Tissue iron content in riboflavin and pyridoxine deficient rats

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The effect of riboflavin and (or) pyridoxine deficiency and repletion on tissue iron content was studied in rats. The iron content in liver, spleen, and kidney and plasma iron concentration of riboflavin deficient (RD) rats was lower, but hematocrit was not. In pyridoxine deficient (PD) rats versus control rats, the iron content in liver was significantly higher but not in spleen and kidney. In PD rats hematocrit was lower but plasma iron concentration was not. Although combined riboflavin and pyridoxine deficient (CD) rats had lower iron content in liver and spleen compared with control rats, these values were intermediate between those of RD rats and PD rats. After RD and PD rats were repleted, the iron content in liver, spleen, and kidney returned to that of control rats, and the hematological indices were improved significantly. These results suggest that riboflavin and pyridoxine deficiency may impair the absorption and utilization of iron and may result in altered tissue iron content.

Keywords: riboflavin deficiency; pyridoxine deficiency; tissue iron

Introduction

Over the past 20 years, research has suggested that humans need iron for growth[1] and replacement of physiological losses[2] and uterine losses due to menstruation and pregnancy.[3] The absorbed iron is used for the synthesis of the iron-porphyrin proteins, hemoglobin, myoglobin, cytochromes, and cytochrome oxidase; and the excess iron is stored in cells of liver, spleen, and bone in the form of two compounds, ferritin and hemosiderin.[3] This iron store provides a rapidly available supply of iron in response to anemia.[4]

It has been suggested that flavin dependent enzyme is involved in the mobilization of iron from storage compartment,[5] and riboflavin deficiency is associated with a decrease in absorption or an increase in post-absorption loss.[6,7] Also, it has been reported that vitamin B-6 deficiency is associated with a microcytic hypochromic anemia in animal and sideroblastic anemia in men.[8]

The purposes of this study were: (a) to investigate the effect of riboflavin and pyridoxine deficiency on iron content in various tissues, and (b) to determine if riboflavin and pyridoxine repletion improves the iron storage in various tissues.

Materials and methods

Animals

Weanling male Sprague–Dawley rats (Seoul Natl. Univ. Exp. Animal Lab) of 40 to 60g were divided into 4 groups of 10 each: pair-fed control group, riboflavin deficient (RD) group, pyridoxine deficient (PD) group, and combined riboflavin and pyridoxine deficient (CD) group. Animals were housed individually in wire-bottomed stainless steel cages.

Diets

The control diet was a vitamin-free, casein-based semisynthetic diet which met AIN-76 recommendation for the rat.[9,10] The diet contained 20% casein, 65% carbohydrate, and 5% fat by weight. The composition of experimental diet was the same as that of control diet except that riboflavin and (or) pyridoxine were not added to the vitamin mixture.

Feeding

All animals were adapted to the control diet for 1 week. At the end of the adaptation period, the rats fed

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their respective diets for 6 weeks. At the end of week 6, 5 rats from deficient groups were repleted with the control diet for 2 weeks, and 5 rats from the control group were continued on the control diet. Each group was pair-fed against the intake of the PD rats through the whole feeding period.

Collection of samples

At the end of the deficient and repletion period, animals were anesthetized with ether and sacrificed by decapitation. Following decapitation, blood was collected in heparinized tubes, and liver, kidneys, and spleen were removed rapidly, blotted dry, and weighed. Tissue samples were frozen and stored at −20°C until they were analyzed.

Analysis

All animals were weighed weekly and the food intake was measured daily. Total iron in tissues was measured by a modified atomic absorption spectrophotometer (AAS) method.[11] Tissues laid in ashes at 500°C for 24 hours. The ashes were dissolved in a concentrated nitric acid and then diluted with 5% nitric acid to appropriate volumes. Total iron concentration was measured by using AAS (Perkin-Elmer 2380, USA). Microhematocrit was determined by reading the percent red cell after centrifuge. Plasma iron concentration was determined by a modification of the colorimetric method.[12] For statistical analysis, all data were first evaluated by analysis of variance. For those F values which were significant, the least significant difference test was performed.[13] A P value < 0.05 was considered to be statistically significant.

Results

The effect of riboflavin and (or) pyridoxine deficiency on body weight is shown in *Figure 1*. The mean body weight of RD rats and PD rats was lower than that of control rats although they were pair-fed. The mean body weight of CD rats was the lowest among all groups. The other clinical symptoms, i.e., denudeness of head and neck, eczematus condition of skin around nostrils and eyes for riboflavin deficiency, and characteristic skin lesions and enlarged liver for pyridoxine deficiency, were observed. After the deficient groups were repleted with a control diet for 2 weeks, the body weights of prior RD, PD, and control group were essentially identical.

The effect of riboflavin and pyridoxine deficiency on organ weights is shown in *Table 1*. The liver, spleen, and kidney weights of RD rats were significantly lower than those of controls. When expressed per gram body weight, the weights of liver, spleen, and kidney were not different from those of control. Although the liver weight of PD rats was 20% higher than that of control rats, this difference was not significant because of the large standard deviation of PD rats. When expressed per gram body weight, this difference was significant. Kidney weight was also higher, but

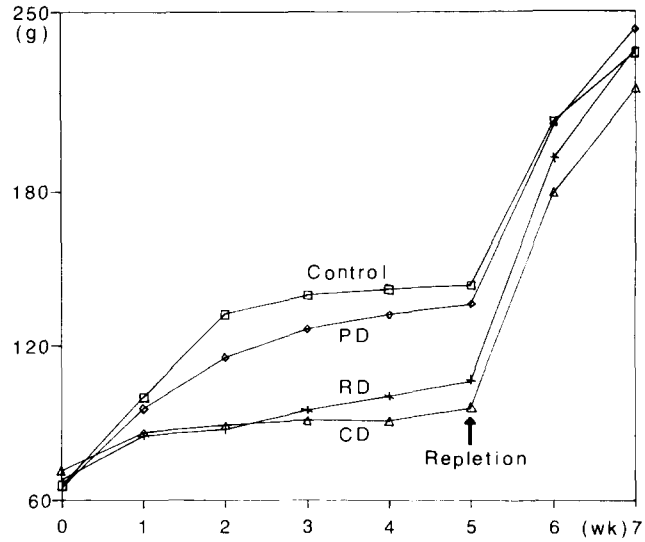

Figure 1 Effect of riboflavin and pyridoxine deficiency on body weight (RD, riboflavin deficient; PD, pyridoxine deficient; CD, riboflavin and pyridoxine deficient).

spleen weight was lower in PD rats than in control rats. Based on the weight of tissues relative to body weight, the weights of liver and spleen in CD rats were not different from those in control rats, but kidney weight of CD rats was higher than that of control.

After 2 weeks' repletion with control diet (*Table 2*), the liver weights of all the deficient groups were heavier than those of the control group. *Table 3* shows the effect of riboflavin and (or) pyridoxine deficiency on tissue iron content. Because there was some difference of organ weights among groups, and this difference may be largely due to the changes of other components rather than iron, the iron concentration per gram of organs could be diluted or concentrated. Thus, the iron content of whole organ which is not affected by the changes of other components in a given organ was calculated. When data were expressed per whole organ, the iron content in liver, spleen, and kidney of RD rats was lower than that of control rats. Compared with control rats, PD rats had higher iron content in liver but similar iron content in spleen and kidney. Although CD rats had lower iron content of liver and spleen than their control rats, mean values of CD rats were intermediate between those of RD rats and PD rats.

Table 4 shows iron contents in various tissues of repleted rats. Iron contents of prior RD and PD rats returned to those of control rats in all tissues when values were expressed per whole organ. Iron content in liver of prior CD rats was still lower than that of control rats with 2 weeks' repletion.

Table 5 shows the effect of vitamin B-2 and (or) vitamin B-6 deficiency on hematocrit and plasma iron concentrations. In RD rats, plasma iron concentration was lower than that of control but hematocrit was not. In both PD and CD rats, hematocrit concentration was lower than that of control but plasma iron was not. After 2 weeks' repletion, hematocrit and plasma iron concentrations were similar among all groups except

Table 1 Effect of riboflavin and (or) pyridoxine deficiency on organ weights[a,b]

	Groups			
	Control	RD[c]	PD[c]	CD[c]
Liver (*g*)	4.84 ± 0.19[a]	3.27 ± 0.49[b]	5.81 ± 1.40[a]	2.66 ± 0.38[b]
(*mg/g* BW)	35.0 ± 1.52[a]	31.0 ± 2.29[a]	43.1 ± 12.7[b]	27.9 ± 1.28[a]
Spleen (*g*)	0.36 ± 0.05[a]	0.20 ± 0.03[b]	0.30 ± 0.03[a]	0.21 ± 0.05[b]
(*mg/g* BW)	2.50 ± 0.48[a]	2.30 ± 0.18[ab]	1.90 ± 0.24[b]	2.10 ± 0.25[ab]
Kidney (*g*)[d]	0.60 ± 0.07[a]	0.48 ± 0.07[b]	0.78 ± 0.07[c]	0.51 ± 0.05[ab]
(*mg/g* BW)	3.40 ± 1.92[a]	4.50 ± 0.25[ab]	5.70 ± 0.54[b]	5.40 ± 0.58[b]

[a] Values are mean ± SEM, n = 5.
[b] Within a given row, those values with different superscripts are significantly different ($P < .05$).
[c] RD, riboflavin deficient group; PD, pyridoxine deficient group; CD, riboflavin and pyridoxine deficient group.
[d] Means of two kidneys.

Table 2 Effect of riboflavin and (or) pyridoxine repletion on organ weights[a,b]

	Groups			
	Control	RR[c]	PR[c]	CR[c]
Liver (*g*)	8.65 ± 0.49[a]	11.8 ± 0.63[b]	11.0 ± 1.68[b]	11.3 ± 1.07[b]
(*mg/g* BW)	37.0 ± 2.89[a]	50.0 ± 3.88[bc]	45.1 ± 5.41[b]	51.0 ± 2.94[c]
Spleen (*g*)	0.58 ± 0.07[a]	0.70 ± 0.07[b]	0.56 ± 0.06[a]	0.73 ± 0.12[b]
(*mg/g* BW)	2.50 ± 0.28[ab]	3.00 ± 0.29[bc]	2.30 ± 0.29[a]	3.30 ± 0.80[c]
Kidney (*g*)[d]	0.86 ± 0.04[a]	0.86 ± 0.07[a]	1.07 ± 0.14[b]	0.92 ± 0.06[a]
(*mg/g* BW)	3.80 ± 0.14[a]	3.60 ± 0.15[a]	4.40 ± 0.40[b]	4.20 ± 0.20[b]

[a] Values are mean ± SEM, n = 5.
[b] Within a given row, those values with different superscripts are significantly different ($P < .05$).
[c] RR, riboflavin repleted group; PR, pyridoxine repleted group; CR, riboflavin and pyridoxine repleted group.
[d] Means of two kidneys.

Table 3 Effect of riboflavin and (or) pyridoxine deficiency on tissue iron concentration[a,b]

	Groups			
	Control	RD[c]	PD[c]	CD[c]
Liver (μ*g/g*)	413 ± 26.0[a]	211 ± 20.4[b]	774 ± 58.6[c]	520 ± 96.6[a]
(μg/organ)	995 ± 133[a]	686 ± 78.8[b]	4434 ± 993[c]	1384 ± 344[ab]
Spleen (μ*g/g*)	190 ± 67.6[a]	234 ± 94.1[a]	275 ± 62.6[a]	268 ± 58.1[a]
(μg/organ)	67.7 ± 11.0[a]	48.0 ± 20.1[b]	85.8 ± 14.8[a]	54.1 ± 10.3[b]
Kidney (μ*g/g*)[d]	68.3 ± 12.0[a]	67.7 ± 17.4[a]	50.8 ± 11.9[b]	61.8 ± 5.40[ab]
(μg/organ)	40.4 ± 5.40[a]	32.3 ± 8.50[b]	38.8 ± 5.20[ab]	31.8 ± 4.20[b]

[a] Values are mean ± SEM, n = 5.
[b] Within a given row, those values with different superscripts are significantly different ($P < .05$).
[c] RD, riboflavin deficient group; PD, pyridoxine deficient group; CD, riboflavin and pyridoxine deficient group.
[d] Means of two kidneys.

Table 4 Effect of riboflavin and (or) pyridoxine repletion on tissue iron concentration[a,b]

	Groups			
	Control	RR[c]	PR[c]	CR[c]
Liver (μ*g/g*)	272 ± 102[a]	268 ± 165[a]	268 ± 77.9[a]	117 ± 12.9[b]
(μg/organ)	2234 ± 815[a]	3151 ± 1945[a]	2976 ± 1095[a]	1315 ± 219[b]
Spleen (μ*g/g*)	134 ± 14.1[a]	128 ± 26.2[a]	155 ± 28.5[a]	131 ± 38.8[a]
(μg/organ)	77.1 ± 2.80[a]	88.9 ± 12.6[a]	85.9 ± 14.8[a]	93.0 ± 18.3[a]
Kidney (μ*g/g*)[d]	51.1 ± 7.30[a]	41.9 ± 7.4[ab]	36.6 ± 7.30[b]	43.7 ± 5.90[ab]
(μg/organ)	44.0 ± 5.40[a]	35.7 ± 5.80[a]	38.4 ± 4.60[a]	39.9 ± 4.40[a]

[a] Values are mean ± SEM, n = 5.
[b] Within a given row, those values with different superscripts are significantly different ($P < .05$).
[c] RR, riboflavin repleted group; PR, pyridoxine repleted group; CR, riboflavin and pyridoxine repleted group.
[d] Means of two kidneys.

Table 5 Effect of riboflavin and (or) pyridoxine deficiency and repletion on hematocrit and plasma iron concentration[a,b]

		Groups			
		Control	RD[c]	PD[c]	CD[c]
Hematocrit (%)	deficiency	45 ± 1.2[a]	45 ± 2.0[a]	37 ± 3.0[b]	41 ± 1.4[b]
	repletion	45 ± 0.8[a]	39 ± 1.1[ab]	39 ± 1.4[ab]	38 ± 0.8[b]
Plasma iron (μg/dl)	deficiency	210 ± 37.9[a]	140 ± 28.5[b]	140 ± 28.5[b]	170 ± 42.0[ab]
	repletion	245 ± 28.6[ab]	268 ± 31.4[a]	243 ± 14.9[ab]	203 ± 53.2[b]

[a] Values are mean ± SEM, n = 5.
[b] Within a given row, those values with different superscripts are significantly different ($P < .05$).
[c] RD, riboflavin deficient group; PD, pyridoxine deficient group; CD, riboflavin and pyridoxine deficient group.

CD rats. The hematocrit and plasma iron concentrations of CD rats were still significantly lower.

Discussion

At various points during the study, riboflavin or pyridoxine deficiency of the rats was confirmed using body weight as a long-term measure. Compared to pair-fed control rats, the lower growth rate accompanied with clinical deficiency symptoms was shown in deficient groups, and 2 weeks' repletion on prior RD, PD, and CD rats resulted in improvements in growth rate and clinical symptoms. Thus, animals were considered to be deficient in riboflavin or pyridoxine by 6 weeks.

The liver, spleen, and kidney weights of RD rats was significantly lower than those of control rats. This result is consistent with the finding of Sirivech et al.[14] However, because these organ weights were not significantly lower when the data were expressed on the body weight basis, the lowered organ weights of RD rats may result from the depressed growth due to riboflavin deficiency rather than to riboflavin deficiency per se. In relation to body weight, the weight of liver and kidney was significantly increased in PD rats. This hypertrophy is in agreement with that of other pyridoxine deficiency studies.[15-17] Since there was no significant difference in various organ weights between CD rats and RD rats while there was some difference between CD rats and PD rats, the weights of liver, spleen, and kidney were affected more by riboflavin deficiency than by pyridoxine deficiency.

The iron content of RD rats was lower than that of control rats in all organs measured, and this lower iron content was observed also in riboflavin deficient animal by Adelekan and Thurnham.[11] Also, plasma iron concentration, a more specific indicator of early iron status than hematocrit, was lower in RD rats. Thus, riboflavin deficient rats are assumed to have lower iron stores. Since Powers[7] reported that riboflavin deficiency is associated with both a reduced absorption of iron and an elevated postabsorption loss, RD rats may not be able to maintain the normal iron stores, and further riboflavin deficiency may lead to a lack of iron storage for hemopoietic system. Support for this comes from the repletion study. After RD rats were repleted, the iron contents of various organs returned to control levels.

The higher iron content in liver of PD rats may be due to either an impaired mobilization of iron from storage compartment or to an increased absorption of iron. Because the iron contents of the other organs, except liver of PD rats, were not significantly different from those of control rats, and liver iron content was higher while hematological indices were lower than those of control rats, it can be ratiocinated that pyridoxine deficiency may depress the release of iron from the liver (site of storage), then progressively accumulate iron in the liver throughout the experimental period, and limit the amount of iron available for hemopoiesis. Repletion on prior PD rats resulted in similar iron content in liver and plasma iron concentration and hematocrit of both groups, possibly due to the improvement in the iron release from liver. In addition, the significantly lowered hematocrit in PD rats may be a result of an impaired hemoglobin synthesis itself, because it was reported that pyridoxal 5'-phosphate is a cofactor for aminolevulinate synthetase (EC. 2,3,1,37), the first, limiting enzyme of heme synthesis.[18]

Although the iron absorption was impaired due to riboflavin deficiency as Powers suggested,[7] if the iron release from storage compartment also might be impaired due to pyridoxine deficiency, the amount of iron accumulated could have increased in liver and spleen when animals were fed a diet deficient in both riboflavin and pyridoxine. Support for this comes from the fact that the iron contents in liver and spleen of CD rats was significantly lower than those of PD rats but slightly higher than those of RD rats. Thus, it was expected that CD rats would aggravate further the hematologic indices of PD rats. However, CD rats showed higher hematocrit than the PD rats, although it was not statistically significant due to the large standard deviation. Because the greatest growth retardation was observed in CD rats, depressing growth of CD rats might have lowered demands of iron on hemopoietic system due to the reduced expansion of blood volume and alleviated the effect of pyridoxine deficiency. Therefore, the higher iron content in organs of CD rats cannot be considered as an available iron for hemopoietic system. After repletion on previ-

ous CD rats, the increase in body mass would increase the body's requirement for the increased blood volume and thereby would show the lowered hematocrit and plasma iron concentrations even though the iron contents stored in organs were not different from those of control rats.

Considering the physiological importance of storage iron, the difficulty with the use of iron store or the depletion of iron store would result in a decrease in an available iron even if the diet is normal in iron content. Population groups having low iron intakes tend to have a diet low in riboflavin and pyridoxine. Thus, a lowered intake of riboflavin or pyridoxine may impair the adaptation of animals during metabolic events related to iron utilization and thereby aggravate the anemic situation.

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