Note

Differences in the Effect of Iron-Deficient Diet on Tissue Weight, Hemoglobin Concentration and Serum Triglycerides in Fischer-344, Sprague-Dawley and Wistar Rats

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Summary This study was designed to examine the differences in the effect of an iron-deficient diet on iron metabolism in Fischer-344 (FC), Sprague-Dawley (SD) and Wistar (WT) rats based on hemoglobin (Hb), hematocrit (Hct), serum iron levels, growth rate and organ weight. Hb concentration was higher in FC rats (14mg/100mL) on the initial day than in SD (10) and WT (10) rats. Although the Hb level was significantly decreased in FC rats fed an iron-deficient (ID, 8mg/kg) diet for 33d compared to the FC rats fed an iron-adequate (IA, 50mg/kg) diet, the relative concentration of Hb was high in FC rats fed the ID diet as compared to the SD and WT rats fed the same diet. A similar relationship was detected between Hct and serum iron concentrations. Although serum triglycerides (TG) were significantly increased in each rat strain fed the ID diet as compared to the IA diet, the percentage of the value for the IA diet was lowest in FC rats (119%) fed the ID diet as compared to the SD (328) and WT (394) rats fed the same diet. Retroperitoneal fat pad was decreased in FC, SD and WT rats fed the ID diet as compared to the IA diet. SD rats were particularly sensitive to the reduction of retroperitoneal fat pad. The results suggested that rat strains responded differently to dietary iron inadequacy, and that FC rats were less sensitive to an iron-deficient diet as compared to the SD and WT rats.

Key Words anemia, growth, iron-deficiency, rats, strain

Concerning iron supplementation programs, a supplement every 3d is more efficient than daily supplementation. This finding was based on results in a Sprague-Dawley (SD) rat model of anemia (1). Some studies have used SD rats to examine the iron metabolism in iron-deficient anemia (1–3), while Fischer-344 (FC) rats have been used in studies of iron metabolism connected with iron stores in rat...
tissue (4) and the role of liver xanthine dehydrogenase in the liberation of iron from liver ferritin stores (5). Wistar (WT) rats, meanwhile, have been used in iron-deficient anemic models (6, 7). To our knowledge, no systematic studies have been performed to compare various strains of rats for their responses to iron deprivation.

The aim of the present study was to examine the differences in the effect of an iron-deficient diet on iron metabolism in FC, SD and WT rats based on hemoglobin (Hb) concentration, packed red cell volumes (hematocrit; Hct), serum iron level, growth rate and organ weight. This variability could be exploited to suit specific experimental needs.

Materials and methods

General treatment of animals. Three-week-old male FC, SD and WT rats were purchased from Charles River Japan (Kanagawa, Japan). They were housed in individual cages with screen bottoms of stainless steel in a room maintained at 23±1°C and lighted from 0800 to 2000 h. Body weight and food intake were recorded daily in the morning before replenishing the feed. The study was approved by the Animal Use Committee of Kumamoto University, and all rats were maintained in accordance with the university guidelines for the care and use of laboratory animals.

Experimental diet and procedure. After acclimation to a basal diet based on AIN-93 (8), blood was obtained from the tail vein, and Hb and Hct were determined for each rat. Rats were divided into two groups (n=6) within the same strain on the basis of Hb and body weight. Rats were given free access to an iron-adequate (50 mg Fe/kg diet, IA) or -deficient (8 mg Fe/kg diet, ID) diet for 33 d. On the final day of the experiment, blood was obtained from the tail vein, and Hb and Hct were also determined. Thereafter, rats were sacrificed by cervical dislocation, and blood was collected. Serum was prepared and stored at -20°C until analysis for iron and triglyceride (TG) concentrations. After blood collection, liver, heart, spleen and retroperitoneal fat pad were removed and weighed.

Sample analyses. Hct was determined by centrifugation in a capillary tube system. Hb, serum iron and TG were measured using Hemoglobin-Test Wako, Fe C-Test Wako and Triglyceride E-Test Wako, respectively (Wako Pure Chemical Industries).

Statistical analyses. Values were expressed as means±SE. Student’s t-test was used to compare the mean values between two dietary groups (IA and ID diets) within the same strain. Two-way analysis of variance (ANOVA) was used to compare the effect of the rat strain and iron in the diet. A 5% level of probability was used to define differences as significant.

Results

Body weight gain, food intake and food efficiency in FC, SD and WT rats fed the IA or ID diet. Two-way ANOVA showed significant interaction effects of strain and iron level in diet on final body weight, body weight gain and food intake.
Body weight gain was 50% lower in FC rats fed the IA diet as compared to the SD and WT rats fed the same diet. However, body weight gain in FC rats fed the ID diet were 68% of the control value. This reduction was almost the same as that for the SD and WT rats fed the ID diet. Although food efficiency was significantly decreased in the FC and SD rats fed the ID diet as compared to the FC and SD rats fed the IA diet, food efficiency did not significantly decrease in WT rats fed the ID diet rather than the IA diet.

**Hb, Hct, serum iron and TG concentration in FC, SD and WT rats fed the IA or ID diet.** Two-way ANOVA showed significant interaction effects of strain and iron level in diet on Hb concentration and Hct (Table 2). Hb, Hct and serum iron concentrations were significantly decreased in FC, SD and WT rats fed the ID diet rather than IA diet, respectively. However, FC rats fed the ID diet had the highest Hb, Hct and serum iron concentrations of each strain for rats fed the ID diet. Although TG concentrations were significantly increased in each strain fed the ID diet as compared to the IA diet, respectively, the percentage of the value for the IA diet was low in FC rats fed the ID diet as compared to the SD and WT rats fed the same diet. Serum TG (y) markedly increased when the Hb level (x) was below 10 mg/100 mL (y = 1241 - 167x + 6x^2, r = 0.718, p < 0.0001).

**Liver, retroperitoneal fat pad, heart and spleen weight in FC, SD and WT rats fed the IA or ID diet.** Two-way ANOVA showed significant interaction effects of strain and iron level in diet on liver and heart weight (Table 3). Heart and spleen weights were increased in FC, SD and WT rats fed the ID diet as compared to the IA diet. Although liver weight was increased in FC and WT rats fed the ID diet rather than the IA diet, liver weight was decreased in SD rats fed the ID diet as compared to the IA diet. Retroperitoneal fat pad was decreased in FC, SD and WT rats fed the ID diet as compared to the IA diet. SD rats were particularly sensitive to the reduction of retroperitoneal fat pad.

**Discussion**

Beard (9) reported that fat pad weight was decreased and heart weight was increased in SD rats fed an iron-deficient diet as compared to rats pair-fed. These results were consistent with our results. Beard (9) also reported that the rates of norepinephrine turnover were increased in the fat pad of SD rats fed the iron-deficient diet as compared to rats pair-fed. Dillmann et al (10) reported that iron-deficient SD rats had an increased urinary norepinephrine value, regardless of Hb concentration. In general, norepinephrine is known as a lipolytic hormone, indicating that lipolysis increases in the retroperitoneal fat pad of SD rats fed the ID diet. The weights of retroperitoneal fat pad were also decreased in FC and WT rats fed the ID diet as compared to those rats fed the IA diet.

In contrast to fat pad weight, serum TG level differed among the strains. Serum TG was low in FC rats fed the ID diet as compared to that in SD and WT rats fed the ID diet. This result might be responsible for the high Hb level (10 mg/100 mL) in FC rats fed the ID diet. Serum TG was dependent on the Hb concentration and
Table 1. Final body weight, body weight gain, food intake and food efficiency in Fischer-344, Sprague-Dawley and Wistar rats fed an iron-adequate or -deficient diet.1

| Strain      | Diet          | Initial body weight (g) | Final body weight (g) | Body weight gain (g/d) | Food intake (g/d) | Food efficiency (%) |
|-------------|---------------|--------------------------|-----------------------|------------------------|-------------------|---------------------|
| Fischer     | Iron adequate | 48 ± 1                   | 184 ± 5               | 4.1 ± 0.2              | 10.9 ± 0.1        | 37.7 ± 1.1          |
|             | Iron deficient| 48 ± 1                   | 141 ± 7 (77)*         | 2.8 ± 0.2 (68)*        | 9.2 ± 0.2 (84)*   | 30.6 ± 1.5 (81)*    |
| Sprague-Dawley | Iron adequate | 47 ± 1                   | 322 ± 7               | 8.3 ± 0.2              | 18.3 ± 0.4        | 45.7 ± 0.9          |
|             | Iron deficient| 47 ± 1                   | 207 ± 7 (64)*         | 4.8 ± 0.2 (58)*        | 12.5 ± 0.4 (69)*  | 38.5 ± 0.7 (84)*    |
| Wistar      | Iron adequate | 50 ± 1                   | 321 ± 7               | 8.2 ± 0.2              | 18.0 ± 0.4        | 45.7 ± 1.0          |
|             | Iron deficient| 50 ± 1                   | 231 ± 6 (72)*         | 5.5 ± 0.2 (67)*        | 12.8 ± 0.2 (71)*  | 42.8 ± 1.0 (94)     |

ANOVA (p-value)**

| Source      | p-value | Source      | p-value | Source      | p-value | Source      | p-value |
|-------------|---------|-------------|---------|-------------|---------|-------------|---------|
| Strain      | <0.001  | Diet        | <0.001  | Strain × Diet | <0.001 | Strain × Diet | <0.001 |

1 Data are expressed as the mean ± SE (n=6). Values in parentheses are expressed as a percentage of the value for the iron-adequate diet group.

* Significantly different from the iron-adequate diet-fed group, p < 0.05.

** Two-way ANOVA was used to compare the effect of strain and diet.
Table 2. Hemoglobin, hematocrit, serum iron and triglyceride in Fischer-344, Sprague-Dawley and Wistar rats fed an iron-adequate or -deficient diet.1

| Strain      | Diet            | Hemoglobin (g/100 mL) | Hematocrit (%) | Serum iron (µg/100 mL) | Triglyceride (mg/100 mL) |
|-------------|-----------------|-----------------------|----------------|------------------------|--------------------------|
|             |                 | Initial | Final | Initial | Final | 190 ± 26 | 64 ± 2 |
| Fischer     | Iron adequate   | 14.4 ± 0.3 | 17.8 ± 0.2 | 44.2 ± 0.9 | 51.2 ± 0.5 | 190 ± 26 | 64 ± 2 |
|             | Iron deficient  | 14.5 ± 0.2 | 10.9 ± 0.7 (61)* | 46.3 ± 1.1 | 36.2 ± 1.1 (71)* | 50 ± 4 (26)* | 76 ± 6 (119)* |
| Sprague-Dawley | Iron adequate | 10.3 ± 0.3 | 16.6 ± 0.5 | 32.7 ± 0.7 | 48.2 ± 1.1 | 131 ± 39 | 104 ± 10 |
|             | Iron deficient  | 10.7 ± 0.4 | 6.5 ± 0.1 (39)* | 32.4 ± 1.8 | 20.2 ± 0.9 (42)* | 18 ± 4 (14)* | 340 ± 98 (328)* |
| Wistar      | Iron adequate   | 10.3 ± 0.2 | 16.8 ± 0.5 | 34.8 ± 1.4 | 50.5 ± 1.5 | 185 ± 50 | 86 ± 5 |
|             | Iron deficient  | 10.9 ± 0.5 | 6.3 ± 0.2 (38)* | 33.8 ± 1.1 | 19.3 ± 0.7 (38)* | 32 ± 4 (17)* | 341 ± 77 (394)* |

ANOVA (p-value)**

| Strain     | <0.001 | <0.001 | 0.259 | 0.010 |
| Diet       | <0.001 | <0.001 | <0.001 | <0.001 |
| Strain × Diet | <0.001 | <0.001 | 0.763 | 0.050 |

1 Data are expressed as the mean ± SE (n = 6). Values in parentheses are expressed as a percentage of the value for the iron-adequate diet group.

* Significantly different from the iron-adequate diet-fed group, p < 0.05.

** Two-way ANOVA was used to compare the effect of strain and diet.
Table 3. Liver, retroperitoneal fat pad, heart and spleen weight in Fischer-344, Sprague-Dawley and Wistar rats fed an iron-adequate or -deficient diet.1

| Strain    | Diet           | Liver (g/100 g BW) | Retroperitoneal fat pad (g/100 g BW) | Heart (mg/100 g BW) | Spleen (mg/100 g BW) |
|-----------|----------------|-------------------|-------------------------------------|---------------------|---------------------|
| Fischer   | Iron adequate  | 3.00 ± 0.09       | 1.28 ± 0.17                         | 338 ± 5             | 238 ± 6             |
|           | Iron deficient | 3.37 ± 0.21 (112) | 0.53 ± 0.04 (41)*                  | 439 ± 7 (130)*      | 266 ± 8 (113)*      |
| Sprague-Dawley | Iron adequate  | 3.50 ± 0.20       | 1.75 ± 0.23                         | 385 ± 5             | 255 ± 42            |
|           | Iron deficient | 2.93 ± 0.05 (83)  | 0.59 ± 0.15 (34)*                  | 801 ± 26 (207)*     | 369 ± 77 (143)      |
| Wistar    | Iron adequate  | 3.04 ± 0.12       | 1.62 ± 0.12                         | 362 ± 15            | 246 ± 5             |
|           | Iron deficient | 3.12 ± 0.06 (102) | 0.96 ± 0.08 (59)*                  | 668 ± 32 (184)*     | 395 ± 14 (160)*     |

ANOVA (p-value)**

|          | Strain | 0.587  | 0.037  | <0.001 | 0.139 |
|----------|--------|--------|--------|--------|-------|
|          | Diet   | 0.705  | <0.001 | <0.001 | 0.002 |
|          | Strain × Diet | 0.005 | 0.215  | <0.001 | 0.242 |

1 Data are expressed as the mean ± SE (n = 6). Values in parentheses are expressed as a percentage of the value for the iron-adequate diet group.

*Significantly different from the iron-adequate diet-fed group, p < 0.05.

**Two-way ANOVA was used to compare the effect of strain and diet.
might not increase by increasing lipolysis through the norephinephrine metabolism. Sherman et al (2) reported that serum TG level was increased in SD rats fed an iron-deficient diet as compared to that in rats fed a control diet. They suggested that elevated serum TG was due to the increased endogenous production of triglycerides. Hulse et al (11) focused on enzymes in the synthesis of carnitine that required ferrous iron as a cofactor in liver, and reported a significant relationship between carnitine metabolism and iron deficiency.

Beard et al (12) reported that iron-deficient anemia was severe in normal growth rats as compared to low growth rate rats when they tested normal protein and low protein levels, suggesting that the restriction of growth moderates iron-deficient anemia. Maeda (13) reported that body weight gain was about 50% lower in FC rats than in SD and WT rats, and food intake was also low. The same result was obtained in the present study, indicating that sensitivity to the ID diet might be low in FC rats. FC rats may be useful as a mild iron-deficient anemic model because energy or protein intake would not have to be restricted.

We conclude that rat strains respond differently to dietary iron inadequacy, and that FC rats are less sensitive to an iron-deficient diet, possibly as a result of their low growth rate.

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