Growth in Iron-Deficient Rats (43879)

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Abstract. **Poor growth in iron deficiency is commonly observed in animal studies. Previous studies from our laboratory showed that iron-deficient rats are metabolically inefficient and have less body fat than controls and proposed that iron deficiency was related to increased metabolic rates and heat loss. To examine these points more completely, we examined growth and metabolic rate of iron-deficient rats at two environmental temperatures, 25°C and 32°C, and feed efficiency in separate groups of rats during a period of rapid growth. Iron deficiency (hemoglobin [Hb] ≈60 g/liter) was associated with a systematic elevation of metabolic rate over the 24-hr day with animals at 25°C. This did not occur in animals living in thermoneutrality. Iron deficiency affected growth of animals at 25°C but not at 32°C. Feed efficiency (kcal retained/kcal absorbed) was 25 ± 4.2 and 31 ± 4.9 kcal ($P < 0.0001$), respectively, in iron deficient rats and animals were not anorexic. Use of food-restricted animals allowed the direct calculation that iron deficiency was associated with a 10%–15% increased requirement for growth. We conclude that iron deficiency anemia is associated with a poor feed efficiency and that it is attenuated when nonshivering thermogenesis is minimized by a thermoneutral environment.** [P.S.E.B.M. 1995, Vol 209]

Poor growth rates are common in iron-deficient postweanling rats (1–4). The problem of separating the direct nutritional effects of iron deficiency from possible indirect effects of decreased food consumption is difficult. That is, is there a specific lesion that exists in iron deficiency that leads to poor growth, or is the poor growth simply a product of "poor nutritional status" leading to anorexia? There is a perception as well as a published report that iron deficient rats have a decreased growth rate due to decreased food consumption when fed a basal low-iron diet (4). The poor growth rate however, might be due to a poor energy efficiency, which is related to an impaired sympathetic nervous system in iron deficiency (2).

Catecholamines of the sympathetic nervous system and T_3 from thyroid hormone, thyroxine, play major roles in nonshivering thermogenesis (NST), which is activated when the environmental temperature drops below thermoneutrality and in response to meals (5). The thermoregulatory capacity of animals during cold exposure relies on their ability to increase metabolic rate with sympatho-adrenal and thyroid responses and to decrease heat loss (6–8). We and other researchers have demonstrated that iron deficiency in humans and rodents attenuates this capacity to respond to severe cold while retaining the capacity for NST to proceed at an accelerated rate at less severe temperatures (1, 9–12). An accelerated heat loss is thus likely related to a metabolic rate that has been stimulated to maintain thermal homeostasis.

Based on our earlier studies of poor feed efficiency of iron deficient anemic rats at 25°C (2) and observations of decreased fatness and increased metabolic rate in other groups of anemic animals (13), we became interested in repeating our earlier study with the addition of food restricted groups of animals and a meal eating protocol. In addition, we wanted to conduct examinations of growth and metabolic rate of iron deficient anemic rats at thermoneutrality. Our rationale was that if NST is increased in iron-deficient anemic animals at room temperature, then allowing them to live at near thermoneutrality would improve growth.

The working hypothesis of these descriptive studies was that iron deficiency anemia results in increased

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sympathetic nervous system activity, increased metabolic rate, and decreased feed efficiency. To test this hypothesis we conducted several studies with the following research questions: (i) Does environmental temperature affect the growth curves equally between iron deficient and control animals? (ii) Are the previous results of poor feed efficiency reproducible in a more controlled setting? (iii) Are iron-deficient anemic animals anorectic with a resulting decrease in growth once one has controlled for body size?

Materials and Methods

Experiment 1. Male Harlan Sprague-Dawley rats were obtained at 21 days of age from a commercial supplier (Harlan Sprague Dawley, Indianapolis, IN) and were housed in wire mesh cages at room temperature (25° ± 2°C) with a 12:12-hr light:dark cycle. All animal procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. The animals were randomly divided into two groups and were provided either an iron-deficient (ID) diet (<5 ppm Fe) or an iron-sufficient (CN) diet (50 ppm Fe as ferrous sulfate). The composition of the purified diet was based on recommendations by The American Institute of Nutrition (AIN) Ad Hoc Committee on Standard for Nutritional Studies (14) modified by replacing sucrose with cornstarch (15). The diets are described in detail elsewhere (16). The iron content of each batch of diet was verified by acid digestion of four random aliquots of the diet followed by measurement of iron content by flame atomic absorption spectrophotometry (17). Reproducibility was within 1% with a minimum sensitivity of 0.1 mg/liter.

Resting oxygen consumption and carbon dioxide production was determined in all rats at 2, 9, 16, and 24 days after commencing their respective dietary treatments using rapid flow calorimetry (18). In order to obtain resting values for oxygen consumption and carbon dioxide production, the animals were introduced individually into one of four cylindrical plexiglass chambers and allowed to rest comfortably for 1 hr before measurements were made. These chambers were housed within a thermally stable cabinet (±5°C). Food was withheld for the previous 12 hr. Analyzers were calibrated daily with a standard gas mixture. Animal chamber temperatures were monitored individually, recorded to ±0.1°C, used in all metabolic determinations. Oxygen consumption, carbon dioxide production, and chamber temperature were obtained at 15-sec intervals for a total of 3 min per animal per data pass and according to previously described and well-documented methods (18). To compensate for response time of the calorimeter, only data obtained during minute 2 and 3 were used in metabolic determinations. Following each complete data pass of all four animals (12 min) room air was sampled to recalibrate data for analyzer drift. This drift was less than a 0.02% change in gas composition over the entire time period. Determinations of resting oxygen consumption and carbon dioxide production were made during a 2-hr period in which each animal had eight passes of his chamber. The resting metabolic rate was determined for each animal as the minimum mean value obtained during those eight passes. This value represented the arithmetic mean of eight samples averaged during that pass. The humidity of the inspired air was 40%–60% and flowed through the chamber at 2 liters/min.

After data accumulation on this group of animals, a second group of animals was obtained from the same supplier and were treated as above except that the animals were housed in identical wire mesh cages in an environmental chamber set at 30° ± 2°C. Oxygen consumption and carbon dioxide production measurements were made at 2, 9, 16, 24, and 29 days after starting their respective dietary treatments. All calorimetry data in both phases of the study were obtained while rats had food withheld for 12 hr and during the midmorning hours of the light cycle.

In a subset of these animals, we wanted to test the hypothesis that the thermic effect of food was affected by iron status. Thus, we fasted four of the iron-deficient rats and four of the control rats for 12–14 hr and then put them into the metabolic chambers as previously described and collected fasting metabolic rate measurements for 2 hr. We then provided access to their respective diets for 1 hr and measured metabolic rates during that hour and for the subsequent 3 hr. "Fed" metabolic rate was calculated as the mean of that 4-hr period, while "fasted" metabolic rate was the mean of the preceding 2-hr period.

Body weight was obtained every 3 days and 100 μl blood obtained for hemoglobin determination by tail vein puncture and subsequent conversion to cyanmethemoglobin (16).

Experiment 2. Male weanling rats were provided iron-deficient or iron-adequate diets as previously described, starting at 21 days of age, and were housed at an environmental temperature of 24°–25°C for 3–4 weeks. Feed efficiency studies were conducted after 21 days of dietary treatment (42 days of age) and lasted for 5 days using methods described in our previous study of feed efficiency (2). These methods calculate the efficiency of retention of calories based on the careful quantification of calories consumed by animals and the gain in caloric content of the carcass over a specified period of time. Animals were sacrificed by exsanguination from the abdominal aorta after anesthesia by overdose of pentobarbital (50 mg/kg ip). As noted in the method reference, five to eight additional animals are killed at the beginning and at the end of the feeding study to determine carcass caloric content per unit of body weight over a wide range. These regression equations are then used to calculate the caloric value of animals in the feeding study (2).

Experiment 3. Male Sprague-Dawley rats were again obtained at 21 days of age and were randomly assigned to one of three dietary treatment groups: (i) an iron-deficient diet, (ii) an iron-adequate diet, and (iii) an iron-adequate diet fed in restricted amounts to match growth rates with that of the rats on the iron-deficient diet. Food-restricted control rats were provided an amount of control diet (50 ppm Fe) 5%–15% less than that consumed by rats provided a low-iron diet (<5 ppm Fe) on the preceding day. Preliminary and previous studies showed that this would maintain growth rates similar to that of iron-deficient animals. The third group was a group comprised of *ad libitum*-fed controls (50 ppm Fe) who were allowed to eat as much food as they wanted. All rats in each treatment group were trained to be meal eaters by providing them access to food between 9:00AM–11:00AM and 7:00PM–9:00PM in a reversed dark cycle (lights off at 9:00AM and on at 9:00PM). This design feature was included to insure that all animals were "meal eaters." This was not the case in our previous feed efficiency study and was a design flaw (2). All rats were weighed daily to insure that body weight of food-restricted rats matched that of iron-deficient anemic rats. The rats were provided access to their respective diets for 6 weeks. Food intake and growth were determined throughout this feeding period and body fatness determined at the end. Animals were terminated by abdominal exsanguination while under pentobarbital anesthesia (50 mg/kg ip).

Measures

Body fatness. The determination of rat body fatness was based on a modification of a standard Folch lipid extraction and our previous proximate analysis method (2, 13). The rat carcass was frozen in a sealed plastic bag for less than 4 months and then partially thawed in hot water, eviscerated, and reweighed. The carcass was cut into 1-inch cubes, and quantitatively ground with a meat grinder into a preweighed container. A weighed amount of woodchips (about 20 g) was ground to clean the grinder, and the ground woodchips were added to the ground carcass and weighed. Two-gram aliquots of the ground carcass were placed in triplicate into 50 ml conical centrifuge tubes, and 20 ml chloroform:methanol (2:1) was added to each tube. The tube contents were homogenized and shaken in a horizontal shaker at room temperature for 2 hr. The homogenate extracts were filtered through Whatman #4 filter paper into preweighed calibrated saponification flasks, and the volume in each flask was brought up to 20 ml with chloroform:methanol (2:1) solution. Next, 2.75 ml salty wash mix (0.05% $CaCl_2 \cdot 2H_2O$) was added to each flask. The flasks were vortexed, allowed to stand until the contents separated into two layers, and the top layer was aspirated off and discarded. Upper phase salty wash mix (2% H_2O, 3% chloroform, 48% methanol, 47% of 0.05% $CaCl_2 \cdot 2H_2O$), 9.75 ml, was added to each flask. The flasks were vortexed and allowed to stand until the contents separated into layers, and the upper phase was aspirated off and discarded. The upper phase salty wash was repeated. The lower phase was evaporated under a stream of dry nitrogen gas at a bath temperature of 60°C until they were of constant weight, at which time the weight was recorded. Triplicate samples of lard and vegetable oil (180 to 200 mg each) were run with each set of carcass extractions. Recovery exceeded 98% with a coefficient of variation of <5%.

Hematology. Hematocrit was determined by centrifugation of blood collected into heparinized microcapillary tubes while hemoglobin concentration was measured calorimetrically by the cyanmethemoglobin method (16).

Statistical Analysis. The values are presented as the mean ± SD. Data were normally distributed and outliers were examined by using the normality test and boxplot program on Minitab statistical software (MINITAB Corp., State College, PA). Analysis of variance (ANOVA) was used to determine whether characteristics of rats were significantly different between dietary treatment groups. This was a simple one-way ANOVA, a repeated measures ANOVA, or a two-way ANOVA, depending on the experiment. When ANOVA indicated significant ($P \leq 0.05$) F values, Tukey's test for multiple comparisons was performed to determine significant ($P \leq 0.05$) differences between group means. Between-group and across-time differences was assessed by using a two-factor analysis of variance. When significant ($P \leq 0.05$) F ratios were found, Tukey's test for multiple comparisons was performed to determine significant ($P \leq 0.05$) differences between group means. Due to the unbalanced design of the studies, these statistical analyses were performed using the General Linear Models (GLM) procedure (MINITAB Corp., State College, PA).

Results

Experiment 1. As noted in Figure 1, iron-deficient anemic rats gained less body weight than controls over a 3- to 4-week period at either 30°–32°C or 24°–25°C. Living at an environmental temperature of 30°–32°C, however, significantly diminished this gap between anemic and control animals, and improved growth rates of iron-deficient anemia (IDA) animals significantly compared with iron-deficient anemics at 24°–25°C. The mean differences in body weight between groups was 86 g at 28 days of dietary treatment when rats lived at the cooler temperature and 48 g when they lived at the near-thermoneutral temperature. Growth of controls was unaffected by environ-

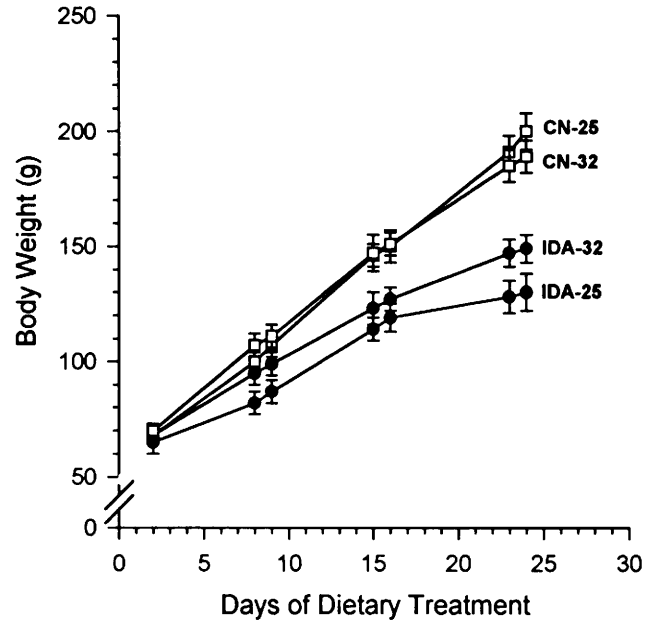

Figure 1. Body weight of iron-deficient anemic (IDA) and control (CN) rats living at either 25°C or 32°C for a period of up to 4 weeks starting at 21 days of age. Body weights of CN rats (CN-25 and CN-32) were significantly higher ($P < 0.05$) than IDA rats (IDA-25 and IDA-32) after 8 to 24 days of dietary treatment. After 24 days, IDA rats at 32°C (IDA-32) weighed significantly ($P < 0.05$) more than IDA rats at 25°C (IDA-25). Values represent the mean ± SEM.

mental temperature. Hematocrits were 17% ± 1% and 20% ± 1%, respectively, for IDA rats at 32°C and 25°C after 14 days of dietary iron restriction and stayed at those levels for the duration of the experiment. Controls had average hematocrits of 46% ± 2%.

Resting metabolic rates of iron-deficient anemic and control animals were measured weekly and are shown in Figure 2. Iron-deficient anemic rats had higher metabolic rates ($P < 0.01$) than controls at 25°C after 2 weeks of dietary treatment. Diet was also a significant main effect at 32°C ($P < 0.05$) and a significant interaction term for temperature and iron status, but specific cell post hoc comparisons were not significant. We also measured metabolic rate in rats for 4 hr either after 12 hr of food withdrawal in the dark or after 12-hr access to their normal respective diets (data not shown). The ratio of the "fed metabolic rate" over "fasted" metabolic rate in these 42-day-old animals

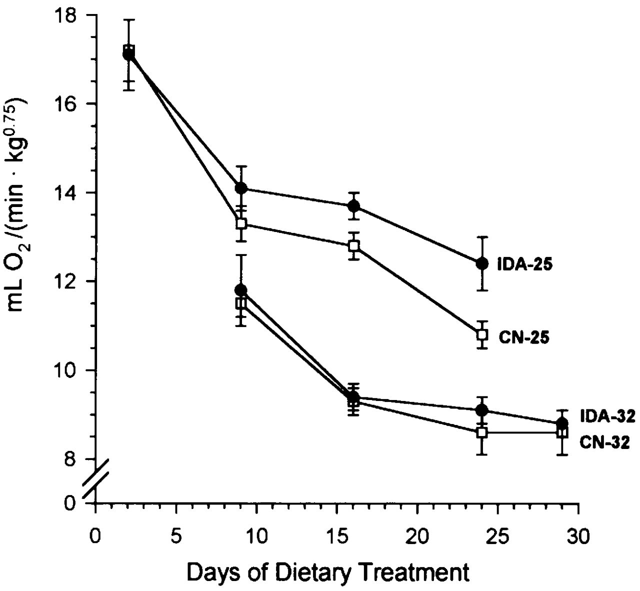

Figure 2. Fasting oxygen consumption rates of iron-deficient anemic (IDA) and control (CN) rats (n = 6 per group) raised at either 25°C or 32°C for 4 weeks starting at 21 days of age. Values represent the group mean ± SEM collected over a 2-hr period on the designated day. Hematocrits of IDA rats averaged 17% ± 1% over the last 3 weeks while CN rats averaged 46%–48% ± 2%.

was 1.15 ± 0.07 in iron deficient anemic animals and 0.91 ± 0.12 in controls and differed significantly by dietary treatment. There was no effect of temperature.

Experiment 2. Feed efficiency studies were conducted in a large cohort of iron deficiency anemic and control rats after 4 weeks of dietary treatment. While the animals were subsequently used in other investigations of iron deficiency anemia and thermoregulation (19), we are concerned with the feed efficiency data in this report. Feed efficiency is defined as the number of calories that are stored divided by the number of calories that are absorbed over a defined period of time. As noted in Table I, iron-deficient anemic rats were significantly less efficient at storage of calories, 25.6% versus 31% ($P < 0.0001$) than were control animals. This poor efficiency was not related to grossly low food intake since iron-deficient anemic rats consumed an average of 44.3 g/100 g body wt and controls 47.6 g/100 g body wt over this 5-day period.

Twenty-four-hour metabolic rates were examined in a randomly chosen group of 10 of these iron-

Table I. Feed Efficiency of Iron-Deficient and Control Rats over 7 Days

	Hb (g/dl)	Body Weight (g)	Body Weight Change (g)	Food Consumed (g)	kcal Consumed (kcal)	Fecal Energy (kcal)	kcal Retained (kcal)	Feed Efficiency (%)
IDA (n = 64)	6.5 ± 0.6	136.5 ± 5	30.5 ± 8.4	66.5 ± 12.4	285 ± 52.7	3.4 ± 0.9	72.9 ± 19.8	25.6 ± 4.2
CN (n = 32)	13.0 ± 0.1	162.5 ± 7	42.5 ± 10.1	87.2 ± 14.1	373 ± 59.1	6.4 ± 1.6	116 ± 29.0	31.0 ± 4.9
Probability	$P < 0.001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Note. Table of mean ± SD and probability of a difference between iron-deficient anemic (IDA) and control rats (CN) after 3 weeks of previous dietary treatment.

deficient anemic and 10 control rats to test the hypothesis that daily metabolic rate was altered in ID and may affect caloric balance (Fig. 3). Iron-deficient rats had significantly higher (main effect of diet significant at $P < 0.0001$) metabolic rates than controls with the exception of two isolated time points in the dark cycle. The respiratory exchange ratios (RER) were similar between anemic and control rats (data not shown).

Experiment 3. Figure 4 shows weekly body weights of iron-deficient anemic, food-restricted, and *ad libitum* control rats during 6 weeks of dietary treatments. After 1 week of the dietary treatment, a significant difference in mean body weight appeared between iron-deficient anemic rats and *ad libitum* control rats ($P < 0.05$). Meal feeding did not affect growth of the *ad libitum* controls, evident from the fact that their growth was not different from that of control given free access to food in Experiment 1 (25°C animals). By the end of 6 weeks of dietary treatment, the mean body weight was significantly depressed by 34% in the iron-deficient anemic group compared with the *ad libitum* control group. There was no significant difference between the iron-deficient anemic group and the food-restricted group except in the 5th and 6th week of dietary treatment.

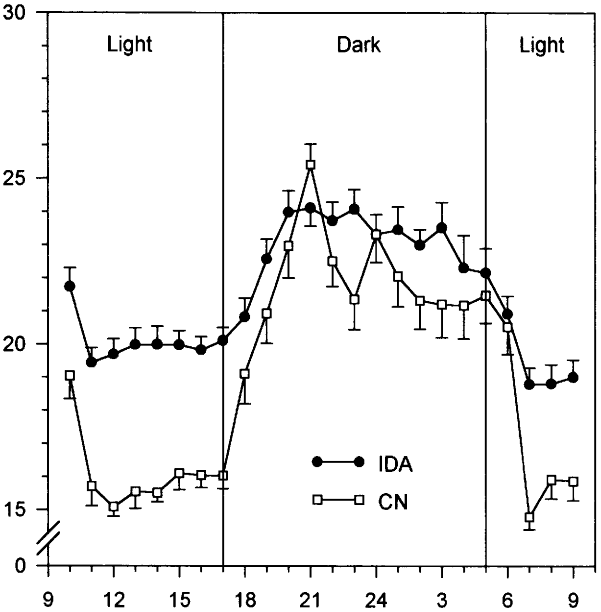

Figure 3. Twenty-four-hour metabolic rates of iron-deficient anemic (IDA) and control (CN) rats (n = 10 per group) collected in metabolic chambers at 24°–25°C. Data were collected each 15 min, aggregated into data points at 30-min intervals, and presented as group mean ± SEM. IDA rats had significantly higher metabolic rates ($P < 0.001$) than CN rats at nearly all times in the light cycle.

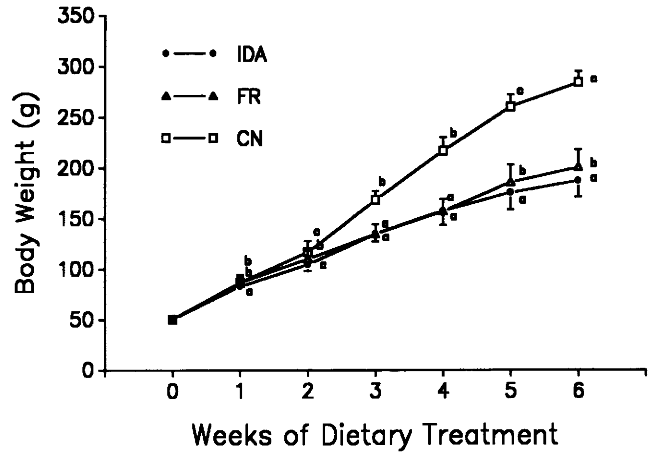

Figure 4. Weekly body weights of rats in three dietary treatment groups. Iron-deficient anemic rats (IDA ●-●, n = 16, mean Hb = 4.3 g/dl); food-restricted rats (FR △-△, n = 15, mean Hb = 13.9 g/dl); *ad libitum* control rats (CN □-□, n = 15, mean Hb = 14.7 g/dl). Values are mean ± SEM. Two-way analysis of variance indicated a significant ($P \leq 0.0001$) group and time interaction for mean body weights. Mean body weights within a group are significantly ($P \leq 0.0001$) different for each week of dietary treatment. Between groups, mean body weights with different superscripts within a week are significantly different at $P \leq 0.05$ by Tukey's test.

In the first 2 weeks of dietary treatment, mean food intakes per gram of body weight was significantly higher in the *ad libitum* control group than in iron-deficient anemic and food-restricted groups. In the 3rd week, there was no significant difference between the iron-deficient anemic group and the *ad libitum* control group. However, in the 4th and 5th weeks, food intake per gram of body weight was significantly higher in the iron-deficient anemic group than in *ad libitum* control group. Iron-deficient anemic rats generally had a 5%–15% greater intake per gram of body weight than weight-matched control rats (food-restricted controls). A reexpression of the data in Figure 5 as grams of food

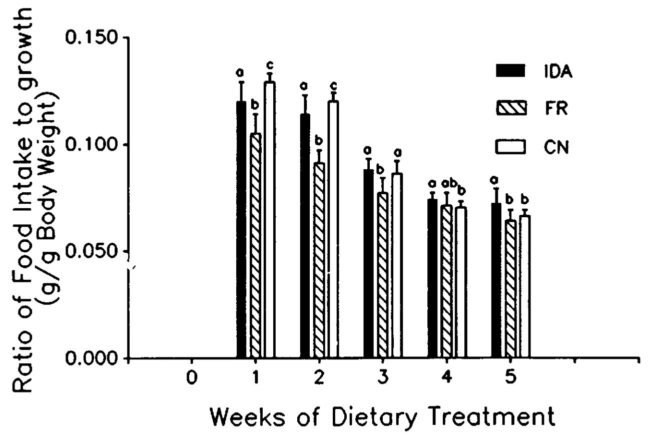

Figure 5. Weekly food intake per gram of body weight for each group: iron-deficient anemic rats (IDA, n = 6); food-restricted rats (FR, n = 14); *ad libitum* control rats (CN, n = 5). Values are mean ± SD. Two-way analysis of variance indicated a significant ($P \leq 0.0001$) group and time interaction for mean food intake per gram of body weight. Mean food intake per gram of body weight within a group are significantly ($P \leq 0.0001$) different for each week of dietary treatment. Between groups, mean food intake per gram of the body weights with different superscripts within a week are significantly different at $P \leq 0.05$ by Tukey's test.

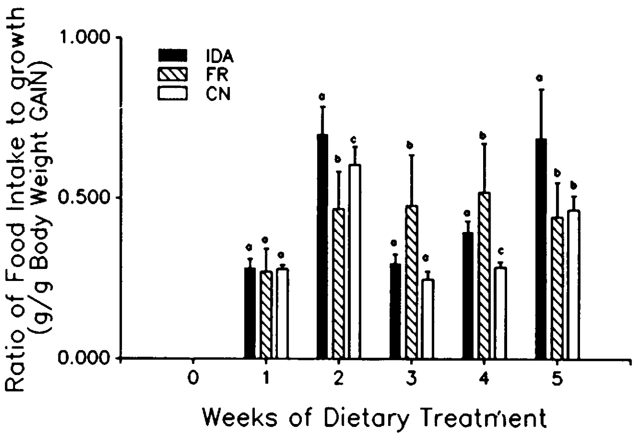

Figure 6. Weekly food intake per gram of body weight gain for each group: iron-deficient anemic rats (IDA, $n = 6$); food-restricted rats (FR, $n = 14$); *ad libitum* control rats (CN, $n = 5$). Values are mean ± SD. Two-way analysis of variance indicated a significant ($P \leqslant 0.0001$) group and time interaction for mean food intake per gram of body weight gain. Mean food intake per gram of body weight gain within a group are significantly ($P \leqslant 0.0001$) different for each week of dietary treatment. Between groups, mean food intake per gram of body weight with different superscripts within a week are significantly different at $P \leqslant 0.05$ by Tukey's test.

intake per gram of body weight gain demonstrates that anemic rats did in fact require 20%–30% more food per gram of weight gain than controls in Week 4 and 5 when they were very anemic (Fig. 6).

The mean final body weights, hemoglobin, hematocrit, body fatness, and metabolic rate of rats in the three dietary treatment groups are shown in Table II. There was no significant difference between food-restricted and *ad libitum* control rats with regard to hematologic status, though the low-iron diet was clearly effective. Resting metabolic rates of these animals, as in the previous studies, were significantly higher in anemic rats than in either age- or size-matched controls. As expected, the food-restricted animals as well as the anemic animals were leaner than the *ad libitum*-fed controls.

Discussion

The present findings clearly confirm that growth is significantly slower in severely anemic rats than in *ad libitum* control rats. This growth failure is evident in as little as 1–2 weeks and is independent of meal feeding or *ad libitum* feeding of an iron-deficient diet. Moreover, changes in environmental temperature to decrease the amount of NST led to improved growth, but did not erase the growth differential between young iron-deficient and iron-sufficient rats. These data are consistent with the findings of other investigators (4, 20) and with our previous studies (1, 2, 16). Pronounced growth failure in male weanling rats fed a low-iron diet is commonly reported but is unexplained (3). A decreased growth rate was proposed to be due to a decreased consumption of the basal iron-deficient diet (4). In contrast, our studies demonstrate that iron-deficient animals not only eat as much as controls, but actually eat significantly more. Despite the 15%–30% increase in food intake per gram of body weight, poor growth is still the result. The extra food intake is clearly insufficient for these growing animals even when compared with animals whose body size was nearly equivalent. The information presented in Figure 5 and Table I shows that at the end of 5 weeks of dietary treatment, ID rats could not attain the same efficiency of conversion of dietary calories into growth with a resulting decreased body weight and body fatness. These observations reaffirm our previous observations of decreased feed efficiency (2) with a much larger number of animals and also reaffirm the observations that ID animals have a decreased body fatness (2, 13) to go along with their overall decreased growth. While not measured in this particular study, we have measured blood flow using radioactive microspheres in iron-deficient animals during cold stress and have measured both skin and core temperatures (21). Anemic animals do have higher apparent rates of heat loss as indicated by an increased flow of blood to the skin and an increased gradient of temperatures from core to surface (unpublished data). Thus, the lower amount of body fatness (a decreased insulative barrier) and an increased skin blood flow likely contribute to an increased rate of heat loss with a corresponding increase in heat production, or metabolic rate, to maintain core temperature.

Table II. Characteristics of Rats

Parameter	IDA Group ($n = 13$)	FR Group ($n = 11$)	CN Group ($n = 10$)
Body weight (g)	182 ± 20^a	204 ± 22^b	282 ± 16^c
Hemoglobin (g/dl)	4.3 ± 0.3^a	13.9 ± 1.3^b	14.7 ± 1.1^b
Packed cell volume (%)	20 ± 3^a	43 ± 2^b	46 ± 2^b
Body fat (%)	6.2 ± 1.1^a	7.1 ± 1.3^a	9.9 ± 5^b
Metabolic rate (kcal/hr/kgFFM)	9.0 ± 0.5^a	8.2 ± 0.6^b	7.3 ± 0.7^b

Note. Values are Mean ± SD. Iron-deficient anemic group (IDA group); food-restricted group (FR group); ad libitum control group (CN group). Means with different superscripts within a row are significant at $P \leqslant 0.05$ as determined by one-way analysis of variance and Tukey's test.

One of the components of this poor energy efficiency is likely the increased sympathetic nervous system (SNS) activity in iron deficiency (2, 9, 18). Several studies have indicated that iron-deficient animals have increased excretions of norepinephrine (NE) (22), increased plasma norepinephrine levels (23), and increased rates of norepinephrine turnover in interscapular brown adipose tissue (2, 9, 10). Such findings support the suggestion that a hypernoradrenergic state, characteristic of animals showing significant dietary or cold-induced thermogenesis, may be responsible for the significant 34% decrease in metabolic efficiency in the iron-deficient anemic rats. While we did not measure SNS activity in the animals in the current studies, the consistent elevation in NE turnover in animals with this level of anemia provides a reasonable linkage (9, 10). The increased SNS activity documented in those studies is supported by the observations of increased resting metabolic rates in anemic animals when either expressed per unit of LBM (Experiment 3) or per unit of metabolic body size (Experiment 1). It is important to note that ID animals have normal metabolic rates when the anemia and tissue iron deficiency is not as severe as in this study and that their growth rates improve tremendously (16, 19). It is also worth noting that an ambient temperature of 25°C likely constitutes a hypothermic stress to the rats in both groups.

Daily energy expenditure measurements revealed several new and interesting observations. First, there was no indication of the reversal of the diurnal light dark activity cycle in iron deficiency as has been suggested by others (24). When the lights went out, both anemic and control animals increased metabolic rate and then decreased this energy expenditure when the lights came back on. While not a direct measure of physical activity, these data agree strongly with the spontaneous motor activity measures of Hunt and colleagues (25).

In summary, we feel the most striking findings of these studies are that food intake per gram of body weight or per gram of body weight gain was increased and not decreased in iron-deficient anemic rats. This observation was supported by the observation of decreased energy efficiency of iron-deficient anemic rats compared with control rats. The anemic animals are hypermetabolic and have a decreased body fatness. Attempts to improve growth by increasing temperature were successful and again suggest that dysfunctional SNS activity regulation is a key factor causing poor growth. It is certainly not the entire reason for poor growth, since growth was not normalized at thermoneutrality. While iron deficiency does not lead to a decreased food intake per se; the reason for the failure to completely compensate for an apparent increase in energy expenditure by an even greater food intake is not clear. We can only speculate that the known alterations in central nervous system dopamine metabolism (26) may be one of the factors that alters normal regulation of food intake.

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