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Feed Efficiency and Norepinephrine Turnover in Iron Deficiency (42488)

JOHN BEARD

With the Technical Assistance of Brian Tobin

Nutrition Program, The Pennsylvania State University, 202 Henderson Human Development, University Park, Pennsylvania 16802

Abstract. Norepinephrine turnover and energetic efficiency studies were conducted in three groups of male Sprague–Dawley rats placed on low iron diets for 5 weeks on weaning. Iron-deficient rats had significant anemia (hematocrit < 20%) and growth retardation relative to pair-fed and *ad libitum* fed controls who received the same diet plus weekly iron dextran injections. Energetic efficiency over a 7-day period was nearly 30% less in anemic animals. This was associated with significantly higher rates of norepinephrine turnover in brown adipose tissue (110%) and heart (330%) with significant hypertrophy in both tissues. There was no difference in body composition in *ad libitum* groups. Plasma triiodothyronine and thyroxine were reduced by 37% in iron deficients compared to controls. Thus 39% increase in caloric requirements in iron deficiency is associated with increased sympathetic and perhaps thermogenic activity in brown adipocytes.

Nutritional iron deficiency continues to be one of the most prevalent nutrient deficiency diseases in the world. Numerous metabolic alterations have been shown secondary to iron deficiency (1), but clear relationships of biochemical abnormalities to body dysfunction have been difficult to demonstrate. It is now clear that both the neuroendocrine and hormonal components of thermoregulation and thermogenesis are affected by iron deficiency (2–5). Norepinephrine (NE) levels in plasma and urine are elevated in iron deficiency in rats (5, 6) and humans (7). Additionally, triiodothyronine (T3) and thyroxine (T4) metabolism are both significantly affected by iron deficiency (2, 5, 8) such that severely anemic animals are unable to thermoregulate at cold temperatures and become hypothermic.

Extensive literature (see (9, 10) for review) show the sympathetic nervous system (SNS) and thyroid hormones act in concert to regulate facultative thermogenesis in mammals in response to environmental temperature changes and certain dietary alterations and hence to alter energy metabolism. Poor growth rates are also a common observation of those who study iron deficiency in postweanling young rats (11, 12). These observations prompted us to examine rates of NE turnover in iron-deficient (ID) animals relative to their energetic efficiency. The present study provides evidence that iron deficiency is related to high rates of NE turnover in certain highly innervated tissues which may result in poor feed efficiency and slow growth.

Methods. Male Sprague–Dawley rats, 3 weeks of age, were obtained from Hilltop Laboratories (Denver, PA). Animals were divided into three dietary treatment groups: iron deficient, control pair fed, and control *ad libitum* fed. They were housed individually in stainless steel cages with wire mesh bottoms and maintained at 25°C on a 12-hr light/dark cycle. Room lights were on from 0800 to 2000 hr daily. Water was available *ad libitum.* These animals were fed a purified diet that contained 5–8 mg iron/kg diet and was prepared to the specifications for the low iron diet of ICN Nutritional Biochemicals (Cleveland, OH). This diet contained 27% vitamin-free casein, 56% cornstarch, 14% corn oil, 1% AIN-76 vitamin mix (ICN Nutritional Biochemicals), and 3% mineral mix (HMW formulation without ferric phosphate, ICN Nutritional Biochemicals). Control animals received the same diet, but received weekly injections (5 mg ip) of iron dextran starting at 4 weeks of age. Earlier studies (13, 14) with this diet and iron dextran injections showed animals to be similar to chow-fed controls when hematologic, biochemical, hormonal, and growth data are compared. Individual pair feeding was done on a daily basis after accounting for food spillage. Pair feeding was initiated after the ani-

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mals had been on the diet for 1 week and was continued for 5 weeks. Initial body weights of animals were not significantly different.

Analyses. The digestible energy value of the purified diet was determined by measuring the energy content of the diet and of feces collected from the rats for the 7-day period. Energy content was measured by bomb calorimetry (Parr Instruments Co., Moline, IL). The gross energy content of the purified diet was 5.14 kcal/g and apparent digestible energy averaged 90 ± 0.5%. Body composition was measured by the method of Hartsook and Hershberger (15). The body energy content of 10–12 homogenized carcasses in each group was used to predict the energy content of the carcasses at the beginning of the energy retention study (7 days previous). The difference in energy content is taken to represent the body energy gain.

Hematocrit was measured by the microhematocrit method and hemoglobin by conversion to cyanmethemoglobin. Thyroid hormone levels were measured by RIA (Monobind Corp., Costa Mesa, CA).

Norepinephrine turnover. Norepinephrine turnover studies were performed using α-methylparatyrosine injection (250 mg/kp ip in 0.9% saline) in unanesthetized nonfasted animals. Experiments were begun at 0800 hr. Rats were killed by cervical dislocation at 0, 3, and 9 hr after injection of the tyrosine hydroxylase inhibitor. Intrascapular brown adipose tissue (IBAT) and heart were rapidly removed and frozen in liquid nitrogen. All samples were frozen at −75°C until the time of analyses. Turnover studies were performed in each group of animals on consecutive days.

Norepinephrine content was measured by high-pressure liquid chromatography with electrochemical detection (HPLC-EC) (Bioanalytical Systems (BAS) Technical Bulletin 14, West Lafayette, IN). Briefly, tissues were homogenized (1:10 w/v) in iced .4 M perchloric acid (PCA), the protein precipitate removed by low speed centrifugation, and the internal standard was added (dihydroxybenzylamine, DHBA, Bioanalytical Systems, West Lafayette, IN). The catechols were extracted onto 50 mg of acid-washed alumina using 1.5 *M* Tris buffer, pH 8.6. Catechols were eluted from the alumina with 200 μl of 0.1 *M* PCA and quantitated by HPLC-EC (Bioanalytical Systems). We used a reverse-phase column (250 × 4 mm, 5 μ, Biophase, Bioanalytical Systems) with a monochloroaceatic acid, EDTA, and 2.0 m*M* sodium octyl sulfate mobile phase flowing at a rate of 1.2 ml/min. The detector potential was set at +0.65 V vs Ag–AgCl reference electrode. The extraction efficiency of the internal standard averaged 84%. Peak areas were integrated using a RC3a Integrator (Shimadzu Corp., Tokyo, Japan) and corrected for internal standard recovery. Slopes of the linear regression equations relating log NE tissue concentration to time were used to calculate the fractional turnover rate constant (k) for NE concentration ($k = b/0.434$). Rates of turnover were calculated by multiplying k for each experimental group times the NE content of the organs of those animals at zero time.

Data were subjected to analysis by one-way analysis of variance (ANOVA). Slopes and fractional turnover rates were compared statistically using ANOVA. Reagents for the mineral mix and laboratory procedures were reagent grade and purchased from Sigma Chemical Co. (St. Louis, MO).

Results. Studies using iron-deficient and *ad libitum* controls showed significantly slower growth in iron-restricted animals and prompted us to conduct more strict feeding studies using control pair-fed animals (Fig. 1). Iron-deficient animals grew more slowly than their pair-fed and *ad libitum* fed counterparts over a 4-week period. Body weight was significantly depressed by 20% in the ID animals by the third week on the low iron diet and at a time when packed cell volume was near 23%. The clear differentiation of pair-fed controls versus ID animals occurred after 4 weeks on the diet and the animals were very anemic.

Mean gross energy intake per gram of weight gain was considerably higher in the ID animals compared to pair-fed (20 kcal/g of weight gain versus 13 kcal/g) during Week 3 on the low iron diet and is reflected in the 25-g mean body weight gain in pair-fed compared to 16 g in iron deficients. Feed efficiency studies conducted on the three groups of animals over a 7-day period and after 4 weeks of dietary treatment expanded these gross energetic efficiency studies (Table I). There was a 28% decrease in energetic efficiency in ID animals compared to *ad libitum* controls and a 36% decrease in efficiency compared to food-re-

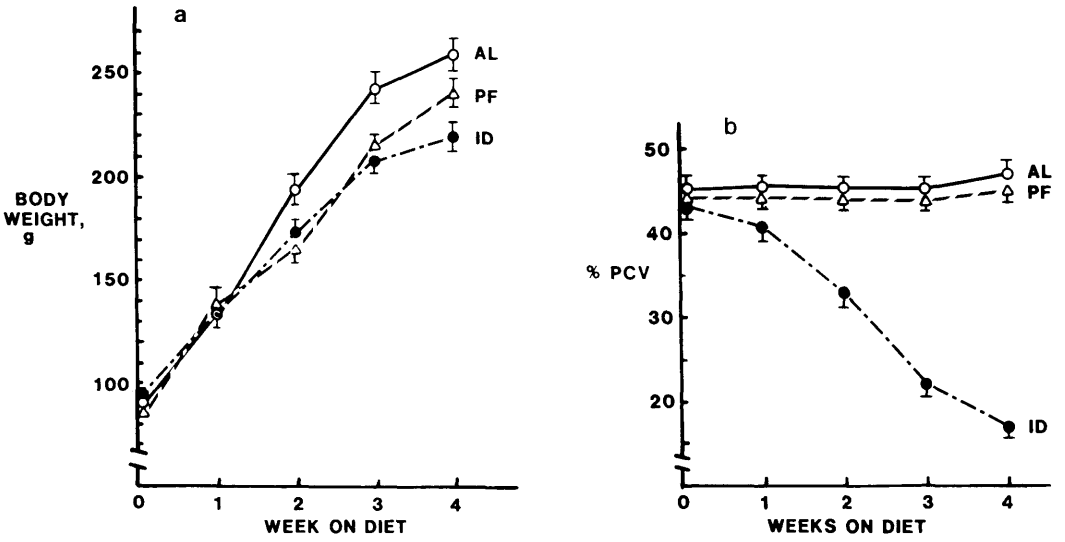

FIG. 1. Growth and hematocrits of iron-deficient (ID—●), pair-fed (PF—△), and *ad libitum* (AL—○) animals during 4 weeks on a low iron diet. Controls received 5 mg iron dextran ip per week. Significant group differences ($P < 0.05$) by ANOVA were observed at Weeks 3 and 4 for growth and by Week 2 for hematocrit. Symbols represent the means for six animals per group and bars the SE.

stricted animals. ID animals gained significantly less body weight per kilocalorie consumed (27 ± 7 mg/kcal for ID versus 42 ± 5 mg/kcal in *ad libitum* controls). The comparison to pair-fed animals is also striking (62 ± 10 mg/kcal) but is complicated by the research design induced changes in feeding behavior, that is, induced meal eating versus the normal rodent pattern of nibbling.

Body composition analysis conducted on animals in each group (Table II) demonstrated few significant differences between *ad libitum* fed groups with respect to major body fractions. Bomb calorimetry showed no significant differences between groups in the mean caloric value of the carcass.

Iron-deficient animals also spilled significantly greater amounts of food than either control group. While *ad libitum* controls spilled less than 400 mg of powdered diet on

TABLE I. IRON DEFICIENCY AND ENERGETIC EFFICIENCY TRIAL[a]

Group	Hct. (%)	Body Weight (g)	ME (kcal)	Energetic efficiency (%)	Conversion inefficiency (kcal ME/g gained)
Iron deficient	17	219	420	6.34	34.2
Pair-fed controls	45	241	423	14.5	16.5
Ad libitum fed controls	47	259	508	9.0	24.6
ANOVA					
Pooled SD	2	5	27	2.15	3.7
F ratio	152	15	16	22	18
Probability	<0.001	<0.001	<0.001	<0.001	<0.001
Number of animals	18				

[a] ME, total metabolized energy for 7 days. Energetic efficiency equals the percentage of calories retained over 7 days. Weight gain or food conversion inefficiency equals kcal ME consumed/g of body wt gained. Regression equations relating body weight to kcal were kcal = −269 + 2.39 g for iron-deficient animals ($n = 12$, $R^2 = 0.824$), kcal = −378 + 4.14 g for pair-fed animals ($n = 12$, $R^2 = 0.896$), and kcal = −136 + 2.75 g for *ad libitum* fed animals ($n = 10$, $R^2 = 0.646$).

TABLE II. BODY COMPOSITION OF RATS IN FOOD EFFICIENCY TRAIL

Group	Moisture (%)	Fat (%)	Protein by difference (%)	Ash (%)
Iron deficient	64.3	10.7	21.4	3.71
Pair-fed controls	61.5*	13.2*	21.9	3.45
Ad libitum fed controls	62.8	10.4	22.9*	3.06
ANOVA				
Pooled SD	1.6	2.1	1.1	.57
F ratio	7.8	5.3	4.5	3.35
Probability	<0.005	<0.025	<0.025	NS
Number of animals	18			

* Group significantly different ($P < 0.05$) than other two groups by post hoc Tukey analysis.

the average, food-restricted controls spilled <100 mg out of the weighted amount (approximately 15–17 g) and iron deficients spilled 1–4 g per day.

Norepinephrine turnover studies conducted in groups of ID, pair-fed, and *ad libitum* fed animals offer some possible explanation for these observations of metabolic inefficiency. The turnover of NE in interscapular brown adipose tissue (IBAT), a key thermogenic organ in the rat, was over 50% greater in iron-deficient animals than in pair-fed controls and more than twice as fast as *ad libitum* controls after 4 weeks on the dietary treatments (Fig. 2 and Table III). The fractional turnover rate (%/hr) in IBAT was nearly 330% greater in ID than *ad libitum* fed controls and 120% greater than food-restricted controls. Since the NE pool is significantly smaller (35%) in ID animals the dramatic increase in fractional turnover accounts for the much greater overall NE turnover (ng/hr). A mild hypertrophy of the interscapular brown fat pad (2.65 ± .2 mg/g body wt in iron-deficient versus 2.19 ± .2 mg/g in *ad libitum* controls) further accentuates the calculated turnover rates when expressed as a function of tissue weight. The data are presented with regard to both total turnover

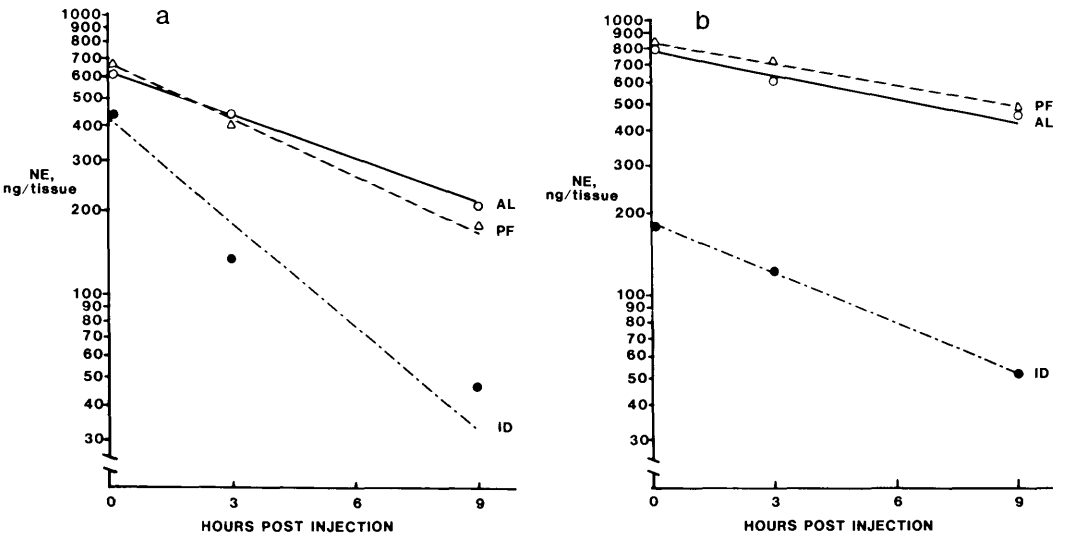

FIG. 2. Least-squares regression lines for norepinephrine turnover for iron-deficient (ID—●), pair-fed (PF—△), and *ad libitum* controls (AL—○) in heart and interscapular BAT. Calculated turnover and fractional rate constants are given in Table III.

TABLE III. EFFECT OF IRON DEFICIENCY ON BODY AND ORGAN WEIGHTS AND ON NE TURNOVER

Group	n	Body wt (g)	Tissue wt (mg)	Fractional turnover (%·hr^{-1})	NE content (ng)	Calculated turnover (ng·hr^{-1})	Calculated turnover (ng·g^{-1}·hr^{-1})
Interscapular brown adipose tissue							
Iron deficient	16	227*	580*	28.9*	418*	121	208
Pair-fed controls	16	260	634**	12.1**	663	80	127
Ad libitum fed controls	16	271	683	8.8	607	53	78
ANOVA							
Pooled SE		4	24	1.4	88		
F ratio		420	158	5.82	18.8		
Probability		<0.001	<0.001	<0.005	<0.001		
Heart							
Iron deficient	16		1287*	13.9*	182*	25	20
Pair-fed controls	16		900	5.8	837	48	54
Ad libitum fed controls	16		987	6.9	799	55	56
ANOVA							
Pooled SE			44	1.9	27		
F ratio			79	7.4	115		
Probability			<0.001	<0.005	<0.001		

* Significantly different from control group by post hoc Tukey analysis, $P < 0.01$.
** Significantly different from *ad libitum* controls by post hoc Tukey analysis, $P < 0.01$.

per pool and turnover per gram of tissue because it is not clear that the pool size does not affect the rate of turnover. Food-restricted controls showed increased (37%) fractional turnover compared to *ad libitum* controls but with no significant differences in content.

Heart NE turnover studies showed an even more dramatic depletion of tissue norepinephrine in ID animals. These significantly hypertrophied hearts (56% increase in heart: body wt ratio) had less than 23% of concentrations of norepinephrine observed in either control group. This depletion of NE was associated with greater than a 200% increase in fractional turnover rate in iron-deficient animals compared to control *ad libitum* animals and an even greater increase compared to pair-fed animals. There were no significant changes in NE content in hearts of control animals with food restriction compared to *ad libitum* fed controls.

Thyroid hormone levels were significantly lower in ID animals (Table IV). Food restriction in the control animals did not appear to have a significant effect on the level of T3 but was associated with a significant increase in circulating thyroxine levels compared to the *ad libitum* fed control animals.

Discussion. The results of this study indicate that iron deficiency is associated with elevated rates of NE turnover in tissue and with significant decreases in energetic efficiency and slowed growth. Altered thyroid hormone status is again observed and shown not to be related to the smaller body size of the ID animals.

Several groups (11, 12) have noted that iron-deficient animals have slower rates of growth than control animals, and that they have increased excretions of NE (6, 16), increased plasma NE levels, and decreased rates of disappearance of ^{3}H-NE from the plasma pool (5). Indeed, Goeneveld (6) has recently shown

TABLE IV. PLASMA THYROID HORMONE LEVELS IN IRON DEFICIENT, PAIR-FED, AND *ad Libitum* FED CONTROLS

Number of animals	Iron deficient	Pair fed	*Ad libitum*
	16	16	16
T_3 (ng/dl)	51 ±4*	83 ±2	85 ±3
T_4 (μg/dl)	3.4± .3*	5.4± .4**	4.2± .2
T_3/T_4 (ng/μg)	17.5±2.0	16 ± .4**	20.3±1.0

* Significantly different than controls by one-way ANOVA, $P < 0.001$.
** Significantly different than *ad libitum* controls, $P < 0.001$.

that urinary NE levels are already increased by the second week on a low iron diet and at a time when we observe growth rates declining (Fig. 1). Several hypotheses have been advanced to explain this hypernoradrenergic state. One is that there is a metabolic blockade in degradation of NE due to a decreased activity of monoamine oxidase (17), an iron-dependent enzyme (16), and there is subsequent "spillover" of nonconjugated or metabolized metabolites into urine. A more recent hypothesis (5) suggests increased sympathetic nervous system activity in partial compensation for decreased conversion of tetraiodothyronine (T4) to triiodothyronine (T3) and in an attempt to maintain body temperature by increased thermogenesis. This is based on the observations we (2, 3) and others (4, 5) have made that iron deficiency anemia is associated with decreased levels of T3 and an inability to thermoregulate at cold temperatures. The current experiment conducted at a constant temperature of 24–25°C is consistent with the latter hypothesis and points to a probable source of the high plasma NE concentrations, namely, high rates of turnover in highly innervated organs and perhaps a limited catabolic capacity.

Alterations in thermogenesis were previously examined in extreme conditions by examining thermoregulatory capacity at 4°C (3). Those experiments clearly showed that maximal heat production was related to anemia, and not to tissue iron deficiency. Exchange transfusion experiments have not yet been conducted in the current paradigm, so we cannot imply that the compensatory SNS activity suggested in this experiment is anemia or tissue iron deficiency dependent. We (J. Beard and W. Green, unpublished data) have observed in other studies that acute exchange transfusion does not normalize the urinary metabolite pattern of iron deficiency within the first 24 hr at either 24 or 4°C. This suggests the SNS hyperactivity we observe is not entirely dependent on anemia. The anemia-related limitation in maximal heat production may be a reflection of blunted brown adipose tissue function rather than the changes in control mechanisms suggested here.

Thermogenesis in IBAT is metabolically important during cold stress and acts as an energy buffer during variations in nutrient intake (9, 10). High rates of NE turnover in IBAT are observed in rats fed low protein diets for 4 weeks and are postulated to be strongly linked to the 40% decrease in energetic efficiency observed in those rats (19). Others (20) have demonstrated that NE turnover increases with low protein feeding in both heart and IBAT. This increased peripheral sympathetic function appeared consequent to increased central sympathetic outflow. There is an increase in metabolic rate and a decreased rate of weight gain, associated increases in IBAT thermogenesis, and BAT hypertrophy (21, 22). These high rates of NE turnover, characteristic of animals showing significant dietary or cold-induced thermogenesis, thus provide a plausible explanation for the significant 30% decrease in metabolic efficiency in iron deficient animals.

Leveille and co-workers showed 15 years ago that meal eating itself is associated with a significant increase in lipid storage and percentage body fat in the rats. The food-restricted animals in our study not only have this increased body fat and feed efficiency, but also significantly increased fractional NE turnover in IBAT compared to *ad libitum* controls: Apparently, this reflects an attempt by diet-induced thermogenesis to buffer this growing adiposity. Although we have not measured tissue iron in our current studies, previous studies by ourselves and others using this diet (2–5, 11) show a significant depletion of iron-dependent enzyme activity in liver, skeletal muscle, IBAT, and other tissues. The use of the same diet, but with injected iron dextran for controls, also removes any effects of dietary variation on metabolic efficiency.

Iron-deficient animals have significant decreases in NE pool size in both tissues examined along with increases in the proportion of that pool disappearing per unit time. The increased fractional turnover and modest decrease in pool size translates into increased mass turnover in IBAT but not in myocardium where the NE pool is very depleted. The greater mass turnover in IBAT implies greater receptor cell function and possibly greater heat production as well. The decreased NE content in presynaptic neurons in heart suggests an inability of iron-deficient animals to match

reuptake, catabolism, and synthesis rates to SNS firing with probable decreases in functional capacity of the myocardium.

Other studies in our laboratory (16) show these increased rates of NE turnover persist even after 5–7 days at a near thermoneutral temperature of 30°C. Thus, a portion of this increase in NE turnover may be explained on the basis of temperature-dependent thermogenesis and in compensation for a decreased thyroid state (21). Mackler (24) has shown a significant effect of iron deficiency on *in vitro* IBAT oxidative capacity but *in vivo* estimates of heat production are lacking. The data gathered in this experiment support the idea that IBAT hypertrophy and increased metabolic activity (as measured by increased NE turnover) contribute to an increased metabolic rate and decreased efficiency.

Iron-deficient animals have elevated resting oxygen consumptions (ROC) at 25°C (4, 5, 25). Perhaps this reflects the significant and profoundly increased NE turnovers observed in our current study. Other possible explanations may involve the increased use of energetically inefficient pathways.

Henderson and co-workers (25) have elegantly shown increased use of glucose as a fuel source with the attended decrease in feed efficiency for growth and weight maintenance. Although the heart work rate is increased in anemic animals to maintain the hyperkinetic blood flow state of anemia, this increased work is probably not sufficient to account for this increased ROC. The very great decreases in heart NE content and increases in turnover are especially interesting since the myocardium is relatively "protected" from iron depletion during iron deficiency anemia (14, 23). This pattern is characteristic of physiologic left-ventricular hypertrophy associated with increased hemodynamic loading (26). Indeed, Rossi *et al.* (27) claim a critical role for norepinephrine in the pathogenesis of cardiac hypertrophy induced by iron deficiency anemia.

One final unaddressed observation in this experiment was the significantly larger "food spillage" by iron-deficient animals. There was easily 4–10× greater spillage than *ad libitum* or pair-fed controls. ID has been linked repeatedly to abnormalities in central nervous system function, behavioral abnormalities, and altered cognition (1). Perhaps the anorexia of iron deficiency, increased peripheral SNS activity, decreased feed efficiency, and poor growth are all secondary to a central CNS mediated defect. Studies are underway to examine this aspect.

In summary, we have observed that iron-deficient anemic animals have a dramatic decrease in feed efficiency and increased caloric requirement for growth when their deficiency becomes quite severe and at a time when there is a very significant increase in NE turnover rate. Other studies (5) have clearly shown the hypernorepinephrinemia is due to tissue iron deficiency and not anemia. We conclude that the poor feed efficiency in iron-deficient animals is associated with a hypernorepinephrenemic state and the frequently observed increased resting oxygen consumption. Further studies are being conducted on the temperature dependence of this hypermetabolic state and the potential role of facultative thermogenesis.

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