Effect of latent iron deficiency on the levels of iron, calcium, zinc, copper, manganese, cadmium and lead in liver, kidney and spleen of growing rats

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Summary. Feeding a marginally low iron content diet (18–20 mg iron/kg diet) to weaned (21-day-old) rats for 8 weeks produced a significant decrease in liver non-heme iron (66%, $p < 0.001$) but no change in blood hemoglobin. Total iron contents of liver (56%, $p < 0.01$), spleen (20%, $p < 0.05$), and kidney (19%, $p < 0.05$) were also found to decrease along with increased zinc, copper, calcium, manganese lead and cadmium in various organs. The magnitude of alteration of a metal was different in different organs. However, liver was found to be the most affected organ. Two weeks of rehabilitation with iron-sufficient diet (390 mg iron/kg diet) normalized these altered levels.
Key words. Latent iron deficiency; liver; kidney; spleen; metals; rehabilitation.

Along with many other abnormalities, severe iron deficiency has been reported to alter the levels of various trace elements in different tissues[1–4]. As the disturbances in the levels of these trace elements are known to produce a variety of physiological disorders[5–8], it can be suggested that such alterations may have an important role in producing certain deleterious effects of this nutritional disorder. Although much knowledge is available regarding the effect of severe iron deficiency on metal levels in body, the information on latent or early iron deficiency in this regard is negligible. Latent iron deficiency is an early stage of iron deficiency characterized by normal hemoglobin and depleted tissue iron[9]. Keeping in mind the importance of metals in various regulatory processes in biological system, the present investigation has been carried out to study the levels of certain metals in storage organs of rat with latent iron deficiency. The levels of two environmental metallotoxins, lead and cadmium, were also studied as their accumulation has been reported to be higher in severe iron deficiency[8, 10].

Dietary treatment and biochemical procedures

21-day-old female albino rats (C–F strain) were housed in plastic cages and kept in standard animal housing conditions. The animals were divided in 2 groups, one group received an iron-deficient (experimental) diet and the other was fed an iron-sufficient (control) diet. For preparation of the iron-sufficient diet, adequate amounts of $FeSO_4 \cdot 7\ H_2O$ was added. When the diets were analyzed for their iron contents by the procedure recommended by the Association of Analytical Chemists[11], the iron-deficient diet was found to contain 18–20 mg iron/kg diet and the iron-sufficient diet had 390 mg iron/kg diet (table 1). After 8 weeks of dietary treatment, half of the rats from both groups were randomly selected and killed by decapitation. After collecting the blood, liver, kidneys and spleen were taken out, rinsed in chilled saline, weighed and stored at −20 °C for various biochemical analyses. For rehabilitation studies, the rest of the 8-week-old iron-deficient rats were given an iron-sufficient diet for 2 weeks and the control group received iron-sufficient diet for 10 weeks.

Table 1. Composition of iron-deficient diet

Dietary constituent	g/kg
Skimmed milk	500
Lactose	300
Potato starch	100
Groundnut oil	50
Salt mixture (iron free)	40
Vitamin mixture	10

Salt mixture contained sodium chloride, 22 g; calcium phosphate 130 g; tripotassium citrate, 125 g; magnesium sulfate, 30 g; trace element mixture, 0.7 g[12]. Vitamin mixture was according to Williams and Mills[13].

Seven different metals, viz. total iron (Fe), calcium (Ca), copper (Cu), manganese (Mn), zinc (Zn), cadmium (Cd) and lead (Pb) were estimated in liver, spleen and kidney using a direct current plasma spectrophotometer after wet digestion of tissue with 5:1 nitric acid and perchloric acid solution[14]. Blood hemoglobin[15] and liver non-heme iron[16] were measured spectrophotometrically.

Results and discussion

Eight weeks of iron deficiency (18–20 mg iron/kg diet) produced no significant change in body and organ (liver, kidney, spleen) weights and in blood hemoglobin level. However, the levels of hepatic non-heme iron in experimental (43 ± 2.5 µg/g tissue) rats were found to be significantly lower (66%, $p < 0.001$) as compared to controls (126 ± 7.8 µg/g tissue). Total iron levels decreased in all the organs studied with a maximum decrease (56%, $p < 0.01$) in liver followed by spleen (20%, $p < 0.05$) and kidney (19%, $p < 0.05$) (table 2). Iron deficiency was found to increase the levels of all the studied metals, i.e. Ca (22%, $p < 0.025$), Cu (22%, $p < 0.005$), Mn (19%, $p < 0.02$), Zn (21%, $p < 0.02$), Cd (16%, $p < 0.05$) and Pb (18%, $p < 0.01$) in the hepatic tissue. The iron-deficient rats also showed significantly higher levels of Cd (22%, $p < 0.02$) in renal tissue and Cu (17%, $p < 0.05$) and Ca (19%, $p < 0.02$) in spleen. All the values normal-

Table 2. Effect of latent iron deficiency on levels of iron, calcium, copper, zinc, manganese, lead and cadmium in liver, kidney and spleen of rats

Metals (μg/g)	Liver		Kidney		Spleen	
	Control	Iron-deficient	Control	Iron-deficient	Control	Iron-deficient
Iron	159.70 ± 7.21	86.25 ± 5.17***	86.37 ± 5.01	70.23 ± 4.07*	308.51 ± 18.70	246.80 ± 15.10*
Calcium	35.79 ± 1.83	43.65 ± 2.01*	54.93 ± 2.21	57.77 ± 2.61	38.58 ± 1.64	45.91 ± 1.89**
Zinc	31.34 ± 1.32	37.92 ± 1.67**	25.57 ± 1.17	27.01 ± 1.23	19.77 ± 0.86	20.11 ± 0.91
Copper	3.852 ± 0.130	4.707 ± 0.162***	5.461 ± 0.207	5.611 ± 0.260	1.600 ± 0.071	1.872 ± 0.089*
Manganese	2.026 ± 0.097	2.389 ± 0.088**	1.461 ± 0.061	1.571 ± 0.072	0.301 ± 0.024	0.331 ± 0.022
Lead	0.225 ± 0.009	0.266 ± 0.007***	0.299 ± 0.012	0.317 ± 0.016	0.101 ± 0.005	0.110 ± 0.006
Cadmium	0.131 ± 0.005	0.152 ± 0.007*	0.148 ± 0.006	0.181 ± 0.008**	0.061 ± 0.002	0.067 ± 0.003

Values represent mean ± SEM for five rats per dietary group. Significance of difference between two groups was evaluated by using t-test. Difference from control; *$p < 0.05$, **$p < 0.02$, ***$p < 0.01$. p values less than 0.05 were considered to be significant. Control = rats received 390 mg iron/kg diet; iron-deficient = rats received 18–20 mg iron/kg diet.

ized following 2 weeks of iron therapy (data not presented).

Feeding a low iron diet for 8 weeks produced a significant increase in various metal levels in different organs. The observed increase in the levels of metals in tissues of iron-deficient rats may be a result of their enhanced absorption as certain metals including Zn, Pb and Mn [10, 17] have been reported to be absorbed at an increased rate in the gut in cases of iron deficiency. The data on rehabilitation, in the present study, have shown that the alterations in the levels of these metals following latent iron deficiency could be normalized after 2 weeks of iron therapy. It is clear from many reports [1, 10, 18, 19] that there seems to exist an inverse relationship between iron and other divalent metals in terms of their dietary availability and tissue concentration. These changes can be explained through a common carrier system. Transferrin, a single chain polypeptide is the main serum protein responsible for the transport of iron [1, 9]. Since transferrin is known to bind with Zn (II), Cr (II), Co (II), Mn (II) and Cu (II) apart from iron, it is possible that this protein may take an active part in transporting other metals as well, particularly in the presence of reduced iron concentration [21, 22]. This may result in a redistribution of metals and eventually a shift from their normal levels in various organs. The observation of enhanced levels of metals in iron deficiency is probably because of interaction of different metals with iron at absorption, transportation and storage sites. The normalization of metal levels in these organs following recovery of their iron levels in rehabilitated animals further supports this notion. It may be concluded that the latent iron deficiency was able to produce significant alterations in certain heavy metal levels which may be responsible for some of the metabolic disorders seen in this form of nutritional disorder. Furthermore, increased accumulation of Pb and Cd in liver and Cd in kidneys showed higher suceptibility in latent iron-deficient animals towards hepato- and nephrotoxicity of these metallotoxins.

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