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ORIGINAL CONTRIBUTION

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Effect of different degrees of moderate iron deficiency on the activities of tricarboxylic acid cycle enzymes, and the cytochrome oxidase, and the iron, copper, and zinc concentrations in rat tissues

Einfluß moderater Eisenmangelsituationen auf die Aktivitäten der Citratzyklusenzyme und der Cytochrom-Oxidase sowie die Eisen-, Kupfer- und Zinkgehalte in Rattengeweben

Summary Severe iron deficiency results in complex systemic disorders e.g., including metabolism of energy and minerals. To investigate whether also moderate iron depletion may alter the activities of citric cycle enzymes and the cytochrome oxidase, the trace element status, and serum enzymes indicative of cell damage, this experiment was carried out with rats supplied with sub-optimal iron (9, 13 and 18 mg iron per kg diet) over a total of 5 weeks. The study included 3 pair-fed groups and an ad libitum group, fed with 50 mg iron/kg diet.

All iron-restricted rats were classified as iron-deficient on the basis of reduced iron concentrations in body and iron-depending blood parameters. Body weight gain and catalase activity in kidney were lowered in rats receiving the lowest dietary iron level, exclusively.

Rats fed 9 and 13 mg iron per kg diet had nearly 6- and 3-fold, respectively higher platelet counts in blood than their corresponding pair-fed controls. The activities of transaminases ASAT and ALAT, alkaline phosphatase, glutamate dehydrogenase and lactate dehydrogenase in serum which are indicative of cell damage were also markedly influenced by moderate dietary iron restriction, in which the enzyme levels in serum increased with intensifying iron depletion. Although, moderate iron restriction to young male rats was associated with marked alterations in iron status and serum enzymes, the activities of tricarboxylic acid cycle enzymes including malic dehydrogenase, fumarase, and isocitric dehydrogenase as well as cytochrome oxidase in liver remained largely unaffected. Only hepatic aconitase showed a somewhat reduction with iron depletion. Moreover, iron restriction was also accompanied with an accumulation of copper in liver which was significant for rats fed 9 and 13 mg iron per kg diet, whereas zinc status remained completely unaffected by moderate iron deficiency.

It can be concluded, that a short-term moderate iron deficiency with ranging hemoglobin concentrations from 66 and 121 g/L, was accompanied with altered platelet counts, serum enzyme activities indicative of cell damage, and hepatic copper concentrations, but the activities of the tricarboxylic acid cycle enzymes and cytochrome oxidase in liver remained largely unaffected.

Zusammenfassung Starker Eisenmangel verursacht im Körper komplexe Störungen, unter anderem auch des Energie- und Mineralstoffwechsels. Um zu prüfen, ob auch moderater Eisenmangel die Aktivitäten von Enzymen des Citratzyklus und der Cytochrom-Oxidase, den Spurenelementstatus sowie Gewebediagnostische Serumenzyme beeinflußt, wurde ein Versuch mit Ratten angelegt, die über einen Zeitraum von 5 Wochen suboptimale Eisengaben erhielten (9, 13 und 18 mg Eisen/kg Diät). Den Mangelgruppen wurden 3 pair-fed Kontrollgruppen und eine ad libitum Kontrollgruppe zugeordnet, deren Rationen 50 mg Eisen/kg enthielten.

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Bei allen Eisen-unterversorgten Ratten ließ sich aufgrund reduzierter Eisenkonzentrationen des Körpers und Eisen-abhängiger Blutparameter ein Eisenmangel diagnostizieren. Der Gewichtszuwachs und die Aktivität der Katalase in den Nieren zeigten sich nur bei Tieren mit niedrigster Eisenversorgung vermindert.

Ratten, die 9 und 13 mg Eisen pro kg Diät erhielten, zeigten nahezu 6-fach bzw. 3-fach höhere Plättchenzahlen im Blut als die entsprechenden pair-fed Kontrolltiere. Die Aktivitäten der Transaminasen ASAT und ALAT, der Alkalischen Phosphatase, der Glutamat- und Laktat-Dehydrogenasen im Serum, die indikativ auf eine Zellschädigung hindeuten, wurden ebenfalls durch den moderaten Eisenmangel deutlich beeinflußt, wobei mit der Stärke des Eisenmangels die Enzymwerte des Serums deutlich zunahmen. Obwohl der moderate Eisenmangel den Eisenstatus und die Serumenzyme junger Ratten deutlich veränderte, blieben die Aktivitäten der Tricarbonsäureenzyme Malat- und Isocitrat-Dehydrogenase, Fumarase und die Aktivität der Cytochrom-Oxidase in der Leber weitestgehend unverändert. Lediglich die Aktivität der hepatischen Aconitase zeigte sich im Eisenmangel leicht vermindert. Darüber hinaus ging die Eisendepletion mit einer Anreicherung von Kupfer in der Leber einher, erreichte jedoch nur bei Ratten mit einer Eisenversorgung von 9 und 13 mg Eisen/kg Diät das Signifikanzniveau. Der Zinkstatus blieb hingegen vom moderaten Eisenmangel gänzlich unbeeinflußt.

Zusammenfassend bleibt festzuhalten, daß ein kurzfristiger moderater Eisenmangel mit Hämoglobinkonzentrationen zwischen 66 und 121 g/L die Zahl der Blutplättchen, die Aktivität Gewebe-diagnostischer Serumenzyme und die Kupferkonzentrationen der Leber beeinflußte, während die Aktivitäten der Citratzyklusenzyme und der Cytochrom-Oxidase in der Leber weitestgehend unverändert blieben.

Key words Moderate iron deficiency – citric acid cycle enzymes – cytochrome oxidase – trace element status – rat

Schlüsselwörter Moderater Eisenmangel – Citratzyklusenzyme – Cytochrom-Oxidase – Spurenelementstatus – Ratte

Introduction

Iron deficiency results in complex systemic disorders that involve nearly all cells. In this respect many reports were focused to characterize iron-induced metabolic defects of the metabolism of energy and minerals (e.g., Cartier et al. (4), Masini et al. (16), Sherman and Moran (25), Sherman and Tissue (26), Yokoi et al. (31)). Some data in the literature revealed that iron restriction is associated with alterations in the oxidative metabolism of liver mitochondria, accompanied with partial uncoupling oxidative phosphorylation process (16), modifications in cytochromes (7, 18), and an impaired activity of succinate dehydrogenase from citric cycle (21). Experiments with animal models during the past decades have also revealed a strong relationship between dietary iron and concentrations of other essential trace elements in body (e.g., Graßmann and Kirchgeßner (12), Sherman and Moran (25), Sourkes et al. (28), Yokoi et al. (31)). Some investigators have observed increased copper levels in liver and altered zinc retention in iron-deficient rats (12, 25, 26, 31). The most experimental models described ascertained their results from severe dietary iron deficiency and severe anemia, respectively. However, clinical iron deficiency is rarely as severe as can be achieved by feeding laboratory animals a diet maximally depleted of iron. Therefore, the present research is aimed to investigate different degrees of moderate iron deficiency in attempt to mimic the human situation more closely. Iron concentrations in serum and tissues, blood parameters and catalase activity in kidney were used to assess the iron status. The additional parameters measured were the hepatic activities of tricarboxylic acid cycle enzymes and the cytochrome oxidase involved in energy metabolism, the copper and zinc concentrations in serum and tissues, and serum enzymes indicative of cell damage.

Moreover, a lot of former iron deficiency studies did not take into consideration that animals deficient in micro-nutrients often reduce voluntary food intake. Iron deficiency is therefore mostly combined with a reduced dietary intake of other micro-nutrients and with an energy deficiency. To eliminate differences in food intake we assigned iron-adequate pair-fed groups to each iron-deficient group. For that purpose, pair-fed rats were fed the iron-adequate diet in amounts equal to the average amount consumed on the previous day by the corresponding deficient group. The importance of such pair-fed groups consists in the fact that some iron effects would be otherwise over- or underestimated.

Materials and methods

Animals and diets

In this experiment, weaned male SPF Sprague-Dawley rats (WIGA GmbH, Sulzfeld, Germany) with an average body weight of 40 ± 2 g were fed diets differing in iron concentration for 5 weeks. For that purpose, the rats were divided into 7 groups of 12 each. The rats were fed diets containing sub-optimal iron: 9, 13 and 18 mg/kg. The basal diet analyzed had 2.6 mg/kg iron. The experiment

included 3 pair-fed control groups and an ad libitum group, fed with 50 mg iron per kg diet. All diets were supplemented with iron as $FeSO_4 \cdot 7H_2O$. All the other components of the diet remained constant. The diet was prepared from natural feedstuffs. All diets were fortified with recommended amounts of vitamins and minerals according to Reeves et al. (23). The composition of the basal semi-synthetic diet is given in Table 1. Rats were housed individually in a controlled environment, in Macrolon cages (Becker GmbH, Castrop-Ruxel, Germany) in a room maintained at 24 °C with a humidity of 60 %. All rats were kept under conditions of controlled lighting with a daily 12-h light:dark cycle, and had free access to drinking water (double distilled water, supplemented with 0.14 g/L sodium chloride to adjust osmolarity to that of tap water). Care and treatment of rats followed recommended guidelines (19). At the end of the experimental period of 5 weeks, 12 h after the last feeding, the rats were killed by decapitation after light anesthesia with diethyl ether. Blood for hematological measurements was collected into EDTA-coated vials. Blood for determination of serum mineral profile, substrates and enzyme activities was collected into untreated tubes. The liver, kidney, femur, and skeletal muscle were promptly excised. Serum and tissue samples were stored at -80 °C until analyzed.

Table 1 Composition of basal diet

Ingredient	g/kg diet
Casein, fat free	200
Corn starch	328
Sucrose	300
Fiber (cellulose)	30
Soybean oil	50
Coconut oil	30
Vitamin mixture[1)]	20
Mineral mixture[2)]	40
DL-methionine	2

[1)]Vitamins per kg diet: 4000 IU *all-trans* retinol; 1000 IU cholecalciferol; 150 mg *all-rac*-α-tocopherol; 1.47 mg menadione sodium bisulfite; 5 mg thiamin · HCl; 7.20 mg riboflavin; 6.00 mg pyridoxine · HCl; 15 mg Ca pantothenate; 30.0 mg nicotinic acid; 1.38 g choline chloride; 0.2 mg folic acid; 0.2 mg D-biotin; 25 mg cyanocobalamin; sucrose to 20 g

[2)]Minerals per kg diet: $Na_2HPO_4 \cdot 2\ H_2O$ 3.15 g; KH_2PO_4 3.82 g; KCl 4.77 g; $MgCl_2 \cdot 6\ H_2O$ 4.24 g; $CaCO_3$ 12.49 g; $NiSO_4 \cdot 6\ H_2O$ 4.48 mg; $ZnSO_4 \cdot 7\ H_2O$ 131.9 mg; $CuSO_4 \cdot 7\ H_2O$ 23.6 mg; $MnSO_4 \cdot 5\ H_2O$ 30.8 mg; KI 0.26 mg; $Na_2MoO_4 \cdot 2\ H_2O$ 0.28 mg; $Na_2SeO_3 \cdot 5\ H_2O$ 0.50 mg; $CrCl_3 \cdot 6\ H_2O$ 9.61 mg; NH_4VO_3 0.23 mg; $Na_2SiO_3 \cdot 5\ H_2O$ 37.76 mg; H_3BO_3 2.86 mg; NaF 2.21 mg; $(CH_3COO)_2Pb \cdot 3\ H_2O$ 0.73 mg; sucrose to 40 g

Hematological measurements and serum enzymes

Hematological variables including erythrocytes, white blood cells and platelets and hematocrit, hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined with a Coulter Counter and a hemoglobinometer (Coulter Electronics GmbH, Krefeld, Germany). Aspartate amino transferase (ASAT, EC 2.6.1.1), alanine amino transferase (ALAT, EC 2.6.1.19), alkaline phosphatase (AP, EC 3.1.3.1), glutamate dehydrogenase (GLDH, EC 1.4.1.4), and lactate dehydrogenase (LDH, EC 1.1.1.27) in serum were determined by standardized procedures using an autoanalyser (Hitachi 704, Boehringer, Mannheim, Germany) and Boehringer kit reagents (Boehringer, Mannheim, Germany).

Mineral analysis

Total femur and aliquots of the liver and muscle were prepared for iron, copper and zinc analyses by dry-ashing the non-homogenized samples. The dry-ashed samples were than dissolved in 0.6 mol/L hydrochloric acid. For each dry-ashing stage, one blank was included. Iron, copper and zinc concentrations in the tissues were quantified by its absorbance at 248.3 nm, 327.4 nm and 213.9 nm by introduction into the flame of an atomic absorption spectrophotometer (model 5100, HGA-600, Perkin-Elmer, Überlingen, Germany). The concentration of iron copper and zinc in serum was measured directly by its absorbance at 248.3 nm, 327.4 nm and 213.9 nm by aspirating a dilute solution (1:5) into the flame of the atomic absorption spectrophotometer. The iron, copper and zinc concentrations of each sample were calculated from standard curves using various dilutions of iron, copper and zinc standard solutions (1000 ± 2 mg/L, Merck, Darmstadt, Germany). All specimens were analyzed in duplicate. In the analysis of iron, copper and zinc, the coefficient of variation for duplicate analysis was typically below 2 %.

Activity of catalase in kidney

For measurement of catalase (EC 1.11.1.6) in tissue, aliquots of the kidney were prepared by a method described in 1970 by Cohen et al. (5) using 10 volumes of isotonic buffer to homogenize the tissue. The homogenate was centrifuged for 10 min at 700 g to remove nuclei and cell debris. To an aliquot of the supernatant fluid, ethanol was added to a final concentration of 0.17 M and samples were incubated for 30 min in an ice-water bath. After that, 10 % Triton X-100 was added to a final concentration of 1.0 % in order to dissolve the catalase. The activity of catalase was measured spectrophotometrically using a method of Aebi (1). The determination of

the enzyme activity was based on the measurement of the rate of converting H_2O_2 at 240 nm and a temperature of 25 °C in the presence of the enzyme. Protein in the supernatant used for enzyme determination was measured by a method of Smith et al. (27) using bicinchoninic acid.

Activities of hepatic enzymes

For determination of enzymes from citric acid cycle aliquots of liver were homogenized in 0.1 M sucrose buffer before centrifugation at 700 g to remove the cell nuclei. The supernatants were then centrifuged at 10,000 g for 10 min to obtain the mitochondria and the supernatant with the microsomes.

Malic dehydrogenase activity (E.C. 1.1.1.37) in liver mitochondria was determined according to a method reported by Ochoa (21). The determination of the enzyme activity was therefore based on the measurement of the rate of oxidation of NADH at 340 nm and 25 °C in the presence of the enzyme and excessive oxalacetate. One unit of enzyme is defined as the amount which causes an increase in optical density of 0.01/min under the above conditions.

The activity of fumarase (E.C. 4.4.1.2) in liver mitochondria was determined according to the method reported by Massey (17), using re-suspended mitochondria. The reaction was based on the chemical determination of *l*-malate, formed from fumarate at 300 nm. One unit of fumarase activity is defined as the amount of enzyme which causes an initial rate of change in optical density at 300 nm of 0.01/min at 25 °C.

The activity of isocitric dehydrogenase (E.C. 1.1.1.41 and E.C. 1.1.1.42) in liver was determined by a method reported by Ochoa (20), using the hepatic mitochondria. The early rate of reduction of $NADP^+$ in the presence of enzyme, Mn^{++}, and an excess of isocitrate is proportional to the concentration of the enzyme within certain limits at 340 nm and 25 °C. One unit of enzyme is defined as the amount which causes an increase in optical density of 0.01 per min under the above conditions.

Aconitase activity (E.C. 4.2.1.3) in liver was determined according to the method given by Anfinsen (3), using the liver supernatant. The method is based on the chemical determination of citrate formed from *cis*-aconitate at 240 nm and 25 °C. One unit of enzyme is defined as the amount which causes an initial rate of increase in optical density of 0.001 per min under the above conditions.

Cytochrome oxidase activity (E.C. 1.9.3.1) was determined on approximately 1–2 g of homogenized liver mitochondria which were obtained by centrifuging the hepatic nuclei-free supernatant three times at 10,000 g for 10 min (at 4 °C). The re-suspended mitochondria pellets were used for the enzyme assay. Cytochrome oxidase activity was spectrophotometrically determined by the rate of cytochrome c oxidation (6). The production of reduced cytochrome c as substrate was carried out using a method of Wharton and Tzagoloff (29). The activity of cytochrome oxidase was expressed as changes in optical density per mg protein and per min at 550 nm and 25 °C.

All enzyme measurements were run in duplicate. The protein concentration in the hepatic mitochondria and supernatant was determined by the Biuret method.

Statistical analysis

The effect of iron concentration in the diet was evaluated by analysis of variance and compared for statistical significance ($P < 0.05$) by the Fisher's multiple range test. All data in the present text are means ± SD of the single values.

Results

Weight gain, feed intake and feed efficiency

Weight gain, feed intake and the feed efficiency defined as ratio between total feed intake (g) and total weight gain (g) of iron-deficient and iron-adequate rats are shown in Table 2. Rats receiving 9 mg iron per kg diet had a lower body weight gain than the corresponding pair-fed controls, and daily food intake was depressed compared to the ad libitum-fed control group. Body weight gain and food intake of the groups fed 13 or 18 mg/kg dietary iron did not differ from their pair-fed controls and the ad libitum-fed group, respectively. The ratio between feed intake and weight gain was significantly higher in rats fed the lowest dietary iron level relative to the pair-fed controls, whereas a slight iron deficiency induced by 13 or 18 mg iron per kg diets did not alter the feed efficiency.

Hematological variables

Iron deficiency did not influence the amount of white blood cells (Table 3). Rats supplied with 9 and 13 mg/kg iron had markedly higher platelet counts in blood than the corresponding pair-fed controls, whereas a dietary iron concentration of 18 mg/kg did not elevate platelet level in blood. All iron-depending hematological parameters mainly including red blood cells, hemoglobin concentration and hematocrit decreased markedly by week 5 in rats on the iron-deficient diets demonstrating that the moderate iron deficiency was well developed 5 weeks after the experiment started. The amount of erythrocytes was significantly lower in rats fed 9 mg iron per kg diet compared to their pair-fed controls, but did not differ between the other groups. Hematological variables including hemoglobin concentration, hematocrit, MCV,

Table 2 Feed intake, weight gain and feed efficiency of moderate iron-deficient rats and their pair-fed controls[1),2)]

Performance variable	Group/Iron supply[3)]						
	Fe-9	Fe+PF9	Fe-13	Fe+PF13	Fe-18	Fe+PF18	Fe+ ad libitum
Daily feed intake (g)	15.3±1.4[b]	15.3±0.1[b]	17.3±2.1[a]	17.3±0.9[a]	18.0±1.0[a]	17.6±1.3[a]	17.2±1.4[a]
Total weight gain (g)	226±13[d]	265±7[c]	286±33[b]	294±16[ab]	306±15[a]	301±22[ab]	292±27[ab]
Feed efficiency (g/g)[4)]	2.64±0.23[a]	2.25±0.05[c]	2.36±0.10[b]	2.29±0.07[bc]	2.30±0.07[bc]	2.28±0.06[bc]	2.30±0.12[bc]

[1)]Data are means ± SD, n = 12 for each group
[2)]means were compared by the Fisher's multiple range test; significantly different means ($p < 0.05$) are marked with superscripts
[3)]Fe- represents iron-deficient groups, Fe+ represents iron-adequate groups, PF represents control group pair-fed to the iron-deficient group
[3]Feed efficiency is defined as total feed intake/total weight gain

Table 3 Blood parameters of moderate iron-deficient rats and their pair-fed controls[1,2]

Blood parameter	Group/Iron supply[3)]						
	Fe-9	Fe+PF9	Fe-13	Fe+PF13	Fe-18	Fe+PF18	Fe+ ad libitum
WBC (10^9/L)	19.6±5.0	15.6±6.4	15.0±5.4	17.9±7.4	16.8±5.2	16.9±7.4	16.7±5.1
RBC (10^{12}/L)	6.27±0.70[d]	7.10±0.27[bc]	7.88±1.02[a]	7.18±0.54[abc]	7.55±1.25[ab]	7.10±0.45[bc]	6.71±1.48[cd]
Platelets (10^9/L)	2134±470[a]	366±287[c]	1242±412[b]	406±267[c]	376±175[c]	317±267[c]	321±279[c]
Hemoglobin (g/L)	66.3±8.40[d]	152± 5[a]	96.6±12.5[c]	150±10[a]	121±21[b]	146±10[a]	145±21[a]
Hematocrit	0.23±0.03[d]	0.44±0.01[a]	0.32±0.04[c]	0.44±0.03[a]	0.38±0.06[b]	0.44±0.03[a]	0.42±0.09[a]
MCV (μm^3)	36.0±1.5[d]	62.4±2.0[a]	40.3±1.8[c]	61.3±1.8[a]	49.9±3.7[b]	61.8±1.4[a]	61.0±1.8[a]
MCH (pg/cell)	10.6±0.4[e]	21.4±0.8[ab]	12.3±0.7[d]	20.9±0.7[ab]	16.0±1.6[c]	20.6±2.0[b]	22.3±4.0[a]
MCHC (g/L)	294±5[e]	343±5[ab]	305±9[de]	341±3[abc]	320±10[cd]	333±33[bc]	359±60[a]

[1)]Data are means ± SD, n = 12 for each group
[2)]means were compared by the Fisher's multiple range test; significantly different means ($p < 0.05$) are marked with superscripts
[3)]Fe- represents iron-deficient groups, Fe+ represents iron-adequate groups, PF represents control group pair-fed to the iron-deficient group

Table 4 Serum enzymes of moderate iron-deficient rats and their pair-fed controls[1,2]

Enzyme	Group/Iron supply[3)]						
	Fe-9	Fe+PF9	Fe-13	Fe+PF13	Fe-18	Fe+PF18	Fe+ ad libitum
ASAT (U/L)	362±89[a]	228±35[bc]	269±45[b]	221±28[c]	238±48[bc]	231±57[bc]	259±28[bc]
ALAT (U/L)	62.1±11.3[a]	38.3±5.8[c]	53.6±11.0[ab]	52.7±29.2[ab]	48.5±7.5[bc]	46.7±7.9[bc]	48.4±7.1[bc]
AP (U/L)	539±126[a]	385±50[c]	484±78[ab]	419±78[bc]	470±86[ab]	429±96[bc]	464±129[abc]
GLDH (U/L)	8.48±1.13[a]	6.66±0.79[b]	8.54±1.54[a]	8.14±1.60[a]	7.72±0.99[ab]	7.84±1.85[a]	8.13±1.26[a]
LDH (U/L)	3585±456[a]	2658±666[bc]	3122±660[ab]	2510±531[c]	3056±656[b]	2510±824[c]	3210±358[a]

[1)]Data are means ± SD, n = 12 for each group
[2)]means were compared by the Fisher's multiple range test; significantly different means ($p < 0.05$) are marked with superscripts
[3)]Fe- represents iron-deficient groups, Fe+ represents iron-adequate groups, PF represents control group pair-fed to the iron-deficient group

MCH and MCHC reflected the grading iron status best and were significantly depressed by diets containing 9, 13 and 18 mg/kg. Control rats fed a restricted amount of the diet to keep their food intake similar to those of the iron-deficient animals did not have depressed iron-depending heme parameters.

Serum enzymes

The activities of serum enzymes including ASAT, ALAT, AP, GLDH, and LDH increased with the severity of dietary iron deficiency anemia (Table 4). All iron-deficient groups had significantly higher activities of LDH than their corresponding pair-fed groups. ASAT activity was significantly elevated in groups fed 9 and 13 mg iron per kg diet, and ALAT, AP, and GLDH were only influenced by the lowest dietary iron supply.

Iron, copper and zinc profile in body

The concentration of iron in serum, liver, femur and skeletal muscle was considerably depressed in all moderate iron-deficient groups compared to their corresponding pair-fed controls (Table 5). The maximum reduction of iron concentration occurred in liver was by about 77 %, 66 %, and 60 %, depending on the severity of iron restriction. The copper concentration in serum, femur, and skeletal muscle was not influenced by the different degrees of iron depletion, but hepatic copper concentration was significantly higher in rats fed 9 and 13 mg iron/kg diet than in the corresponding pair-fed control animals. Although serum and tissue zinc concentrations remained unchanged by dietary iron, zinc concentration in skeletal muscle seemed to be influenced by the feed intake and weight gain, respectively.

Hepatic and kidney enzymes

The activities of most enzymes in mitochondria measured including malic dehydrogenase, fumarase, isocitric dehydrogenase and cytochrome oxidase did not depend on the different iron supplies at all (Table 6). However, aconitase activity was somewhat reduced due to iron deficiency by a maximum of 10 %. Also the activity of catalase in kidney was reduced by a maximum of 31 % in rats supplied with 9 mg iron per kg diet.

Discussion

Most of the previous studies on the effects of iron deficiency on mineral status or energy metabolism have been investigated in severe iron-deficiency anemia. We report here that even mild and moderate iron deficiency where hemoglobin concentrations ranges between 66 and 121 g/L, was accompanied with some marked biochemical alterations including platelets, serum enzymes, hepatic copper concentrations, and a slight change in aconitase activity. Hemoglobin concentrations, hematocrit and other iron-depending blood parameters, iron concentrations in serum and tissues, as well as catalase activity in kidney were the parameters used to assess iron status. All iron-depending hematological parameters mainly including red blood cells, hemoglobin concentration and hematocrit and the iron concentrations in serum, liver, femur and skeletal muscle decreased markedly by week 5 in rats on the iron-deficient diets demonstrating that the moderate iron deficiency was well developed 5 weeks after the experiment started.

Since iron is participated in a wide variety of biochemical processes, particularly enzymatic reactions associated with mitochondrial energy production (11), one aspect of the present study was to investigate whether a mild and moderate iron restriction is associated with changes in the activities of citric acid cycle enzymes and the cytochrome oxidase in liver. A partial uncoupling of oxidative phosphorylation observed in an in vitro study gave additional support for the conclusion as to the occurrence of a perturbation of the energy state in iron-deficient liver (16). Although, the results of the present research reveal that slight and moderate iron restriction to young male rats is associated with several alterations in iron status and serum enzymes indicative of cell damage, the activities of tricarboxylic acid cycle enzymes and cytochrome oxidase in liver were largely unaffected. These data are consisting with the results of Willis et al. (30) and Cartier et al. (4) showing that even severe iron deficiency have little or no effect on citric acid cycle enzymes and the levels of other mitochondrial matrix enzymes. The unaffected cytochrome oxidase activity measured might be explicable, inasmuch as concentrations of cytochromes a and a3, which are components of cytochrome oxidase, are less changeable by iron deficiency than other cytochromes (10). Contrary, another study has found that cytochrome oxidase activity in skeletal muscle was even reduced in mild iron deficiency with hemoglobin concentrations of 100 g/L (22). These contradictory results elucidate the difficulty to compare the quality and extent of changes in enzyme activities found in the present iron deficiency study with those from other experiments, because the changeability of these parameters depends besides the severity of iron depletion on other experimental factors, including duration of this deficiency, animal's age, and the composition of the diets used (11). However, it is feasible that a longer time of moderate iron deficiency may render the cells more susceptible to metabolic alterations in the event of impaired enzyme activities. However, the activity of aconitase where the iron molecule participates as cofactor was somewhat reduced by the lowest dietary iron level in the present study. Since, the enterocyte aconitase activity may be important in the regulation of iron metabolism and absorption (9), the slightly reduced hepatic aconitase activity found in the present study might possibly reflect a beginning homeostatic iron regulation.

The results of the present research reveal that iron-deficient rats fed 9 mg iron per kg diet grew more slowly than their pair-fed and ad libitum-fed counterparts over

Table 5 Iron, copper and zinc concentrations in serum, liver, femur, and skeletal muscle of iron-deficient rats and their pair-fed controls[1,2]

Mineral	Group/Iron supply[3]						
	Fe-9	Fe+PF9	Fe-13	Fe+PF13	Fe-18	Fe+PF18	Fe+ ad libitum
Serum							
Iron (µmol/L)	18.6±4.84^{a}	52.7±5.55^{c}	25.1±7.88^{a}	54.8±4.30^{c}	36.2±6.98^{b}	59.6±14.0^{c}	58.2±13.3^{c}
Copper (µmol/L)	17.2±2.83	16.5±3.15	17.6±2.52	17.5±1.89	16.5±1.42	17.6±2.05	18.6±3.78
Zinc (µmol/L)	22.0±2.14^{a}	22.2±1.68^{ab}	23.5±2.75^{abc}	23.7±2.45^{abc}	24.9±1.38^{c}	24.3±1.99^{abc}	24.3±3.06^{bc}
Liver							
Iron (mmol/kg)	0.44±0.03^{c}	1.92±0.36^{a}	0.51±0.05^{c}	1.49±0.29^{b}	0.64±0.08^{c}	1.59±0.25^{b}	1.62±0.28^{b}
Copper (µmol/kg)	89.4±15.6^{d}	64.7±3.0^{bc}	71.3±9.6^{b}	60.1±7.2^{c}	67.7±4.4^{bc}	65.2±10.2^{bc}	62.0±4.9^{bc}
Zinc (mmol/kg)	0.37±0.02	0.41±0.02	0.38±0.02	0.36±0.01	0.38±0.03	0.37±0.04	0.37±0.02
Femur							
Iron (mmol/kg)	0.37±0.18^{d}	0.94±0.15^{a}	0.41±0.06^{d}	0.81±0.08^{b}	0.54±0.12^{c}	0.79±0.15^{b}	0.85±0.14^{ab}
Copper (µmol/kg)	45.5±9.0	47.7±7.1	47.8±9.0	45.8±5.8	46.0±4.2	45.0±5.0	45.6±2.8
Zinc (mmol/kg)	2.19±0.47	2.24±0.25	2.35±0.37	2.07±0.52	2.17±0.61	1.92±0.61	2.12±0.19
Skeletal Muscle							
Iron (µmol/kg)	45.7±6.6^{e}	136±16^{b}	75.9±17.9^{d}	143±19^{ab}	106±12^{c}	151±16^{a}	138±16^{ab}
Copper (µmol/kg)	11.6±11.2	11.5±5.0	13.9±9.9	14.9±5.0	12.9±5.2	14.0±8.2	12.1±6.5
Zinc (µmol/kg)	129±18^{c}	135±15^{bc}	150±25^{ab}	143±21^{abc}	149±17^{ab}	153±23^{a}	149±19^{ab}

[1] Data are means ± SD, n = 12 for each group
[2] means were compared by the Fisher's multiple range test; significantly different means ($p < 0.05$) are marked with superscripts
[3] Fe- represents iron-deficient groups, Fe+ represents iron-adequate groups, PF represents control group pair-fed to the iron-deficient group

Table 6 Activities of enzymes from citric acid cycle[1] and the cytochrome oxidase[2] in liver and the activity of catalase[3] in kidney of moderate iron-deficient rats and their pair-fed controls[4,5]

Enzyme	Group/Iron supply						
	Fe-9	Fe+PF9	Fe-13	Fe+PF13	Fe-18	Fe+PF18	Fe+ ad libitum
	U/mg protein[6]						
Malic dehydrogenase	15.3±9.3	14.3±8.4	20.3±11.8	15.4±9.8	20.1±13.0	15.0±8.3	15.3±6.5
Fumarase	31.6±16.7	38.0±15.4	32.8±11.5	39.5±9.9	41.1±9.0	38.2±11.5	36.3±10.5
Isocitric dehydrogenase	106±19	99±23	119±23	107±29	111±14	111±22	104±17
Aconitase	27.0±4.8^{c}	30.1±4.5^{abc}	30.7±4.8^{abc}	33.1±5.7^{a}	28.2±6.0^{bc}	31.4±3.4^{ab}	33.9±5.5^{a}
Cytochrome oxidase	0.48±0.13	0.47±0.33	0.54±0.28	0.45±0.16	0.43±0.22	0.43±0.21	0.35±0.17
Catalase	110±42^{a}	159±30^{b}	143±30^{b}	150±23^{b}	162±43^{b}	159±29^{b}	163±34^{b}

[1] 1U of malic dehydrogenase, fumarase and isocitric dehydrogenase is defined as a change in optical density of 0.01/min at 25 °C; 1U of aconitase is defined as a change in optical density of 0.001/min at 25 °C
[2] 1U of cytochrome oxidase is defined as the change in optical density/min at 25 °C
[3] 1U of catalase is defined as 1 µmol peroxide substrate decomposed/min at 25 °C
[4] Data are means ± SD, n = 12 for each group
[5] means were compared by the Fisher's multiple range test; significantly different means ($p < 0.05$) are marked with superscripts
[6] Fe- represents iron-deficient groups, Fe+ represents iron-adequate groups, PF represents control group pair-fed to the iron-deficient group

a 5-week period. However, on the basis of data measured it can be excluded that the impaired growth gain and feed efficiency in moderate dietary iron restricted growing rats was due to altered activities of citric acid cycle enzymes and the cytochrome oxidase. The effect on growth rate therefore might be explained by the reduction in iron-depending ribonucleotide reductase activity which is involved in DNA synthesis (14).

The present results demonstrate that moderate iron deficiency altered fundamentally the activities of serum

enzymes including the transaminases ALAT and ASAT, GLDH, LDH and AP. An increase of these enzymes may be indicative of cell damage, especially liver parenchymal cells. Degeneration of parenchymal liver cells along with ultrastructural abnormalities including enlarged, rounded, and electron-lucent mitochrondria in the hepatocyte has been formerly reported in iron-deficient rats (2, 7, 8). The present data therefore give no rise to the suspicion that there was a close relationship among mitochondrial respiratory function and hepatic cell damage, and this is in agreement with the results of Dallman and Goodman (8) showing that no close relationship among mitochondrial respiratory function, changes in mitochondrial morphology, and a decreased cytochrome content is apparent.

Moderate iron deficiency was also accompanied with decreased liver iron, while levels of copper increased. This phenomenon has been even shown during iron deficiency with dietary iron levels between 5 and 8 mg/kg (12, 25, 26, 31). In the present work, an increase in copper concentration of the iron-deficient rats was observed only in liver, an organ which was directly associated with erythropoiesis. It is speculated that iron deficiency evokes the inefficient utilization of copper and that excess copper accumulated in tissue. However, iron-induced alterations in body zinc concentration, which could be observed in other iron deficiency studies of Sherman and Tissue (26) and Yokoi et al. (31) were not present in this study, although zinc concentration in serum and skeletal muscle was primarily effected by the level of feed intake. The thrombocytosis observed in the iron-deficient rats from this study is probably associated with the impaired erythropoiesis and confirms data from previous iron deficiency studies showing that moderate iron deficiency commonly causes a rise in platelet count (13, 24). It has been suggested that erythropoietin which was increased in iron deficiency might be responsible for the thrombocytosis (15, 24).

In conclusion, this animal model using the rat reflected closely the gradual degrees of iron deficiency as they can be observed in man. From the foregoing observations it is evident that the moderate iron deficiency with ranging hemoglobin concentrations from 66 and 121 g/L, was accompanied with few biochemical alterations including platelet counts, serum enzyme activities indicative of cell damage, and hepatic copper concentrations, whilst the activities of the tricarboxylic acid cycle enzymes and cytochrome oxidase in liver remained largely unaffected. However, we must take into consideration that iron deficiency anemia was induced within a relatively short period of time and our observations are at a relatively early stage of iron depletion. Despite the short experimental period, iron deficiency has been shown to be well developed in these animals. This might be due to the fact that the rats used for this study were during a period of rapid growth, and therefore less resistant to trace element deficiencies than adults. Conclusions about the effects of prolonged iron deficiency can only be made with some reservation. However, it is feasible that a longer time of iron deficiency which may occur for several month or years may render the biochemical processes more susceptible to iron-induced pertubations.

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