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Effect of Iron Deficiency on the Capacity for Peroxisomal and Mitochondrial β-oxidation

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ABSTRACT

In order to determine the effect of iron depletion and subsequent supplementation on the muscle capacity for peroxisomal (PO) and mitochondrial (MO) β-oxidation during high fat feeding, weanling rats were fed a 44% (HF) or 2.5% (LF) fat diet with (+Fe) or without (−Fe) iron for 6 or 9 weeks. After 1 week rats fed HF+Fe or HF−Fe had 50~100% more PO and MO in heart, soleus, psoas and gastrocnemius than did rats fed low fat, but after 3 weeks rats fed HF−Fe had lower muscle PO and MO. In muscles of iron depleted rats PO and MO were not increased by supplementation with iron for 3 weeks. After 6 weeks MO and PO in skeletal muscles of rats fed HF+Fe were lower than after 3 weeks. It is concluded that adequate iron is necessary for maximum response of muscle PO and MO to high fat feeding. However, after 6 weeks both PO and MO have returned to levels similar to those of rats fed low fat diets, hence, the elevated catalase activities seen at this time do not reflect peroxisomal β-oxidation.

KEY WORDS : iron deficiency · fatty acid oxidation · peroxisome · catalase · skeletal muscle.

Introduction

Iron deficiency is a worldwide nutritional problem and usually occurs among populations that are at high risk, such as infants, children and women during the child-bearing years[1)2)]. Iron deficiency has been associated with biochemical abnormalities in essential iron compounds. In iron deficient rats, skeletal muscle myoglobin concentration was decreased by 20~50%, especially during the growth period[3-9)]. It has been reported that iron enzymes of mitochondria, such as cytochrome C and iron-sulfur proteins, can be most severely affected in the skeletal muscles of young, iron deficient rats[6)9-18)].

Previously we found that iron depletion in rats resulted in decreased iron concentration and catalase activity in skeletal muscles[19)]. Rats fed a high fat diet had elevated muscle catalase activity, but when the diet was deficient in iron, elevated activity of this enzyme was not sustained[19)]. We have also found that peroxisomal β-oxidation capacity is increased in some muscles from rats fed a high fat diet[20)21)]. Therefore, we questioned whether the normal increase in peroxisomal fatty acid oxidation in response to high fat feeding

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could be impaired by iron depletion. Furthermore, we questioned whether peroxisomal fatty acid oxidation could affect catalase activity in muscles from rats fed a high fat diet.

It was the purpose of this study to determine the effect of iron depletion and subsequent supplementation on the muscle capacity for peroxisomal and mitochondrial β-oxidation during high fat feeding.

Materials and Methods

Male Sprague-Dawley rats, weighing about 50g each, were purchased from Bio Labs, Inc., St. Paul, MN. The animals were housed individually in stainless-steel wire-floored cages and allowed free access to food and water. The room was maintained at 23℃ with a light cycle between 8 : 00 and 20 : 00 h. The animals were fed an unrefined diet (Purina Rat chow, St. Louis, MO) for 4 days. Six animals were then killed to provide baseline values and the remaining animals were randomized into four dietary groups. Two groups served as iron-supplemented controls and were fed either high- (HF+Fe) or low-fat (LF+Fe) diets, while the remaining two groups were fed high- or low-fat diets without iron (HF−Fe and LF−Fe, respectively) for 6 weeks. The composition of the diets is shown in Table 1. Because food intake was different among groups, diets were fed in amounts such that each group had isoenergetic intake. The animals were given deionized water for the duration of the experiment. The high-fat diet contained 44% by weight of 2 : 1 mixture of corn oil and lard, while the low-fat diet contained 2.5% of this mixture. Iron($FeSO_4 \cdot 7H_2O$) was added in the high-fat diet (90 ppm) and low-fat diet (60 ppm). Animals were killed after 1week, 3weeks and 6weeks. After 6weeks, the animals in the iron-depleted groups were fed an iron-supplemented diet for 3weeks.

Blood was taken by cardiac puncture from rats anesthesized with ether. Blood was diluted with Drabkins reagent and hemoglobin was determined, using the cyanomethemoglobin method, with a Sigma Diagnostic kit[22].

Table 1. Composition of experimental diets

Ingredients	High fat		Low fat	
	g	g/100 Cal	g	g/100 Cal
Fat[1]	440	8.48	25	0.72
Glucose[2]	-	-	605	17.46
Cellulose[2]	157.5	3.03	105	3.03
Casein[2]	300	5.78	200	5.77
DL-Methionine[3]	4.5	0.087	3	0.087
Arginine-HCl[2]	3	0.058	2	0.058
BHT[2]	8.25	0.159	5.5	0.159
AIN vitamin mix[4]	22.5	0.43	15	0.43
Mineral mix(-Fe)[5]	60	1.16	40	1.15
added Fe(+Fe)	0.09	0.0017	0.06	0.0017

1) Fat was 2 : 1 corn oil and lard(University of Minnesota, Food Service, ST. Paul, MN)
2) U.S.Biochemical Corporation, Cleveland, OH
3) Sigma Chemical Co., St. Louis, MO
4) ICN Nutritional Biochemicals, Cleveland, OH
5) In mg/100 Cal, NaH_2PO_4 368.2 ; $CaCO_3$ 368.2 ; KCl 144.4 ; $MgSO_4$ 118.8 ; $ZnSo_4$ 4.2 ; $MnSO_4H_2O$ 4.5 ; $CuSO_45H_2O$ 2.3 ; KIO_4 0.04

After blood sampling, the unconscious animals were killed by decapitation. Liver and muscles were immediately removed to ice-cold medium (pH 7.5) containing 100mM KCl, 50mM Tris-HC l, 5mM $MgCl_2$ 1mM ATP and 1mM EDTA. The tissues were finely minced with scissors, suspended in the medium(10% or 20%) and homogenized with a cornical ground-glass homogenizer (Kontes, Evanston, IL).

The peroxisomal and mitochondrial β-oxidation capacities were determined using [1-^{14}C] palmitate as substrate by a method described previously[20]. The assay conditions are shown in Table 2. The reaction was started by addition of sample and incubation was carried out for 10min at 37℃ in a Dubnoff metabolic shaker waterbath. When measuring mitochondrial oxidation the reaction was performed in 10ml vials capped with rubber septums with a center well containing a piece of filter paper. The reaction was stopped by injecting 0.3ml of 3M perchloric acid into each vial. Immediately 0.3ml of a mixture of ethanolamine/ethylene glycol (1/2 v/v) was injected into the center wells to collect $^{14}CO_2$. The vials were then incubated overnight at 4℃. The contents of each well were rinsed into 5ml of toluene/methanol (2/1 v/v) containing 0.4% Omnifluor(New England Nuclear, Boston, MA). The acid solution was centrifuged at 2000×g for 15min and an aliquot of the supernatant was pipetted into 5ml of Aquasol (New England Nuclear, Boston, MA) /toluene (2/1 v/v). Radioactivity was determined by scintillation spectrometry in a Beckman LS-2 scintillation counter. Effeciency was determined by the external standard ratio. When measuring peroxisomal oxidation a similar procedure was used except the reaction was carried out in 15×75 mm uncapped tubes and CO_2 was not collected, as it had been previously determined that no $^{14}CO_2$ was produced. Redioactive substrate was obtained from New England Nuclear, Boston, MA. The palmitate was bound to a fatty acid-free bovine serum albumin (Sigma, St. Louis, MO) in a 5 : 1 palmitate : albumin molar ratio.

Table 2. Assay conditions for peroxisomal and mitochondrial β-oxidation

	peroxisomal	mitochondrial
pH	8.5	7.5
Rotenone	10.0μM[1)]	–
Triton-X-100	0.01%	–
FAD	10.0μM	–
L-carnitine	–	1.0mM
L-malate	–	0.5mM
Cytochrome C	–	25.0μM
ATP	5.0mM	5.0mM
NAD	0.2mM	1.0mM
CoA	0.1mM	0.1mM
Dithiothreitol	1.0mM	–
Tris-HCl	45.0mM	75.0mM
Sucrose	–	25.0mM
K_2HPO_4 $3H_2O$	–	10.0mM
$MgCl_2$	–	5.0mM
EDTA	–	1.0mM

1) Final concentration

All reagents from Sigma Chemical Co., St. Louis, MO

Catalase activity was measured using the method described by Baudhuin et al[23], except that Triton X-100 was added to determine the total enzyme activity. Data were expressed as EU/g tissue. One EU(Enzyme Unit) is the amount of enzyme which oxidized one mole of H_2/O_2 under the conditions of the assay, based on a standard curve using bovine liver catalase(Sigma, St. Louis, MO) as the standard. Mineral analysis was done by plasma emission spectroscopy as described previously[19]. Data were expressed as means± S.E (n=6) and analyzed using analysis of variance followed by the Honestly Significant Differeñce test(T-method)[24]. Paired comparisons were made by Student's t-test[25].

Results

Rats fed the LF−Fe diet for 6weeks showed expected lower growth rate than did the controls (Fig. 1)[3)19)26)27)]. Upon supplementing the iron depleted groups with iron for 3weeks, after six weeks of iron depletion, it seems that there was some increase in body weight.

During the first 3weeks of the experiment, hemoglobin values in the iron-depleted rats fed either the high-or low-fat diets failed to increase with age at the normal rate(Fig. 2). When the iron-depleted rats were supplemented with iron for 3weeks, after the 6week depletion period, there was rapid increase in hemoglobin.

The iron concentration of the gastrocnemius muscle was not significantly different between control groups fed either high-or low-fat diets after 6weeks(Table 3). In contrast, there was significantly lower iron concentration in muscles of both iron-depleted groups. After 6 weeks, the iron concentration of the gastrocnemius was lowered by about 50~60% in rats fed the iron-depleted diets. After supplementing both iron-depleted groups with iron for 3weeks, the iron concentration of the gastrocnemius in rats fed the high-fat diet reached that of controls, but did not in rats fed the low-fat diet. The copper concentration of the gastrocnemius in rats fed the low-fat diet was significantly lower than that in control group. In rats fed high fat diet, however, there was no significant difference in the copper concentration between iron-depleted and control group. The concentra-

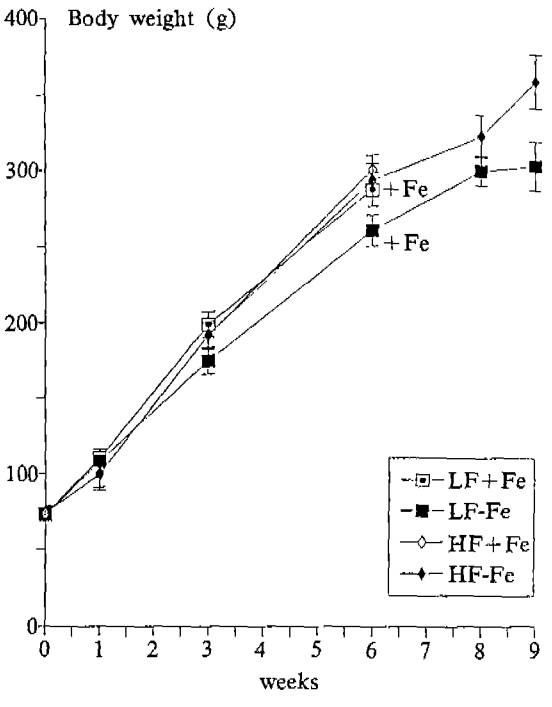

Fig. 1. Growth rate of rats fed low fat with iron (LF+Fe) or without iron (LF−Fe), or high fat with iron (HF+Fe) or without iron (HF−Fe) diets.

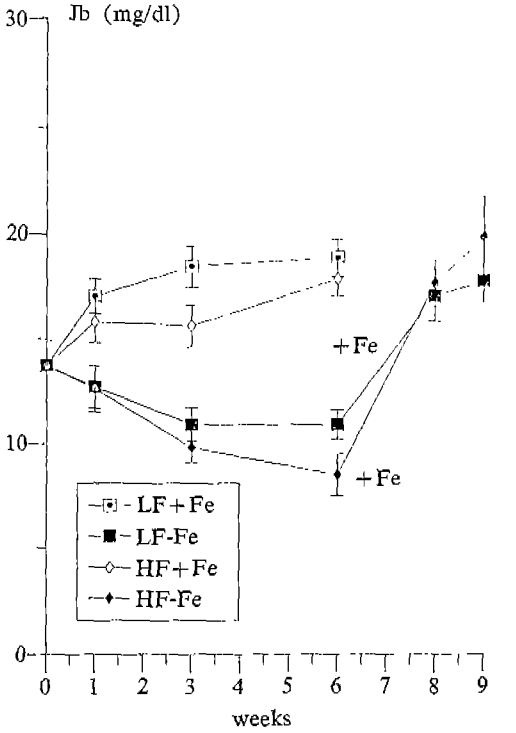

Fig. 2. Hemoglobin concentration of rats fed low fat with iron (LF+Fe) or without iron (LF−Fe), or high fat with iron(HF+Fe) or without iron (HF−Fe) diets.

tion of zinc and calcium in the gastrocnemius was not significantly different among all control and iron-depleted groups.

The capacity of the heart, psoas, soleus and gastrocnemius for peroxisomal β-oxidation in rats fed the low-fat diets was significantly lower than that in rats fed the high-fat diets for 1week, but the capacity of the heart and gastrocnemius was not different from that in rats fed the high-fat diet for 3weeks(Table 4). On the other hand, the liver capacity for peroxisomal β-oxidation was not different between high-fat and low-fat groups after 1week. After 3weeks, however, the liver capacity for peroxisomal β-oxidation in rats fed the low-fat diets was significantly lower than that in rats fed the high-fat diets.

The heart capacity for peroxisomal β-oxidation in rats fed the HF−Fe diet was significantly decreased when compared to HF controls after 3 weeks, while the heart capacity for peroxisomal β-oxidation in rats fed the LF-Fe diet was not significantly different from LF controls after 3 weeks. There was significantly lower capacities of the psoas and gastrocnemius for peroxisomal β-oxidation in both iron-depleted groups fed either high-fat or low-fat diets for 3weeks.

The capacity of the liver, psoas, soleus and gastrocnemius for peroxisomal β-oxidation did not remain elevated in rats fed the HF+Fe diet for 6weeks. Therefore, there was no difference in capacity of the liver, psoas and gastrocnemius for peroxisomal β-oxidation among all control and iron-depleted groups fed either high-fat or low-fat diet for 6weeks. The soleus capacity for peroxisomal β-oxidation, however, in rats fed the low-fat diets was greater than that in rats fed the high-fat diets for 6weeks.

Iron supplementation for 3weeks, after 6weeks of iron depletion, did not result in elevated capacity for peroxisomal β-oxidation in the heart and soleus from rats fed either high-fat or low-fat diet, in the psoas from rats fed the high-fat diet and in the gastrocnemius from rats fed the low-fat diet. On the other hand, the capacity for peroxisomal β-oxidation was decreased in the psoas from rats fed the low-fat diet and elevated in the gastro-

Table 3. Effects of iron depletion and subsequent iron supplementation on rat gastrocnemius muscle mineral concentration

	Fe	Cu	Zn	Ca
Baseline	0.96± 0.09[1)]	0.08± 0.02	0.95± 0.07	8.85± 0.67
6weeks				
LF+Fe[2)]	1.03± 0.02[b3)]	0.08± 0.001[b]	0.89± 0.02[a]	6.46± 0.27[a]
LF−Fe	0.47± 0.07[a]	0.06± 0.01[a]	0.72± 0.02[a]	6.71± 0.31[a]
HF+Fe	0.96± 0.09[b]	0.08± 0.01[b]	0.83± 0.05[a]	5.78± 0.29[a]
HF−Fe	0.58± 0.05[a]	0.09± 0.01[b]	0.76± 0.02[a]	6.73± 0.09[a]
6weeks of iron depletion plus 3weeks of iron supplementation				
(LF−Fe)+Fe	0.78± 0.04*[4)]	0.06± 0.01	0.78± 0.07	6.03± 0.17
(HF−Fe)+Fe	0.93± 0.09	0.07± 0.02	0.88± 0.06	5.65± 0.24

1) Values are means± S.E., expressed as μmoles/g dry tissue

2) Rats were fed low fat with iron (LF+Fe), low fat without iron (LF-Fe), high fat with iron(HF+Fe), or high fat without iron (HF-Fe) diets

3) Values with different alphabetic superscipts within each column are significantly different($p<0.05$) by the Honestly Significant Difference Test (T-method)

4) Student's t-test was used to compare supplemented animals with their 6week controls. *$p<0.01$

cnemius fed the high-fat diet, after subsequent iron supplementation.

The capacity of the liver, psoas, soleus and gastrocnemius for mitochondrial β-oxidation in rats fed the low-fat diets was significantly lower than that in rats fed the high-fat diets after 1week and it remained lower in the liver, heart, psoas and gastrocnemius after 3weeks(Table 5). On the other hand, the soleus capacity for mitochondrial β-oxidation was not different between high-fat and low-fat groups after 3weeks.

The soleus capacity for mitochondrial β-oxidation in rats fed the HF−Fe diet was significantly decreased when compared to HF controls after 1week. There was a significantly decreased capacity of the psoas and gastrocnemius for mitochondrial β-oxidation in rats fed the HF−Fe diet for 3weeks. Also there was a significantly decreased capacity of the soleus for mitochondrial β-oxidation in both iron-depleted groups fed either high-fat or low-fat diets for 3weeks and the capacity of the soleus for mitochondrial β-oxidation in rats

Table 4. Effects of iron depletion and subsequent iron supplementation on peroxisomal β-oxidation capacity

	Liver	Heart	Psoas	Soleus	Gastrocnemius
Baseline	198± 9[1)]	313± 7	87± 3	107± 9	92± 4
1 week					
LF+Fe[2)]	236± 6[a3)]	305±10[a]	89± 8[a]	140±13[a]	70± 6[a]
LF−Fe	216±12[a]	309±21[a]	74± 1[a]	120± 6[a]	68± 8[a]
HF+Fe	201± 9[a]	456±12[b]	171±14[b]	289±17[b]	149± 3[b]
HF−Fe	191± 9[a]	471±27[b]	157± 6[b]	253± 9[b]	131±11[b]
3 weeks					
LF+Fe	149±13[b]	417± 8[a]	107± 2[b]	110± 2[b]	119±15[b]
LF−Fe	106± 7[a]	401±25[a]	89± 7[a]	75± 9[a]	76± 8[a]
HF+Fe	188± 5[c]	410±28[a]	179± 4[c]	141± 8[c]	136±12[b]
HF−Fe	181± 6[c]	333± 5[b]	83± 6[a]	93±15[a]	79±10[a]
6 weeks					
LF+Fe	179±12[a]	350± 4[a]	81± 5[a]	158± 8[b]	85± 6[a]
LF−Fe	147±17[a]	360± 9[a]	65± 4[a]	141±23[b]	74±13[a]
HF+Fe	135±12[a]	405± 5[a]	76± 5[a]	63± 9[a]	71± 5[a]
HF−Fe	114± 1[a]	341± 3[a]	53±11[a]	66± 1[a]	50± 3[a]
6 weeks of iron depletion plus 2 weeks of iron supplementation					
LF−Fe(+Fe)	198±12	317± 6*[4)]	28± 2*	52± 4	43± 5
HF−Fe(+Fe)	150±16	352±13	72± 8	83± 8	86±10
6 weeks of iron depletion plus 3 weeks of iron supplementation					
LF−Fe(+Fe)	198±23	322±15	28± 3*	53± 6	39± 4
HF−Fe(+Fe)	139± 9	333±20	65± 2	81± 6	115±13*

1) Values are means± S.E., expersssed as nmole palmitate oxidized/g tissue/min

2) Rats were fed low fat with iron(LF+Fe), low fat without iron (LF−Fe), high fat with iron (HF+Fe), or high fat without iron(HF−Fe) diets

3) For each tissue, values with different alphabetic superscipts within each column are significantly different ($p<0.05$) by the Honestly Significant Difference Test (T-method)

4) Student's t-test used to compare iron-supplemented animals with their 6 week iron-deficient counterparts. *$p<0.01$

fed the LF−Fe diet was lower than that in rats fed the HF−Fe diet for 3weeks.

After 6weeks the capacity of the liver, heart and gastrocnemius for mitochondrial β-oxidation in rats fed the low-fat diet was not lower than that in rats fed the high fat-diet. The capacity of the psoas and soleus for mitochondrial β-oxidation, however, in rats fed the low-fat diet was greater than that in rats fed the high-fat diet for 6weeks. The decreased capacity for mitochondrial β-oxidation was shown only in the psoas from rats fed the LF−Fe diet for 6weeks when compared to LF controls.

Iron supplementation for 3weeks, after 6weeks of iron depletion, resulted in significantly decreased capacity for mitochondrial β-oxidation in the soleus from rats fed the low-fat diet.

The catalase activity of the liver, heart, psoas, soleus and gastrocnemius in rats fed the high-fat diet was greater than in rats fed the low-fat diet after 1week and the elevated activity was sustained in the heart and skeletal muscle, but not in the liver through the 6week experimental period(Table 6).

The catalase activity only in the psoas from rats fed the LF−Fe diet was significantly lower when compared to LF controls after 1week. On the other hand, the catalase activity in the liver, heart, psoas, soleus and gastrocnemius from rats fed the HF−Fe diet was significantly lower when

Table 5. Effects of iron depletion and subsequent iron supplementation on mitochondrial β-oxidation capacity

	Liver	Heart	Psoas	Soleus	Gastrocnemius
Baseline	481± 11[1)]	585± 7	132± 11	166± 9	134± 11
1 week					
LF+Fe[2)]	447± 2[a3)]	540± 17[a]	136± 16[a]	170± 10[a]	112± 12[a]
LF−Fe	429± 17[a]	540± 12[a]	132± 9[a]	147± 17[a]	117± 9[a]
HF+Fe	643± 20[b]	683± 16[b]	264± 14[b]	332± 11[c]	298± 15[b]
HF−Fe	609± 8[b]	682± 12[b]	255± 9[b]	268± 6[b]	278± 13[b]
3 weeks					
LF+Fe	494± 7[a]	555± 6[a]	219± 13[a]	345± 27[c]	247± 14[b]
LF−Fe	492± 17[a]	544± 13[a]	217± 18[a]	181± 6[a]	239± 8[ab]
HF+Fe	554± 18[b]	627± 14[b]	352± 18[b]	375± 25[c]	335± 13[c]
HF−Fe	515± 14[ab]	611± 10[b]	207± 16[a]	266± 28[b]	205± 13[a]
6 weeks					
LF+Fe	564± 16[a]	579± 9[a]	192± 18[b]	264± 16[b]	158± 15[a]
LF−Fe	526± 20[a]	560± 16[a]	111± 12[a]	257± 19[b]	137± 20[a]
HF+Fe	570± 19[a]	600± 6[a]	148± 13[a]	150± 2[a]	168± 23[a]
HF−Fe	551± 36[a]	584± 13[a]	118± 5[a]	164± 19[a]	132± 9[a]
6 weeks of iron depletion plus 2 weeks of iron supplementation					
LF−Fe(+Fe)	380± 7	366± 23	90± 6	119± 7	102± 16
HF−Fe(+Fe)	445± 23	467± 29	85± 6	82± 5	117± 8
6 weeks of iron depletion plus 3 weeks of iron supplementation					
LF−Fe(+Fe)	424± 22	332± 32	57± 5	57± 5[*4)]	66± 5
HF−Fe(+Fe)	439± 20	500± 16	96± 6	118± 9	147± 4

1)2)3)4) See the legend of Table 4 for abbreviations, unit and letter designators

Table 6. Effects of iron depletion and subsequent iron supplementation on catalase activity

	Liver	Heart	Psoas	Soleus	Gastrocnemius
Baseline	5757± 331[1)]	163± 8	51± 2	46± 9	47± 1
1 week					
LF+Fe[2)]	5139± 456[a3)]	117± 9[a]	37± 2[b]	32± 3[a]	34± 2[a]
LF−Fe	4861± 456[a]	124± 2[a]	22± 1[a]	21± 2[a]	25± 3[a]
HF+Fe	7883± 155[c]	307± 18[c]	135± 6[d]	201± 12[c]	136± 9[c]
HF−Fe	6631± 372[b]	223± 19[b]	99± 6[c]	140± 11[b]	105± 10[b]
3 weeks					
LF+Fe	6872± 572[a]	196± 5[a]	61± 2[b]	69± 6[b]	57± 2[b]
LF−Fe	5440± 164[a]	179± 25[a]	46± 4[a]	34± 2[a]	37± 6[a]
HF+Fe	6636± 980[a]	292± 11[c]	117± 5[c]	201± 12[c]	109± 2[c]
HF−Fe	4710± 438[a]	232± 8[b]	49± 6[ab]	55± 8[ab]	45± 2[ab]
6 weeks					
LF+Fe	6531± 880[b]	140± 4[b]	58± 2[ab]	86± 1[b]	46± 2[a]
LF−Fe	4486± 408[a]	107± 9[a]	37± 2[a]	47± 3[a]	31± 5[a]
HF+Fe	6694± 406[b]	342± 5[d]	109± 14[c]	170± 11[c]	108± 6[c]
HF−Fe	4106± 336[a]	249± 7[c]	79± 5[b]	98± 8[b]	50± 3[a]
6 weeks of iron depletion plus 2 weeks of iron supplementation					
LF−Fe(+Fe)	5474± 866	207± 15*[4)]	46± 3*	78± 5*	39± 2
HF−Fe(+Fe)	6727± 448	298± 41	65± 5	126± 17	71± 7
6 weeks of iron depletion plus 3 weeks of iron supplementation					
LF−Fe(+Fe)	6745± 452	262± 29**	49± 6*	76± 7*	43± 1
HF−Fe(+Fe)	7035± 1703	328± 8**	76± 6	143± 21	71± 5

1) Values are means± S.E., expressed as EU per g tissue
2)3)4) See the legend of Table 4 for abbreviations and denotes

compared to HF controls after 1week. There was a signicantly decreased catalase activity of the heart in rats fed the HF−Fe diet for 3weeks. Also there was a significantly decreased catalase activity in the psoas, soleus and gastrocnemius in both iron-depleted groups fed either high-fat or low-fat diets for 3weeks.

Iron supplementation for 3weeks. after 6weeks of iron depletion, resulted in significantly increased catalase activity in the heart from rats fed either high-fat or low-fat diets and in the psoas and soleus from rats fed the low-fat diet.

Discussion

From the results of the present study, it is clear that adequate iron is necessary for maximum response of muscle peroxisomal and mitochondrial β-oxidation to high fat feeding. It has been reported that concentration of muscle myoglobin[3-9)], cytochrome C and other iron-containing proteins in mitochondria[8)9-18)] are reduced in iron-deficient rats. Therefore, the impairment of the muscle peroxisomal and mitochondrial oxidative capacity may be due to the effect of reduced concentrations of myoglobin, cytochrome C and other iron-containing proteins. The impaired oxygen transport and storage due to reduced concentration of muscle myoglobin is a possible reason for the impaired induction of peroxisomal β-oxidation by high fat feeding in iron-deficient rats because peroxisomal β-oxidation is known to be

closely related to oxygen tension[28]. The finding that iron depletion results in decreased carnitine concentration in liver and heart[29)30] might suggest that impaired mitochondrial oxidative capacity in rats fed the HF−Fe diet is partially due to carnitine deficiency resulted from iron depletion, which prevents the transport of long chain fatty acyl-CoA through the mitochondrial inner membrane.

In the present study, changes in the peroxisomal and mitochondrial capacities for fatty acid oxidation showed a similar pattern to that of hemoglobin value. It can be interpreted from the previous finding that some tissue iron compounds such as muscle myoglobin and cytochrome C become depleted to a similar degree to hemoglobin, even in rats that mildly iron-deficient[4] because changes of these oxidative capacity may reflect changes in the concentrations of tissue iron compounds.

It is known that most of mitochondrial enzymes involved in the oxidative phosphorylation for the production of ATP contain iron[31]. Therefore, iron depletion results in a lower production of ATP and thus growth suppression[3)19)26)27] and impaired thermogenesis[32-34]. It has been found that skeletal muscle capacity for oxidative metabolism is severely affected by iron deficiency[4)6)7)12)15-18)35)36]. In the present study, impaired muscle peroxisomal capacity, in addition to impaired mitochondrial capacity, for fatty acid oxidation was shown and, because of its great mass in the body, impaired muscle capacity for oxidative metabolism may have a major impact on overall energy metabolism.

It has been shown that young iron-deficient rats who grow rapidly are more susceptible to loss of myoglobin[4]. Also skeletal muscles appear to be more susceptible than cardiac muscle to loss of myoglobin[5]. Most studies demonstrated the absence of changes in the concentration of cytochrome C in cardiac muscle during iron deficiency, while it was clearly decreased in skeletal muscles[4)37]. Cardiac muscle has been shown to retain a much higher oxidative capacity compared to skeletal muscles in the iron-deficient rats because of its increased work load associated with anemia[6)9)38]. Therefore, essential heme compounds and mitochondrial enzymes are least affected in the heart during iron deficiency. There is a contradiction concerning changes in the concentration of liver cytochrom C during iron deficiency [37)38]. Indeed, essential iron compound is depressed by iron depletion to a different degree and even the same compound is depressed by iron depletion to a different degree according to its tissue location or age of the animal.

Previously it has been suggested that lower skeletal muscle catalase activity due to iron depletion or elevated skeletal muscle catalase activity due to high fat feeding could be interpreted as reflecting alterations in peroxisomal β-oxidation[19]. In the present study, however, after 6weeks of iron depletion muscle catalase activity remained decreased in rats fed iron-depleted diets but peroxisomal β-oxidation did not. Also catalase activity remained elevated in skeletal muscle from rats fed high-fat diet added with iron for 6weeks but peroxisomal β-oxidation capacity did not. Therefore, the alterations in muscle catalase activity do not reflect that in muscle capacity for peroxisomal β-oxidation. Moreover, reduction in catalase activity due to iron depletion was greater than the fall in hemoglobin values and sustained. Therefore, the priority for iron use appears to be given to the synthesis of hemoglobin and essential iron compounds related to oxidative metabolism over catalase synthesis.

Iron depletion resulted in decreased skeletal muscle iron concentration and decreased catalase

activity. This result is the same that of previous study[19]. The lower catalase activity after 6weeks of iron depletion suggests that iron may be not available for heme synthesis and thus heme-containing enzyme is less produced. The finding that the concentration of iron and other heme-containing proteins in skeletal muscle is significantly lower in iron-deficient rats[3-9] can support this suggestion.

Iron supplementation for 3 weeks, after 6weeks of iron depletion, increased the hemoglobin values to normal, while peroxisomal and mitochondrial oxidative capacities and catalase activity did not completely returned to their control values. It has been shown that myoglobin and cytochrome C did not return to their normal concentrations until long after the return to normal hemoglobin value[4]. Heart catalase activity returned to the activity of controls after 3weeks of supplementation, whereas liver and skeletal muscle catalase activity did not. Therefore, even in recovery from iron depletion, the priority for iron use was given to cardiac muscle over liver and skeletal muscles.

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Iron deficiency and Fatty acid oxidation

= 국문초록 =

철결핍증이 쥐 간과 근육의 퍼옥시좀과 미토콘드리아의 지방산산화에 미치는 영향

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철결핍증이 고지방식이를 먹인 흰쥐 근육의 peroxisome (PO)과 mitochondria의 지방산 산화 (MO)에 미치는 영향을 살펴보기 위하여, 이유기의 Sprague-Dawley 쥐를 철공급군 (HF+Fe, LF+Fe)과 철결핍군(HF−Fe, LF−Fe)으로 각각 나누어 전체식이량의 44%와 2.5%를 지방으로 공급하여 6주간 사육한 후, 철결핍군은 철을 보충하여 3주간 더 사육하였다. 실험 1주후에 고지방식이군 (HF+Fe, HF−Fe)은 저지방식이군 (LF+Fe, LF−Fe)에 비해 심장, psoas, soleus, gastrocnemius 근육에서 PO와 MO가 각각 50~100% 증대되었다. 실험 3주후에 고지방철결핍군 (HF−Fe)은 고지방철공급군 (HF+Fe)에 비해 근육의 PO와 MO가 저하되므로, 고지방식이에 의해 근육의 PO와 MO가 최대한으로 증대되기 위해서는 철분의 적절한 공급이 필수적이다. 실험 6주후에는 고지방철공급군 (HF+Fe)은 근육의 PO와 MO가 3주보다 저하되었지만, catalase활성도는 고지방식이에 의해 상승된 것이 유지되므로, 이 시기의 상승된 catalase 활성도는 PO를 반영하지 않는다.