Hemoglobin Regeneration in Anemic Rats by Feeding Hemosiderin as Source of Iron

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Abstract

Bioavailability of iron in ferrous sulfate and hemosiderin, which was prepared from pig liver in the laboratory, was evaluated in terms of hemoglobin regeneration efficiency (HRE%) in anemic rats. Twenty-four male rats of Wistar strain were made anemic by feeding a casein-based, iron-deficient diet for 21 days. The anemic rats were divided into 4 groups and fed, for additional 30 days, the iron-deficient diet without (negative control group) or with supplementation of ferrous sulfate at 24 or 36 mg Fe/kg diet (24-or 36-reference group), or the experimental diet group receiving hemosiderin iron at 36 mg Fe/kg diet (hemosiderin group). Relative biological values (RBV), evaluated as HRE% by assuming RBV of 100 for the 24-reference group, were 86 and 90% for the 36-reference and the hemosiderin groups, respectively. HRE was 47.5±1.6, 40.1±1.6 and 41.8±1.4 for the 24- and 36-reference and hemosiderin groups, respectively. The results obtained indicate that iron in hemosiderin is biologically available as highly as ferrous sulfate is. This observation is in sharp contrast to the previously held notion of the poor bioavailability of hemosiderin iron.

Introduction

Iron deficiency anemia continues to be a major nutritional problem (1, 2), in both developing and industrialized countries. According to the World Health Organization these disorders affect 15 to 20 % of the world's population (3, 4). Anemia is caused not only by low iron intake, but more often, from the diet of poor iron availability, due to iron interaction with or inhibition by other dietary components (5). Many dietary constituents are known to have both a positive and detrimental effect on iron bioavailability (6, 7, 8). Previous studies on hemosiderin have shown that the iron of supplementary hemosiderin was less available in normal subjects than in anemic subjects about 1.9 and 7.5 %, respectively, but its availability was markedly increased when administered with ascorbic acid (9).

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The objective of the present study was to assess the bioavailability of iron from hemosiderin with that from ferrous sulfate (reference diet) in terms of anemic rats.

Materials and Methods

Hemosiderin : Hemosiderin was prepared from pig liver by a modified method of Stephan et al and Gabric et al. (10, 11). The isolation procedure included homogenization, filtration through gauze, centrifugation, lyophilization, regrinding and storing at −20°C, under nitrogen and sealed in plastic bags until use. Hemosiderin powder was analyzed for proximate composition and iron content. The hemosiderin preparation used in this study contained 66.2 % protein, 9.8 % fat, 15.3 % carbohydrate, and 33.3 mg Fe/100g.

Diets and animals : The basal diet was formulated according to AIN-76 (12). Twenty-four male weanling Wistar albino rats, 21 days old (purchased from Shimizu laboratory Supplies, Ltd., Kyoto) were individually housed in stainless steel wiremeshed cages. Housing was temperature-controlled at 23℃ with a 12 hour light and dark cycle. Before 2 days of iron depletion phase, we gave only redistilled water. Anemia was induced by feeding the rats the iron-deficient casein diet for 21 days. At the end of depletion period, blood was collected from the rats' tails for determing hemoglobin concentration and hematocrit. The rats were then assigned into 4 groups of 6 animals each, on the base of hemoglobin values (45.8 ± 6.4 g/L) and body-weights (132±7 g). During the repletion phase, the rats were given free access to the diets and water for 4 wks. The negative control group continued to receive the iron-deficient diet. The reference groups received the diet containing ferrous sulfate at 24 and 36 mg Fe/kg diet, respectively, and the hemosiderin group received the diet containing hemosiderin at 36 mg Fe/kg diet. After 4 weeks of the repletion phase, blood was collected again for evaluation of hematological parameters.

Chemical analysis : Hematological parameters, hematocrit and hemoglobin concentrations were evaluated for each rat. Hemoglobin concentration was measured by the cyanomethemoglobin method using a commercial kit (hemoglobin-test-Wako, Wako Pure Chemical Ind., Osaka, Japan). Red blood cells were counted under microscope. Hemoglobin regeneration efficiency (HRE) was calculated as follows :

$$\text{HRE} = \frac{\text{mg Hb Fe (Final)} - \text{mg Hb Fe (Initial)}}{\text{mg iron consumed}} \times 100$$

Statistical analysis : The data are reported as the mean ± SEM. The significance of differences means was evaluated by Duncan's multiple range test. The comparison between the initial and the final hemoglobin concentrations and hematocrit was made by using the Student's t test. Difference was considered significant when $p < 0.05$

Results and discussion

Initially body-weight, hemoglobin and hematocrit were the same in all 4 groups. However, after 30 days of iron repletion the final hemoglobin concentration and hematocrit in the 24-or 36-reference group and in the hemosiderin group were significantly greater than initial levels except the rats fed the iron-deficient diet (Figs. 1 and 2). Rats fed the hemosiderin diet grew to a final body-weight (data not shown) of substantially higher than those fed the basal diet, and were somewhat higher than those which were fed the reference diet. The lower body-weight gain has been repeatedly observed for iron-deficient rats (13).

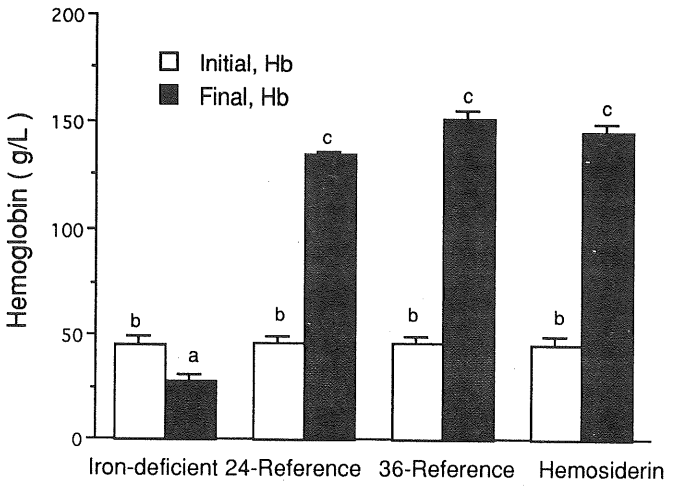

Fig. 1 Hemoglobin concentrations in rats fed ferrous sulfate and hemosiderin.
Values represent the means for six rats and bars the standard errors. Values with different letters are significantly different at $p<0.05$.

HRE value reflects the ability of anemic rats to utilize and retain dietary iron. HRE values after 4 weeks of repletion are compared in Fig. 3. Animals fed 24-reference diet showed HRE value of significantly greater than those of animals fed the 36-reference and hemosiderin diets. The results shown in Fig. 3. indicate that HRE decreases with the iron supplementation level above 24 mg Fe/kg. The rats fed hemosiderin at 36 mg Fe/kg showed a higher HRE value than those of animals fed the 36-reference diet.

The data obtained in this study show that hemosiderin is beneficial to anemic rats by increasing hemoglobin concentration and hematocrit and red blood cell counts to the normal levels (7×10^{12}) and that all rats recovered from anemia. Therefore, hemosiderin can be considered as a source of highly bioavailable iron.

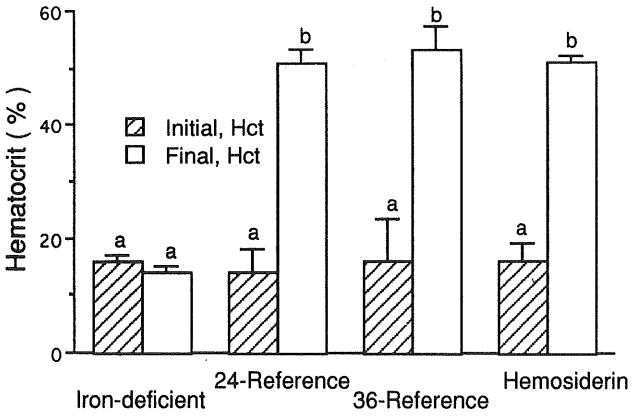

Fig. 2 Hematocrit values for the rats fed ferrous sulfate and hemosiderin. Values represent the means for six rats and bars the standard errors. Values with different letters are significantly different at $p<0.05$.

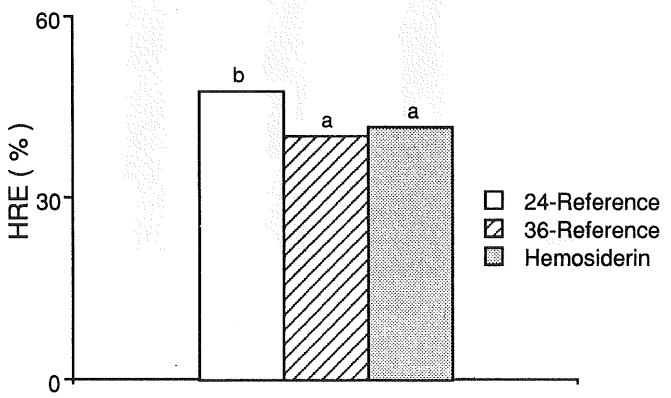

Fig. 3 Hemoglobin regeneration efficiency (%) in rats fed ferrous sulfate and hemosiderin. Values represent the means for six rats. Values with different letters are significantly different at $p<0.05$

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