Effects of Iron Repletion and Correction of Anemia on Norepinephrine Turnover and Thyroid Metabolism in Iron Deficiency (43040)

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Abstract. **The reversibility of the alterations in norepinephrine (NE) content and turnover in interscapular brown adipose tissue and heart of iron-deficient rats has not been demonstrated. We therefore examined NE metabolism in age-matched male Sprague-Dawley rats depleted of iron by dietary means and after repletion with iron dextran. Heart NE content was 58, 61, and 85% of controls at 0, 3, and 7 days after repletion, whereas interscapular brown adipose tissue-NE content was 87, 103, and 104% of controls. Fractional heart NE turnover was 225% greater in iron-deficient anemics than controls but normalized within 3 days. Interscapular brown adipose tissue NE turnover was 58%, 46%, and 20% above controls in iron-deficient rats after 0, 3, or 7 days of iron repletion. Hematocrit returned to 80% of normal in 7 days. Liver triiodothyronine production also increased to 80% of control in this period. A second experiment used isovolemic exchange transfusion to examine the influence of anemia per se on these alterations in organ NE turnover. Acute correction of anemia in iron deficiency did not alter brown fat NE turnover. Heart NE turnover was significantly lower in anemic animals regardless of iron status. Defects in heart and brown fat NE metabolism are reversible within 7 days of iron treatment as are alterations in triiodothyronine production. Anemia per se has little effect on brown fat NE metabolism but does dramatically decrease heart NE content.** [P.S.E.B.M. 1990, Vol 193]

An impaired thermoregulatory competence has been linked to iron deficiency in both humans (1, 2) and in animal models (3, 4). Studies designed to link a biochemical lesion to this phenomenon have focused on the two primary thermogenic systems, the sympathetic nervous system, and the thyroid hormone system. Increased fractional turnover rate of norepinephrine (NE) in both the heart and interscapular brown adipose tissue (IBAT) have been observed in iron-deficient (ID) animals (5, 6), as well as depressed plasma triiodothyronine (T_3) concentration (4–6) and blunted thyrotropin-releasing hormone challenge response (7). It has also been shown that iron deficiency results in a significant decrease in growth rate and energetic efficiency (5).

Studies by Dillman *et al.* (4) demonstrated that this impaired thermoregulatory capacity in iron-deficient rats is repaired within 7 days of iron repletion via intraperitoneal injection of iron dextran. This repair of thermoregulatory capacity coincided with a return of plasma thyroid hormone levels to normal values, a decrease in plasma catecholamines to normal levels, and recovery from anemia. Although the repletion of iron using an intraperitoneal injection of iron dextran is effective in demonstrating reversibility and dependence on iron, it does not clearly differentiate the contributions of iron deficiency anemia from tissue iron deficiency. It is unknown if the repair of this thermoregulatory capacity and the repair of plasma thyroid hormone levels is temporally related to the normalization of NE turnover and peripheral conversion of thyroxine to the active hormone, T_3.

Based on this information, we designed experiments to determine whether the increased rates of fractional NE turnover and diminished tissue NE pool sizes in iron-deficient rats are acutely reversible with iron therapy. To establish this link, we designed additional experiments to distinguish these two aspects of iron deficiency using exchange transfusion.

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Table I. Body Weight, Heart Weight, and IBAT Weight of Control and Iron-deficient Rats Supplemented with Iron Beginning at 0 Days[a]

Group	*n*	Body weight (g)	Hematocrit (%)	IBAT (mg)	IBAT/body weight ($mg \cdot g^{-1}$)	Heart (mg)	Heart/body weight ($mg \cdot g^{-1}$)
ID + 0 days	15	218 ± 10a	23 ± 3a	317 ± 32a	1.45 ± 0.13a	1140 ± 90a	5.22 ± 0.28a
ID + 3 days	15	234 ± 23b	30 ± 2b	346 ± 49a, b	1.49 ± 0.14a, b	1180 ± 60a	4.99 ± 0.40a, b
ID + 7 days	15	234 ± 19b	38 ± 4c	390 ± 35b, c	1.67 ± 0.09b	1150 ± 120a	4.77 ± 0.18b
CN	15	269 ± 16c	47 ± 3d	410 ± 48c	1.52 ± 0.14b	970 ± 80b	3.59 ± 0.19c
	P≤	0.0001	0.0001	0.0003	NS	0.0005	0.0001

[a] Mean ± SD; groups in a column not sharing identical letters differ by $P < 0.05$, analysis by one-way ANOVA with a priori least squares means comparisons.

Table II. Effect of Iron Deficiency and 3 or 7 Days of Repletion on Heart NE Turnover[a]

Group	NE content ($ng \cdot tissue^{-1}$)	Fractional turnover (k) ($\% \cdot hr^{-1}$)	NE Turnover · $tissue^{-1}$	
			($ng \cdot hr^{-1}$)	($ng \cdot hr^{-1} \cdot g^{-1}$)
Heart				
ID + 0	455 ± 232a	13.0 ± 3.1a	76	58
ID + 3	561 ± 87a, c	5.6 ± 1.1b	38	27
ID + 7	775 ± 98b, c	5.8 ± 1.1b	52	39
CN	911 ± 140b	3.9 ± 1.1c	44	36
P[b]	0.012	0.005		
IBAT				
ID + 0	551 ± 84	16.9 ± 1.8a	105	272
ID + 3	659 ± 31	15.4 ± 1.6a, b	125	252
ID + 7	643 ± 127	14.0 ± 1.8b	104	207
CN	633 ± 127	12.9 ± 1.6b	97	173
P[b]	NS	0.015		

[a] Mean ± SD, $n = 15$ in each group except content which equalled five animals per group. NE turnover per tissue or per gram of tissue is calculated as the product of fractional rate (k) times each individual animal.
[b] Probability of a significant difference by one-way ANOVA, groups in a column not sharing identical letters are significantly different (<0.05).

Materials and Methods

Experiment 1. *Animals.* Sixty male weanling Sprague-Dawley rats (Hilltop Laboratories, Denver, PA) were randomly divided into four groups at 21 days of age; 45 animals were assigned to a low iron diet group (AIN 76 diet, without cellulose, corn starch as the carbohydrate source, iron ≤ 5 ppm) and 15 animals to a similar diet except with 50 ppm iron added as ferrous sulfate (CN) (8). Animals were housed individually in stainless steel, rust-free cages and maintained on a 12-hr light/dark cycle. After 4 weeks of *ad libitum* feeding, 15 of the ID rats received no injected iron (ID + 0), 15 rats received 5 mg of iron ip as iron dextran 3 days before sacrifice (ID + 3), and 15 ID rats received 5 mg of iron ip as iron dextran 7 days before sacrifice (ID + 7). The injections of repletion iron were timed so that equal numbers of animals in all four groups (ID-O, ID-3, ID-7, CN) were sacrificed on each of 4 days.

NE turnover protocol. Animals were injected in a nonfasted state with *α-methyl-p*-tyrosine methyl ester (250 mg/kg body wt ip in sterile saline) and sacrificed at 0, 3, or 9 hr after injection. The sacrifice of animals was designed so the four treatment groups and the three time points (0, 3, and 9 hr) were equally distributed across the 4 days of sacrifice to control for any systematic bias due to within day and between day effects on NE content. Animals were killed by cervical dislocation and decapitation, trunk blood was collected without stasis, and tissues were rapidly removed and placed in liquid nitrogen. Hematocrit was determined by microcapillary methods and hemoglobin by conversion to cyanmethemoglobin using standard methods.

Tissue content of NE was determined by reverse phase high-pressure liquid chromatography using electrochemical detection (Bioanalytical Systems, West Lafayette, IN) and published methods (5, 6). Content was corrected to recovery of an internal standard (dihydroxybenzylamine; BAS, West Lafayette, IN).

Liver T_3 production protocol. Hepatic 5′-deiodinase activity was measured by previously published methods (7) with minor modification. Briefly, a 1 to 2-gr section of the fresh liver was immediately homogenized in 100 m*M* (pH 7.2) phosphate buffer (1:3, w:v) and centrifuged for 15 min at 3000 rpm at 4°C. The supernatant was incubated with phosphate buffer (100 m*M*, pH 7.2), dithiothreitol (3.0 m*M*), and thyroxine (T_4, 20 μg/

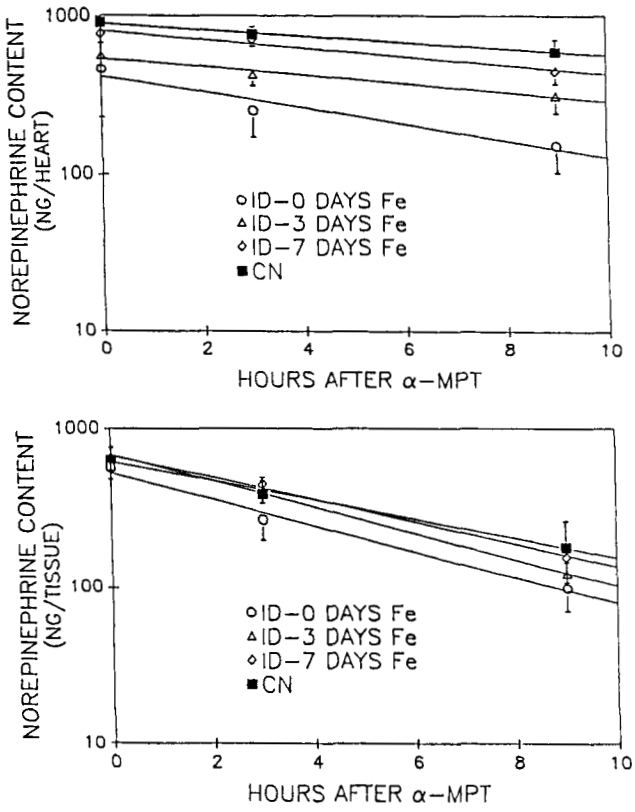

Figure 1. Turnover of norepinephrine in heart (top panel) and brown fat (bottom panel) of control and iron-deficient rats repleted with iron 0, 3, or 7 days. Symbols represent mean ± SD.

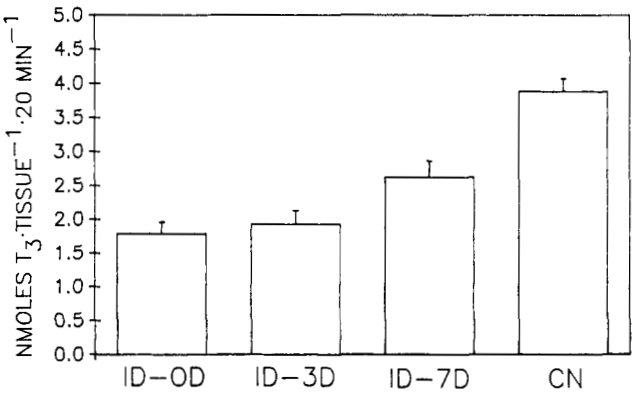

Figure 2. Hepatic thyroxine-5′-deiodinase activity in control and iron-deficient rats repleted 0, 3, or 7 days. One-way ANOVA showed significant differences ($P < 0.001$). Bar height is the mean with bracket 1 SD.

ml) for 20 min at 37°C under flowing N_2. Triplicate 100-μl aliquots were removed at either 0 or 20 min and the reaction was stopped by addition of two volumes of iced ethanol. The denatured protein was pelleted by microcentrifugation, 1 min at 14,000 rpm, and the supernatant frozen. T_3 concentration was determined by radioimmunoassay (Monobind Corp., Costa Mesa, CA) on 0′ and 20′ triplicate aliquots for each sample.

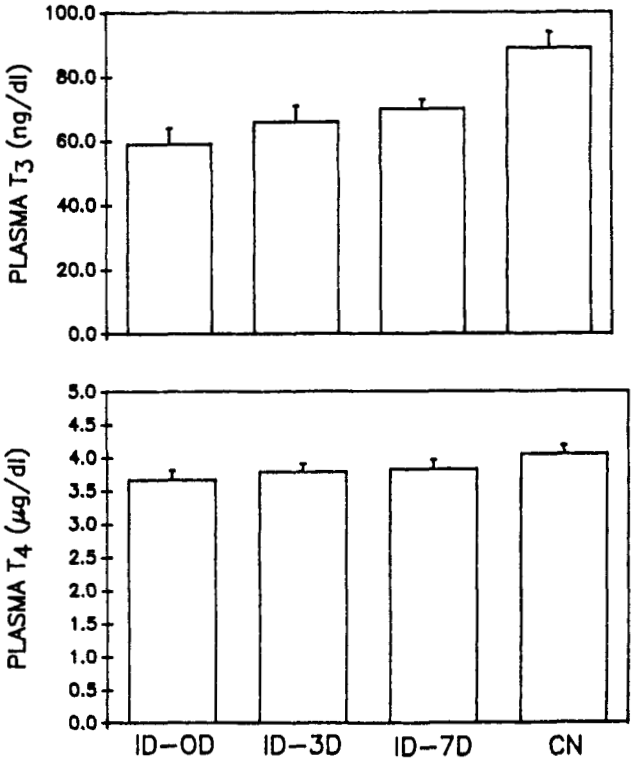

Figure 3. Plasma T_3 and T_4 concentrations. Groups as in Figures 1 and 2. One-way ANOVA showed a significant difference ($P < 0.005$) for T_3 but not for T_4.

Average T_3 production was calculated and expressed per milligram of crude homogenate protein. Protein concentration was determined by a modified Lowry method (9).

Experiment 2. *Animals.* Sixty male Sprague-Dawley rats were divided into ID and CN groups and maintained on diets as described in Experiment 1. After 4 weeks of *ad libitum* feeding, animals were anesthetized using ketamine (6.5×10^{-4} ml/g; Bristol-Meyers, Syracuse, NY) and Rompun (3.25×10^{-4} ml/g, Mobay Corp., Shawnee, KS) and an intra-atrial PE-50 catheter was inserted via the right jugular vein. The catheter was exteriorized and secured in the scapular region with 2–3 cm exposed. Catheters were filled with sterile heparinized (100 units/ml) saline to maintain patency, and animals were allowed to recover for 48 hr. Each group of animals (ID, CN) was then assigned to one of two treatments: exchange or sham transfused, resulting in four treatment groups—iron-deficient nonanemic (ID-NA), iron-deficient anemic (ID-A), control nonanemic (CN-NA), and control anemic (CN-A). ID-NA animals were isovolemically transfused to a hematocrit of 45% packed cell volume using red cells that had been washed three times with sterile saline and plasma from iron-deficient donor animals. CN-A animals were isovolemically transfused to a hematocrit of 15–20% packed cell volume using plasma from donor CN animals. Exchange transfusions in both ID and CN groups were

Table III. Body and Organ Weights of Exchange or Sham-Transfused ID and CN Rats 24 hr after Blood Manipulation[a]

Group	*n*	Body weight (g)	Hematocrit (%)	IBAT (g)	Heart (g)	Liver (g)
ID-A	15	167.8 ± 25.4a	16 ± 2	0.35 ± 0.07a	1.16 ± 0.11a	5.98 ± 0.90a
ID-NA	15	163.7 ± 19.5a	44 ± 3	0.30 ± 0.04b	1.16 ± 0.15a	6.09 ± 0.99a
CN-NA	15	294.5 ± 16.7b	48 ± 3	0.48 ± 0.07c	0.94 ± 0.11b	11.23 ± 1.17b
CN-A	15	270.7 ± 11.0c	18 ± 3	0.45 ± 0.06c	0.97 ± 0.11b	9.91 ± 1.42c
ANOVA						
Iron status		0.001	NS	0.001	0.001	0.001
Anemia		0.036	0.001	NS	NS	0.034
Interaction		0.013	NS	0.011	NS	NS

[a] Mean ± SD; groups in a column not sharing identical letters differ by $P < 0.05$, analysis by two-way ANOVA with a priori least squares means comparisons. Indicated probability is for main effects and interaction terms.

Table IV. Effect of Exchange Transfusion on NE Turnover in Heart, Brown Adipose Tissue, and Liver of Iron-Deficient and Control Rats[a]

	NE Content ($ng \cdot tissue^{-1}$)	Fractional turnover ($\%\cdot hr^{-1}$)	NE Turnover ($ng \cdot hr^{-1}$)
Heart			
ID			
Anemic	129 ± 52[b,c]	9.4 ± 7.4	5
Nonanemic	180 ± 59[b]	13.3 ± 6.4	29
CN			
Nonanemic	775 ± 157	7.6 ± 3.2	39
Anemic	605 ± 174[c]	10.9 ± 3.6	66
IBAT			
ID			
Anemic	421 ± 92[b]	22.5 ± 7.7[b]	95
Nonanemic	472 ± 95[b]	25.1 ± 4.9[b]	119
CN			
Nonanemic	880 ± 177	13.1 ± 2.8	115
Anemic	713 ± 98	19.6 ± 3.4[d]	139
Liver			
ID			
Anemic	223 ± 27[b]	12.0 ± 3.8	27
Nonanemic	388 ± 87[b,d]	25.3 ± 1.2[d]	97
CN			
Nonanemic	683 ± 109	16.4 ± 4.4	96
Anemic	692 ± 187	15.3 ± 3.8	82

[a] Mean ± SD; $n = 5$/group for NE content and 15/group for fractional turnover. See the footnote to Table III for calculation of NE turnover.
[b] ID groups significantly different ($P < 0.001$) from CN by two-way ANOVA.
[c] Anemia is a significant main effect ($P \leq 0.03$).
[d] Transfusion resulted in a significant change ($P < 0.01$) within a diet group.

carried out via three volumetrically equal stepwise stages with less than 3 ml of fluid exchanged per step to minimize trauma to the animals. This was completed within 20 min. Sham-transfused animals had blood withdrawn and reinfused in a similar fashion as exchange transfused except no addition or deletion of plasma or red cell volume occurred. Twenty-four hours following transfusion NE turnover experiments were carried out as described in Experiment 1.

All reagents and dietary components unless otherwise noted were reagent grade and obtained from Sigma Chemical Co. (St. Louis, MO).

Statistics. Most comparisons were conducted by either one- or two-way analyses of variance (ANOVA) with a priori post hoc comparisons. These analysis were conducted on an IBM 370 mainframe computer using SAS software (Statistical Analysis System, Cary, NC) and the general linear models subroutine. We considered a probability of ≤ 0.05 as an indication of statistical significance between groups and performed specific comparisons using the least square means technique. The method for calculating NE turnover typically multiplies the group average fractional turnover (k) times each individual NE content of animals killed at time zero. We did not calculate the variance of each group's NE turnover because the animal to animal variation in

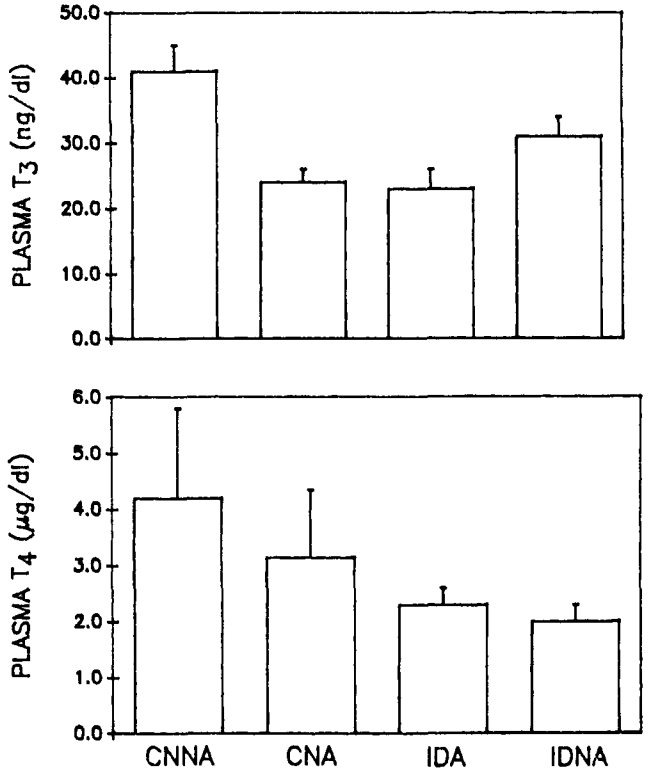

Figure 4. Norepinephrine turnover in heart in control (CN) and iron-deficient (ID) rats that were either anemic (A) or nonanemic (NA). Symbols represent group average concentration (±SD) at each time point.

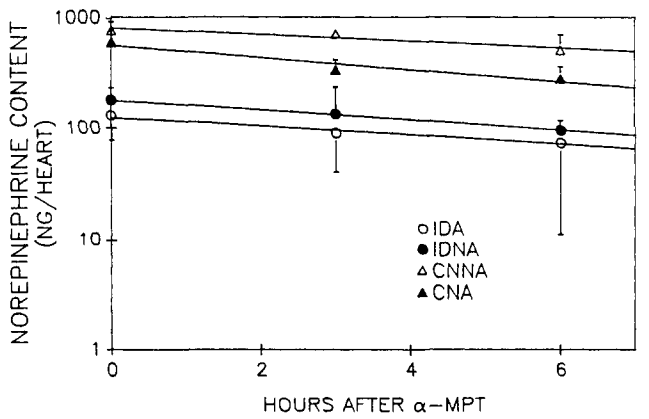

Figure 5. Plasma T_3 and T_4 concentrations (mean ±SD) in exchange transfused rats as described in the legend to Figure 4. Anemia and iron status were significant main effects for T_4 ($P < 0.01$) and <0.001, respectively) but for not T_3. There was a significant ($P < 0.05$) decrease in T_3 with transfusion in CN rats.

fractional turnover would not be included in the variance of the NE turnover and thus underestimate true group variation.

Results

Experiment 1. ID anemic rats had a significantly lower body weight relative to controls (Table I). A prompt recovery of body weight with provision of iron was seen in both the 3- and 7-day repleted groups. The

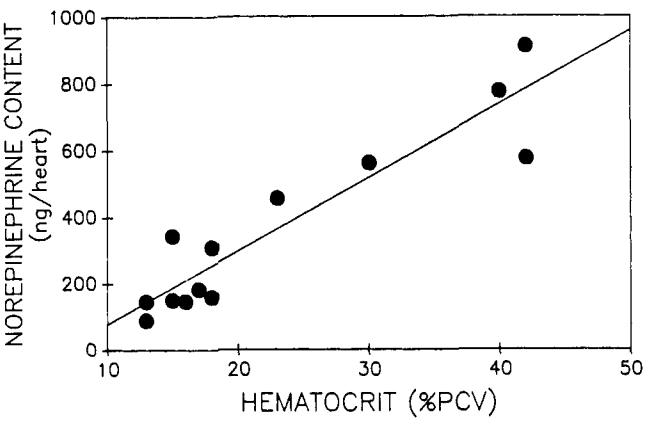

Figure 6. Plot of hematocrit versus heart NE content. Symbols represent group means for animals in the experiment and others (5, 6; unpublished data).

hematocrits of these two supplemented groups were significantly greater than those of the unsupplemented animals but was still below those of the control animals. There was no effect of iron deficiency on IBAT to body weight ratio, although the hearts of the anemic animals were significantly larger than controls (Table I).

Heart NE content in ID anemic animals was only 50% that of controls (Table II) and returned to normal with 7 days of iron supplementation. The 225% greater fractional NE turnover in ID hearts was returned to normal within 3 days of iron repletion and before normalization of the hematocrit or NE content. NE turnover (ng/hr) fell 50% and was similar to that of controls at 3 days (Fig. 1). Brown adipose tissue NE content was statistically unaffected by iron deficiency while fractional NE turnover was 21% higher in unsupplemented ID animals. This rate normalized within 3 days.

The significantly lower concentrations of plasma thyroid hormones in ID anemic animals returned to near control concentrations with supplementation (Fig. 2). Hepatic T_3 production was also dramatically affected by iron deficiency (Fig. 3) and showed a time frame for repair that was similar to that of plasma T_3 concentration. The production of T_3 in liver was only 46% of CN rats' production rates in the iron-deprived animals and changed to 68% of controls after 7 days of iron repletion.

Experiment 2. Iron-deficient anemic animals transfused to nonanemic hematocrits (ID-NA) and controls transfused to anemic hematocrits (CN-A) were hematologically similar to their sham-transfused counterparts at the time of sacrifice, 24 hr after transfusion (Table III). Acute correction of anemia or its induction was without demonstrable effect on organ size.

As seen previously, NE content was significantly lower in tissues of iron-deficient animals than in controls (Table IV). Transfusion of ID anemic rats signifi-

cantly increased NE content only in liver (50%). In contrast, acute induction of anemia in controls was associated only with decreases in NE content in heart. Fractional turnover was affected by iron only in brown fat and correction of the anemia affected only liver with a 110% increase in fractional turnover. Correction of anemia in iron deficiency led to a dramatic increase in cardiac mass turnover and a 3-fold increase in liver turnover (Fig. 4 and Table IV).

The acute correction of the anemia in the tissue of iron-deficient animals did not significantly alter the concentrations of T_3 or T_4 in plasma. Control animals made anemic had a significant decrease in the plasma T_3 concentration from 41 to 23 $ng \cdot dl^{-1}$ (Fig. 5). Likewise, the thyroxine concentration was significantly lower in the anemic control animals.

Discussion

This study demonstrates that some factors associated with thermogenic activity are affected by anemia to a much lesser extent than actual ability to maintain body temperature during a cold stress. Previous studies (3) showed clearly that the ability to maintain body temperature during exposure to 4°C was dramatically improved in iron-deficient rats if the anemia was corrected with exchange transfusion. There was also an improved responsiveness of the thyroid hormone system with alleviation of the anemia. In contrast, Dillman *et al.* (4) demonstrated that exchange transfusion did not alter the elevated urinary norepinephrine concentrations of iron-deficient anemic rats. Since those initial studies, we have further characterized the alterations in the sympathetic nervous system in iron deficiency with demonstrations that tissue norepinephrine turnover varies in iron deficiency in an organ-specific fashion (5 6). Dillman *et al.* (4, 10) demonstrated that plasma concentrations of NE were elevated in ID animals with anemia but that epinephrine was normal. The plasma clearance rate of a ^{3}H-NE dose was considerably slower in ID animals, with the authors suggesting a possible defect in catabolism of the neurotransmitter. Other experiments (5, 6) suggest an imbalance between probable increased nerve traffic and a limited tissue capacity to maintain NE content since ID animals failed to increase NE turnover in brown fat with decreasing temperature. Thus, a generalized hypernoradrenergic state is an oversimplification and more organ-specific effects need to be considered.

The turnover of norepinephrine in brown adipose tissue is a more direct index of activation of the heat-producing machinery in that tissue than are measures of either urinary or plasma catecholamines (11, 12). The current study demonstrates that this indicator is relatively unaffected by acute correction of the anemia or the induction of anemia in control animals. The failure of brown fat norepinephrine turnover to be responsive to acute correction of anemia in iron deficiency does not preclude a possible defect in this portion of the control system for heat production. Indeed, the systematic effect of iron deficiency on lowering tissue norepinephrine content and its ready reversibility with iron therapy demonstrates a clear relationship of iron to sympathetic neural metabolism.

The effects of iron deficiency anemia on norepinephrine kinetics and content in the heart merits some discussion. There is a large difference in the norepinephrine contents in the iron-deficient anemic animals in the two studies presented here. The severity of anemia was greater in the second than in the first study, mean hematocrits of 16% vs 23%. This might seem like a small difference considering that both groups of rats are severely anemic and had considerable hypertrophy of the heart. Nonetheless, this small decrease in hematocrit was associated with a 72% decrease in content of NE and a 93% decrease in turnover rate. Rossi *et al.* (13) demonstrated a linear relationship of heart NE content to hemoglobin but had minimum hemoglobins of 5 g/dl. In order to see if our data are consistent with the study of Rossi *et al.*, we generated a simple plot (Fig. 6) of hematocrit versus NE content. The correlation coefficient is highly significant ($r = 0.784$, $P < 0.005$)) over a considerable range of hematocrits. Despite this linear relationship, we do not know the exact cause of this decrease in NE content. The 6-fold increase in NE turnover with acute transfusion to correct anemia suggests to us that norepinephrine synthesis per se is not significantly impaired in iron deficiency and supports the previous publications relating hemodynamic stress of anemia to NE content (14, 15). Unpublished data from our laboratory showed no effect of iron deficiency on heart tyrosine hydroxylase activity despite Nagatsu's (16) demonstration of an *in vitro* dependence on ferrous iron.

Plasma thyroid hormones had previously been shown to be dramatically increased with the acute correction of anemia and iron repletion in iron-deficient animals (5, 6). The current study extends those observations by noting that hepatic T_3 production rates are systematically increased with iron therapy. Since the enzyme in this organ appears to primarily export the produced cystolic T_3 into the plasma pool (17), a possible link of iron status to T_3 concentrations and plasma T_3 turnover is established.

These studies show that iron therapy readily reverses the alterations in norepinephrine content and turnover in iron deficiency. The differences in rates of recovery of hemoglobin, NE turnover, and T_3 metabolism probably reflect the rates of incorporation of iron into those particular metabolic compartments and the pools of iron within these compartments (3). NE and T_3 recovery may similarly reflect the impact of iron incorporation into other neuroendocrine pathways

which then impact on these hormones (11, 17). It is clear these are not mutually exclusive explanations and we are conducting experiments to address these questions.

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