鉄欠乏貧血ラットの骨格筋および主要臓器中における乳酸代謝とLDH ISOZYMESの適応。

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純系の雌ラットに鉄欠乏飼料を摂食させ，乳酸代謝およびLDH ISOZYMEのPATTERNSに与える影響を調べた。5週間の鉄欠乏食を与えた後，ラットの血中 HEMOGLOBIN 値はCONTROL群の12.2g/dℓに対して欠乏群は6.4と低い。

血漿中の鉄含有量は，162μg/dℓと58μg/dℓ で後者の欠乏群はCONTROL群の約3分の1強しか含有していないことを観察した。

乳酸値について見ると，全血液（WHOLE BLOOD）血漿，および心筋中ともに鉄欠乏群の方がかなりの増加傾向を示した。

LDHの活性は，鉄欠乏によって，とくに腓腹筋・ヒラメ筋中に高く，心筋中は逆に値が低くなっている。

ISOZYMEのSUBUNITについて見ると，ヒラメ筋はM_3HおよびM_2H_2の2つが増加し，腓腹筋ではM_2H_2およびMH_3の2つに有意な増加が見られる。

H_4のSUBUNITは，下肢筋中にとくに活性が低くなる。このことから，HEART TYPEのSUBUNITの活性低下は乳酸産出量を増加させる重要な一因であり，その活性低下は鉄欠乏からの影響を大きく受けていることは明らかである。

SUMMARY

Effects of dietary iron deficiency on lactate metabolism were studied in weanling female rats. Following the iron deficient diet for 5 weeks, mean hemoglobin concentration was lowered to 6.4 g/dl relative to 12.2 in control group. Mean plasma iron levels were 58 and 162 μg/dl, respectively. Significantly elevated resting lactate levels were observed in whole blood, plasma, and heart from iron-dificient anemic relative to control rats. Total activity of lactate dehydrogenase (LDH) was elevated in soleus and gastrocnemius muscles in response to iron deficiency. That in heart was lowered. The M_3H and M_2H_2 isozymes in soleus and M_2H_2 and MH_3 in gastrocnemius were increased. The H_4 isozyme was significantly reduced in the lower leg skeletal muscles. It was suggested that iron deficiency anemia induces an elevation of lactate production following an increase in total LDH activity and change in LHD isozyme patterns.

INTRODUCTION

Servere iron deficiency causes a reduction in iron-containing oxidative enzyme activities (1, 2) as well as hemoglobin (Hb), myoglobin, and cytochromec (3). Thus, aerobic energy production is impaired. Severe iron deficiency anemia also causes lactic acidosis (4, 5, 6). Although resting venous blood lactate is not elevated in anemic humans (5), severely iron-deficient, anemic rats had significantly elevated resting lactate levels (4). It is assumed that insufficient oxygen in blood and tissue leads directly or indirectly to lactate elevation. This study was designed to investigate the concentration of lactate, the activity of lactate hehydrogenase (LDH), and its isozyme patterns in various tissues of iron-deficient rats.

METHODS

Thirteen newly-weaned female Sprague-Dawley rats were randomly diyided 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 $FeSO_4$ (2). 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 (7). 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 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 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 fronzen in liquid nitrogen prior to the determination of lactate, LDH activity, and LDH isozyme pattern. The portion of tissue for lactate determination was homogenized in 6% TCA solution and centrifuged at 3000g for 20 min. The assay was performed as for blood lactate determination.

Whole blood was mixed with distilled water to hemolyze the cells for LDH activity determination. The mixture was centrifuged, and the suspernatant was saved. Tissues were homogenized in 0.25M sucrose/0.01M KH_2PO_4 (pH 7.4). The total LHD 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); 0.10M 2-amino-2-methyl-1, 3-propanediol/0.01M boric acid buffer (pH 10.2); 0.05% TEMED (v/v); 0.0028% ammonium persulfate (w/v). Electrophoresis was carried out at room temperature for 5 hr at constant voltage of 10v/cm. Gels were stained for LDH activity in a solution containing 0.18M Tris-HCl buffer (pH 8.0), 0.045M lactate, 0.36 mg/ml NAD; 0.073 mg/ml mitorblue tetrazolium, and 0.036 mg/ml phenazine methosulfate. Staining reaction was terminated after approximately 1 hr at 37°C by transfering 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 dificient after eating a lowiron diet for 5 weeks, as shown by lower Hb and plasma iron concentrations (Table 1). Tissue iron concentration in response to the same experimental condition have been reported previously (2).

The lactate concentrations in various tissues of resting rats are shown in Table 2. Whole blood and plasma lactate were significantly elevated in iron-deficient animals. Tissue lactate accumulation was significant only in cardiac muscles from iron-deficient anemic rats ($p < 0.05$).

The total LDH activities of muscles, heart, and brain are shown in Figures 1-5 and of blood and liver in Table 3. Soleus (Fig. 1) and gastrocnemius muscles (Fig. 2) from the iorn-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. As a result of iron deficiency the M_3H ($p < 0.05$) and M_2H_2 ($p < 0.01$) were increased in soleus, and M_2H_2 ($p < 0.001$) and MH_3 ($p < 0.05$) 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.

Table 1. Hematological status of iron-deficient and normal rats

	n	Hemoglobin (g/dl)	Plasma iron (μg/dl)	Plasma protein (g/dl)
Iron-deficient	8	6.4 ± 0.4	58 ± 20	5.0 ± 0.5
Control	5	12.2 ± 0.6 ***	162 ± 33 ***	5.6 ± 0.5

Mean ± SD. *** = $P < 0.001$ by unpaired t-tests.

Table 2. Effect of dietary iron deficiency on lactate levels in rats

	n	Blood	Plasma	Soleus	Gast	Plantaris	Liver	Heart	Brain
Iron-deficient	8	5.14±1.94	6.99±2.18	16.3±4.6	41.0±3.4	35.8±6.7	12.5±1.8	27.9±4.4	17.6±2.3
Control	5	1.53±0.30**	2.48±0.45***	16.4±4.1	40.9±2.3	36.8±1.0	11.0±2.4	19.5±6.2	16.7±2.2

Mean ± SD. Unit for blood and plasma is mmol/l and for others is μmol/g. * = $p < 0.05$, ** = $p < 0.01$ by unpaired t-tests.

Gast: gastrocnemius.

Table 3. Effect of dietary iron deficiency on LDH activity in rats

	n	Whole blood ($IU \cdot 10^{-3} \cdot ml^{-1}$)	Plasma ($IU \cdot 10^{-3} \cdot ml^{-1}$)	Liver ($IU \cdot 10^{-3} \cdot g^{-1}$)
Iron-deficient	8	6.54 ± 2.57	1.47 ± 1.31	774 ± 212
Control	5	6.06 ± 1.76	1.30 ± 0.59	752 ± 174

Mean ± SD.

Figure 1. Total LDH activity and isozyme pattern in rat soleus muscle.

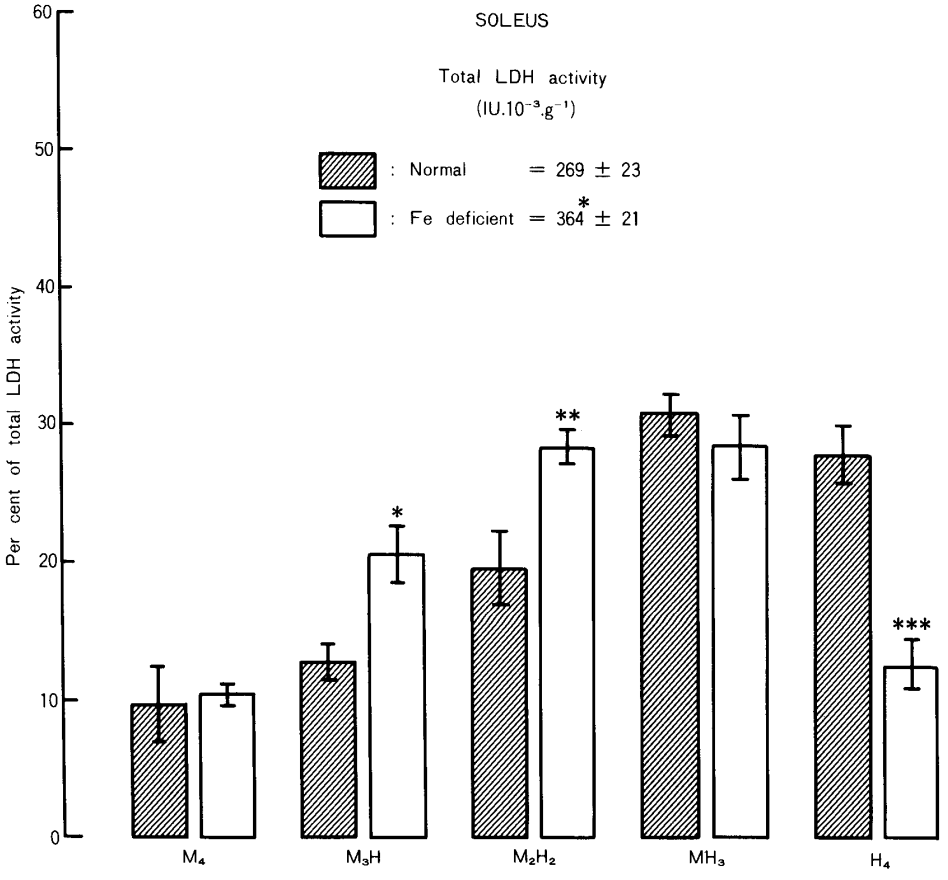


Figure 2. Total LDH activity and isozyme pattern in rat gastrocnemius muscle.

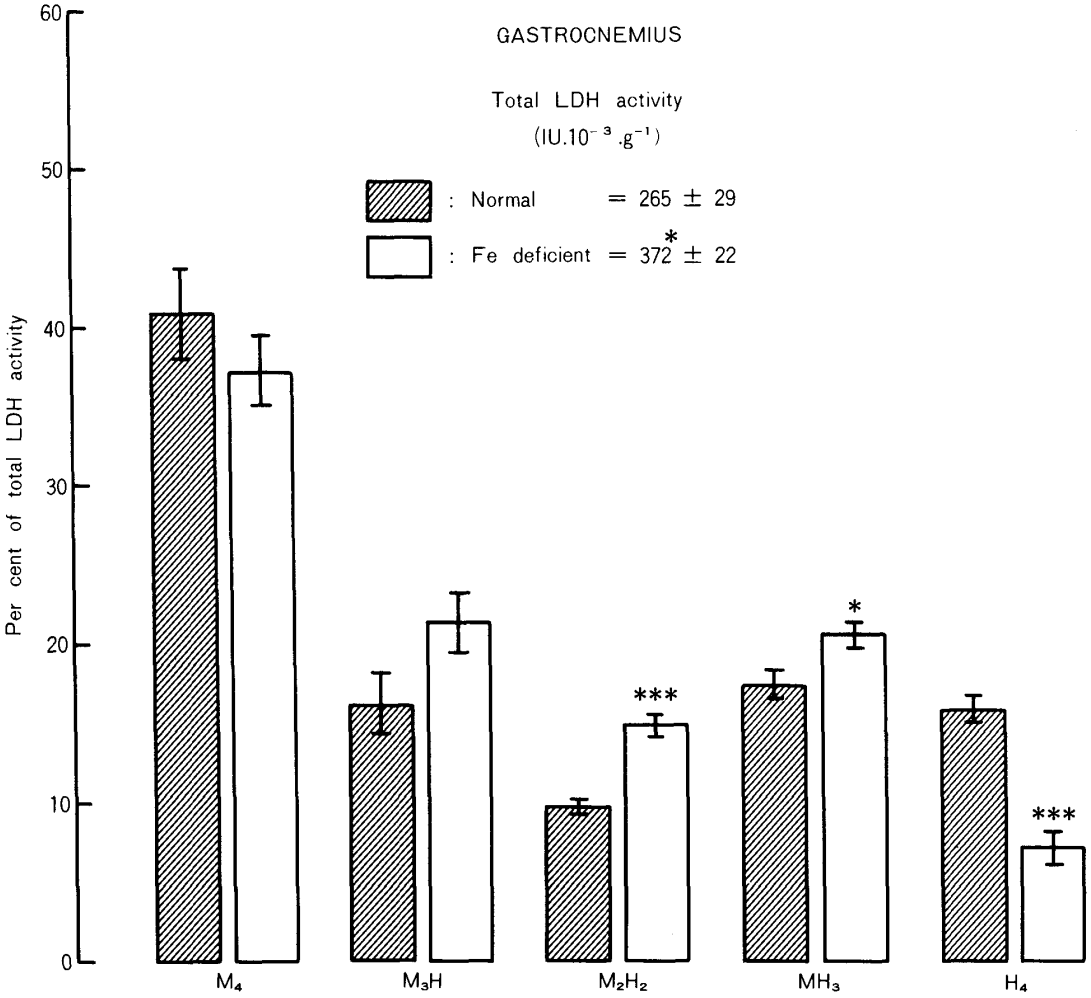


Figure 3. Total LDH activity and isozyme pattern in rat plantaris muscle.

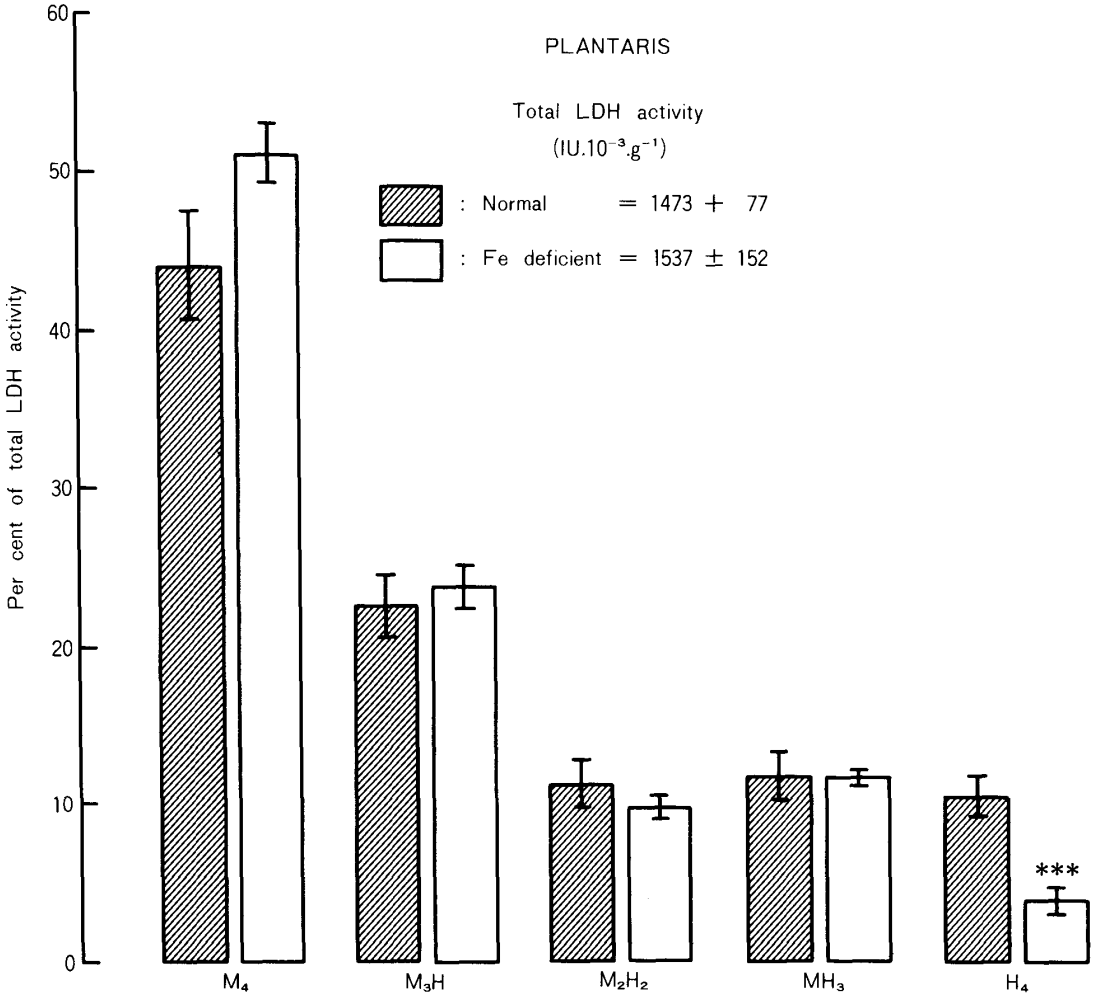


Figure 4. Total LDH activity and isozyme pattern in rat heart.

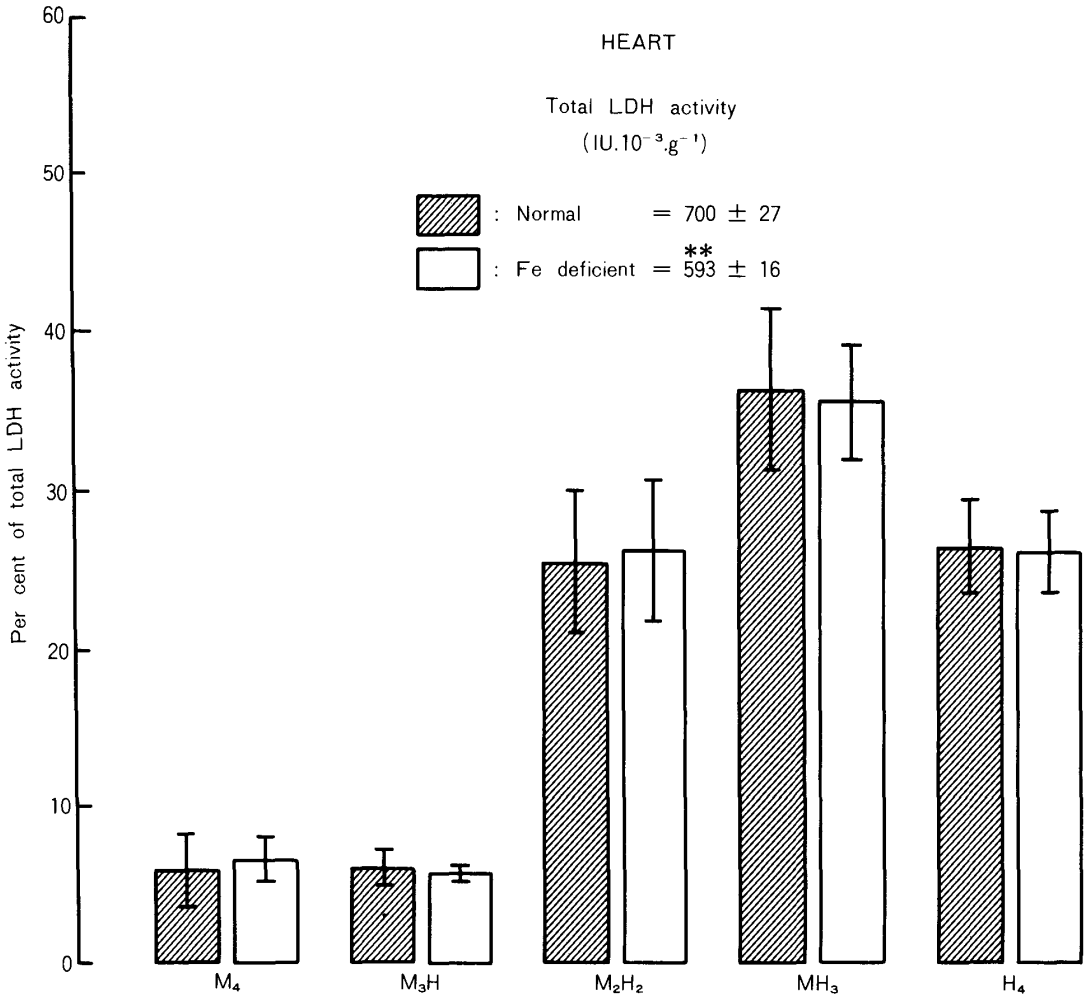
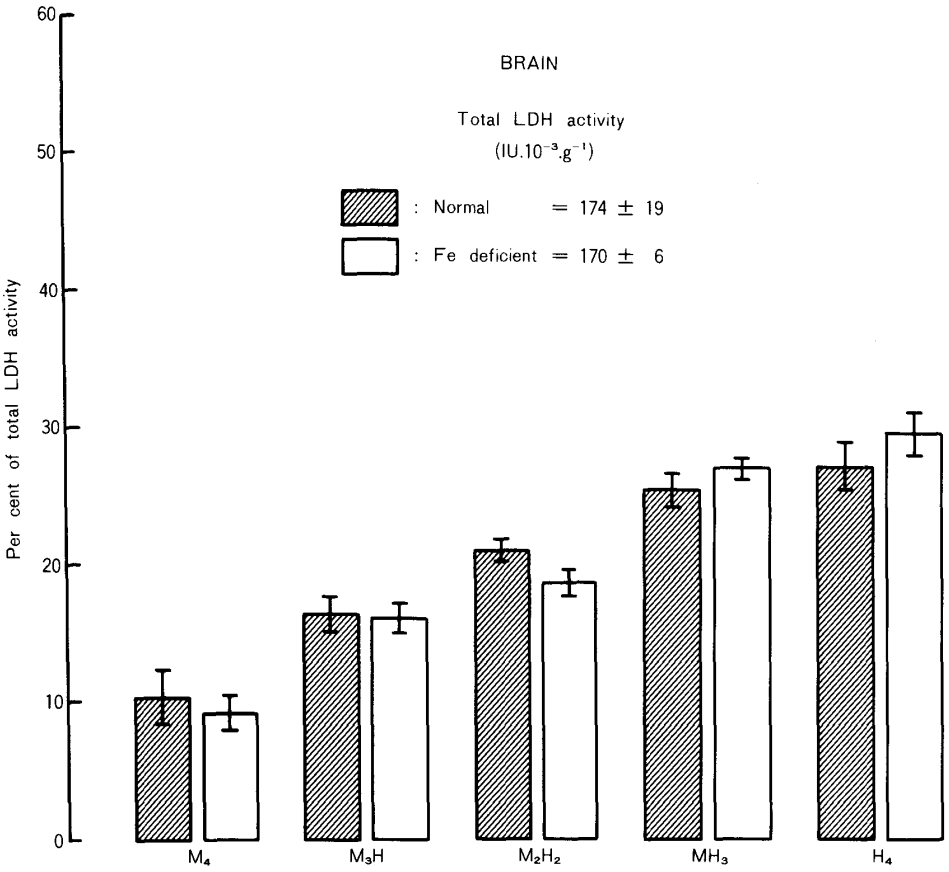


Figure 5. Total LDH activity and isozyme pattern in rat brain.



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 (6). This result suggests 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.

Tissue lactate accumulation in response to iron deficiency anemia was observed only in hearts from resting rats. This is to be expected since the heart must maintain its basal function even at rest. Elevated lactate level in hearts from iron-deficient rats may be due to both higher production of lactate in cardiac muscle and higher uptake from the bolld. Aerobic metabolism is impaired in iron-deficient anemic rats as measured by reduced levels of cytochrome oxidase (1) and succinate dehydrogenase (SDH) activities (2) and Fe-S proteins representing NADH-linked dehydrogenase and SDH (2). The rise in total LDH activity in soleus and gastrocnemius muscles observed in the current study may compensate for decreased oxidative phosphorylation. However, phosphofructokinase activity in soleus and vastus lateralis (both white and red portions) did not decrease when two month old rats were maintained anemic by bleeding and dietary iron deficiency (1). These data suggest some selective adaptation in the gloycolytic 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. Concomitantly, some muscle-type isozymes were significantly elevated. The M_4 isozyme is characterized by a higher V max than the H_4 and is not inhibited by pyruvate. These findings suggest a sequence of events in which iron deficiency anemia leads to an elevation of lactate production which in turn induces a change in the total concentration of LDH in some tissues (e.g., soleus and gastrocnemius), as well as an alteration in the isozyme pattern which can more effectively cope with the increased lactate.

Acknowledgements

This study was supported, in part, by USPHS NTH Research Grant AM-12386.

The authors wish to thank Dr. Y. Ohira for his important assistance for this paper. Dr. P. Saltman, J. Hegenauer too help me a great deal when I was a visiting scholar in UCSD Department of Biology, La Jolla San Diego California U.S.A.

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