrocnemius	265 ± 65	372 ± 61*	15.8 ± 1.9	7.2 ± 2.9***	17.4 ± 2.0	20.5 ± 2.3*	9.8 ± 0.9	14.8 ± 2.0***	16.2 ± 4.5	21.3 ± 5.3	40.9 ± 6.4	37.3 ± 6.3
Plantaris	1473 ± 173	1537 ± 431	10.5 ± 2.9	3.9 ± 2.1***	11.7 ± 3.6	11.7 ± 1.5	11.2 ± 3.6	9.7 ± 2.1	22.6 ± 4.5	23.7 ± 4.1	44.0 ± 7.5	51.1 ± 5.3
Heart	700 ± 61	593 ± 45**	26.4 ± 6.4	26.1 ± 6.8	36.2 ± 11.4	35.5 ± 9.7	25.4 ± 9.9	26.1 ± 11.8	6.0 ± 2.8	5.6 ± 1.4	5.9 ± 5.1	6.6 ± 3.7
Brain	174 ± 42	170 ± 17	27.1 ± 4.1	29.5 ± 3.9	25.3 ± 2.8	26.9 ± 1.8	20.9 ± 1.9	18.6 ± 2.5	16.3 ± 2.9	16.0 ± 2.6	10.3 ± 4.4	9.2 ± 3.3
Liver	752 ± 174	774 ± 212	0 ± 0	0 ± 0	2.1 ± 1.7	2.5 ± 1.8	3.8 ± 3.6	4.2 ± 3.7	11.4 ± 3.2	12.1 ± 4.2	85.8 ± 9.2	72.4 ± 8.1

[a] Mean ± SD.
[b] Unit for whole blood and plasma is IU · 10^{-3} · ml^{-1} and IU · 10^{-3} · g^{-1} for other tissues.
[c] Percentage.
[d] Control, $n = 5$.
[e] Iron-deficient, $n = 8$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$ by unpaired t tests.

carried out at room temperature for 5 hr at constant voltage of 10 V/cm. Gels were stained for LDH activity in a solution containing 0.18 *M* Tris–HCl buffer (pH 8.0), 0.045 *M* lactate, 0.36 mg/ml NAD^+, 0.073 mg/ml nitroblue tetrazolium, and 0.036 mg/ml phenazine methosulfate. Staining reaction was terminated after approximately 1 hr at 37°C by transferring the gel into 7% acetic acid after clear bands had appeared. Activity was quantitated densitometrically by integrating areas under each band.

Results. Weanling rats became severely iron-deficient after eating a low-iron diet for 5 weeks, as shown by lower Hb and plasma iron concentrations (Table I). Tissue iron concentration in response to the same experimental condition has been reported previously (6).

Whole blood and plasma lactate were significantly elevated in iron-deficient animals. The total LDH activities of skeletal muscles, heart, brain, blood and liver are shown in Table II. Soleus (Fig. 1) and gastrocnemius muscles from the iron-deficient rats had significantly elevated LDH activity ($P < 0.05$). The distribution of isozymes with "heart" (H) and "muscle" (M) subunits varied as a function of tissues (Table II). As a result of iron deficiency the M_3H ($P < 0.05$) and M_2H_2 ($P < 0.01$) were increased in soleus (shown graphically in Fig. 1), and M_2H_2 ($P < 0.001$) and MH_3 ($P < 0.05$) were increased in gastrocnemius. The H_4 isozyme was significantly reduced in all of the iron-deficient skeletal muscles. However, no significant change was observed in heart and brain.

Discussion. In moderately anemic subjects with identical Hb levels and subjected to similar exercise intensity, it was reported that blood lactate was more elevated in those subjects with lower iron status (10). Such lactate elevation due to tissue iron deficiency was observed even at rest in iron-deficient rats (8). Those results suggest some metabolic changes in response to iron deficiency anemia and further to latent iron deficiency alone. The present data show that both whole blood and plasma lactate levels in iron-deficient anemic rats were significantly higher than controls. Finch *et al.* (8) suggested that elevated blood lactate concentrations in iron-deficient anemic rats were due not to decreased clearance

but to excessive production of lactate. Their study showed that lactate clearance from the blood was unaffected by iron status.

Aerobic metabolism is impaired in iron-deficient anemic rats as measured by reduced levels of mitochondrial enzyme activities, such as cytochrome oxidase (2, 4, 5), succinate dehydrogenase (SDH) (6), and Fe-S proteins representing NADH dehydrogenase and SDH (2, 6). The rise in total LDH activity in soleus and gastrocnemius muscles observed in the current study may compensate for decreased oxidative phosphorylation. However, in our previous study phosphofructokinase activity in soleus and vastus lateralis (both white and red portions) did not decrease when 2-month-old rats were maintained anemic by bleeding and dietary iron deficiency (4). These data suggest some selective adaptation in the glycolytic pathway.

Data obtained by electrophoresis showed significant changes in isozyme patterns of LDH in skeletal muscles of iron-deficient rats. Soleus, gastrocnemius, and plantaris had significantly lowered H_4 isozyme. However, M_4 isozymes were not affected significantly. It is unclear whether these changes are induced in response to tissue iron deficiency alone, anemia alone, or a combination of the two. Decrease in "H" type LDH isozyme may decrease the probability of converting lactate to pyruvate and could be one of the major causes for greater lactate accumulation. The role of muscle fiber types and voluntary activity in the induction of such adaptations is under further investigation.

These findings are consistent with a sequence of events in which iron-deficiency anemia permits an elevation of tissue lactate which may be due, in part, to a change in the total concentration of LDH as well as an alteration in the isozyme pattern, in some tissues (e.g., soleus and gastrocnemius).

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