Plasma Thyroid Hormone Kinetics Are Altered in Iron-Deficient Rats

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ABSTRACT Iron deficiency anemia is associated with lower plasma thyroid hormone concentrations in rodents and, in some studies, in humans. The objective of this project was to determine if plasma triiodothyronine (T₃) and thyroxine (T₄) kinetics were affected by iron deficiency. Studies were done at a near-thermoneutral temperature (30°C), and a cool environmental temperature (15°C), to determine plasma T₃ and T₄ kinetics as a function of dietary iron intake and environmental need for the hormones. Weanling male Sprague-Dawley rats were fed either a low Fe diet [iron-deficient group (ID), <5 µg/g Fe] or a control diet [control group (CN), 35 µg/g Fe] at each temperature for 7 wk before the tracer kinetic studies. An additional ID group receiving exogenous thyroid hormone replacement was also used at the cooler temperature. For T₄, the disposal rate was >60% lower (89 ± 6 vs. 256 ± 53 pmol/h, P < 0.001) in ID rats than in controls at 30°C, and ~40% lower (192 ± 27 vs. 372 ± 26 pmol/h, P < 0.01) in ID rats at 15°C. Exogenous T₄ replacement in a cohort of ID rats at 15°C normalized the T₄ concentration and the disposal rate. For T₃, the disposal rate was significantly lower in ID rats in a cool environment (92 ± 11 vs. 129 ± 11 pmol/h, P < 0.01); thyroxine replacement again normalized the T₃ disposal rate (126 ± 12 pmol/h). Neither liver nor brown fat thyroxine 5'-deiodinase activities were sufficiently different to explain the lower T₃ disposal rates in iron deficiency. Thus, plasma thyroid hormone kinetics in iron deficiency anemia are corrected by simply providing more thyroxine. This suggests a central regulatory defect as the primary lesion and not peripheral alterations. J. Nutr. 128: 1401–1408, 1998.

KEY WORDS: • iron deficiency anemia • thyroid hormone • metabolic rate • rats • Simulation Analysis and Modeling (SAAM)

Iron deficiency is a major nutritional problem, affecting ~15% of the world’s population (DeMayer and Adiels-Tegman 1985). Iron deficiency anemia impairs the ability of both humans and rats to thermoregulate during cold exposure (see review by Brigham and Beard 1995). In rats, iron deficiency lowers plasma thyroid hormone concentration (Beard et al. 1982) and triiodothyronine (T₃) turnover rates (Beard et al. 1989), resulting in lowered utilization of these hormones by tissues.

Thyroid hormones are essential for mammals to maintain normal body temperature at any temperature below thermoneutrality. During cold exposure, norepinephrine released from sympathetic nerve endings acts on adrenergic receptors in brown adipose tissue (BAT) to increase heat production. Adrenergic receptor activation stimulates the synthesis of mRNA for thyroxine 5'-deidinase, the enzyme that converts plasma-derived thyroxine (T₄) to T₃ (Rasmaja and Larsen 1989). Increased 5'-deiodinase synthesis and the subsequent increased activity of the enzyme result in an increase in BAT intracellular concentration of T₃, leading to virtual saturation of nuclear T₃ receptors (Silva 1988). Nuclear T₃ receptor saturation, acting in concert with adrenergic-dependent signals, induces synthesis of mRNA for uncoupling protein, the mitochondrial protein that is responsible for heat production in BAT (Bianco and Silva 1987, Himms-Hagen 1990). The observed inability of iron-deficient (ID) rats to thermoregulate in the cold may result from a failure to increase metabolic rates sufficiently, as well as an inability to lower heat loss rates to the environment (Brigham and Beard 1995).

Thyroid hormone kinetics in rats are well characterized (DiStefano et al. 1993, Silva et al. 1984, Yamada et al. 1996). The plasma hormones equilibrate rapidly with “fast” equilibrating tissue pools (primarily in liver and kidney) and equilibrate much more slowly with other pools in peripheral tissues (DiStefano et al. 1982b). Fifty-seven percent of the body T₄ and 76% of the body T₃ are contained in slowly turning over pools, with the remainder contained in the fast turning over extravascular pool (17% for T₄ and 19% for T₃) and in the plasma pool (26% for T₄ and 3% for T₃). An enterohepatic circulation of the glucuronide and sulfate conjugates of T₄ and T₃ is quite active, with as much as 43% of T₄ and 34% of T₃ returned to the plasma pool (DiStefano et al. 1982a and 1993). Cold exposure is associated with an increased plasma thyroid hormone pool size and turnover rate (Reed et al. 1992 and 1994) and with virtual saturation of nuclear T₃ receptors (Bianco and Silva 1987).

Specific studies of iron deficiency anemia and thermoregula-

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Abbreviations used: BAT, brown adipose tissue; DR, disposal rate; FCPr, fractional catabolic rate; Hb, hemoglobin; Hct, hematocrit; IBAT, intrascapular brown adipose tissue; ID, iron deficient; rT₃-deiodinase, reverse T₃ deiodinase; T₄, di-iodothyronine; T₃, triiodothyronine; T₄, thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; TTR, transthyretin.
tion in humans demonstrate a clear failure of thermoregulatory systems to maintain a normal body temperature during either a cold water (Beard et al. 1990, Dillman et al. 1980) or a cold air stressor (Lukasik et al. 1990). Although not all of these studies demonstrate a lower concentration of plasma thyroid hormones in iron deficiency, there is certainly a very strong suggestion from all three human studies that this is the case. The importance in determining whether functional rates of hormone production and utilization are affected by iron deficiency resides in their important role in overall regulation of Na+/K+ ATPase pumping, ion cycling, cell growth and thermogenesis (Guernsey and Edelman 1983).

Our research hypotheses in these experiments were as follow: 1) iron deficiency lowers $T_4$ production and utilization rates and, subsequently, $T_3$ production and heat production; 2) a lower environmental temperature (15 vs. 30°C) amplifies the effect of iron deficiency on thyroid hormone kinetics; and 3) addition of exogenous $T_3$ in the form of constant-release pellets sufficient to normalize plasma $T_3$ concentrations likely normalizes plasma $T_4$ and $T_3$ kinetics. The two environmental temperatures (30 and 15°C) constitute very different situations and will elicit potential interaction effects of dietary status and environmental demand for thyroid hormone. Compared with thermoneutrality, the lower temperature (15°C) should increase plasma $T_4$ and $T_3$ turnover rates, concentrations and plasma pool sizes, but we expected that these responses would be blunted in the ID rats. By comparing plasma thyroid hormone kinetics across the two temperatures and two dietary treatments, the role of altered thyroid hormone metabolism in the thermogenic defect observed in ID rats should be apparent and quantifiable.

MATERIALS AND METHODS

Design. Thyroid hormone metabolism was examined in iron-deficient or control rats housed at a warm, thermoneutral environmental temperature (30°C) (Beard et al. 1988), or at a cool, subthermoneutral temperature (15°C). The 15°C temperature is the lowest temperature that can be tolerated by anemic [hemoglobin (Hb) ~60-70 g/L] ID rats without a change in core body temperature (unpublished observations). To explore the possibility that $T_4$ replacement would be beneficial in iron deficiency, we housed ID rats at a cool environmental temperature and provided exogenous thyroid hormone to some ID rats at a dose sufficient to normalize their plasma $T_4$ concentration. Thus, two experimental groups [ID and control (CN)] were used at the thermoneutral temperature, and three experimental treatment groups at 15°C [ID, CN and ID-$T_4$]. There were 12–18 rats in each experimental treatment, but complete kinetic data were obtained only in 6–10 rats in each treatment. The Pennsylvania State University Animal Care and Use Committee approved all experiments.

Rats and dietary treatment. Male weanling Sprague-Dawley rats were obtained at 21 d of age from a commercial supplier (Harlan Sprague Dawley, Indianapolis, IN) and housed individually in stainless steel cages with a 12-h light:dark cycle (light, 0600–1800 h) at a room temperature of either 30 ± 1°C or 15 ± 1°C (mean ± range to each side of the midpoint). Rats were provided either the AIN-76 diet with an inadequate amount of iron (3–5 mg Fe/kg diet, ID group) or with 35 mg Fe/kg diet added as ferrous sulfate (CN group). Diets were adequate in all other nutrients, as defined by the AIN-76 formulation (American Institute of Nutrition 1980). Carstarch rather than sucrose was the dietary carbohydrate source as recommended by the American Institute of Nutrition for studies in which sucrose may affect the dependent variable (American Institute of Nutrition 1980). Nonnutritive cellulose was deleted from both diets because of its variable iron content. Rats were given free access to food and distilled deionized water.

Animal surgery. After 7 wk, sterile indwelling catheters were surgically placed in the right carotid artery while the rats were anestheitized (ketamine HCl, 76 mg/kg, Quad Pharmaceuticals, Indianapolis, IN, and xylazine, 7.6 mg/kg, Haver, Shawnee, KS) using the methods described previously (Beard et al. 1984). The catheters were filled with heparinized sterile saline, exteriorized through the nape of the neck and flame sealed. The rats were allowed 4 d of recovery with daily monitoring of body temperature, food and water intake, and behavior.

Thyroid hormone replacement. As discussed in a earlier section, we normalized plasma thyroid hormone concentrations in one group of ID rats living at 15°C by providing an exogenous source of the thyroid hormone (Innovative Research, Toledo, OH). These rats had a time-release $T_4$ pellet implanted subcutaneously in the interscapular region of the back. The pellet provided 2 mg of $T_4$ per day (~7.5 $\mu$g/kg·d), a dose sufficient to normalize plasma $T_4$ levels in ID rats (preliminary studies, unpublished data). The pellets were implanted 4 d before thyroid kinetics studies were performed, so that $T_4$ levels could reach a new steady state.

Resting metabolic rate. Three days after catheter implantation (and $T_4$ pellet implantation for ID rats undergoing that treatment), resting metabolic rate was measured during the early part of the light cycle (0900–1100 h) by open-circuit indirect calorimetry (Tobin and Beard 1990). All rats were deprived of food for 12–15 h. Then, resting metabolic rate was measured individually for eight rats randomly chosen from each treatment group, over a 2-h period.

Thyroid kinetics studies. Before use, radioactive materials ($^{125}$I-$T_3$, specific activity ~210 GBq/mg; and $^{125}$I-$T_4$, specific activity ~12.1 GBq/mg; DuoRadiol, NEN, Boston, MA) were purified by Sephadex chromatography to remove free iodide derived from spontaneous degradation. Purity was 98% for $T_3$ and >96% for $T_4$. To determine the proportion of free and transthyretin (TTR)-bound $^{125}$-T$_3$ in the injection solution, an aliquot of prepared $^{125}$-T$_3$ injection solution was chromatographed over a TTR affinity column (CNBr-activated Sepharose-4B) with 0.05 mmol/L Tris-HCl (pH 7.4) containing 0.5 mol/L NaCl as the affinity buffer and H$_2$O as the elution buffer. We determined that 11.9% of the isotope was bound to TTR and 88.1% was free hormone at the time of injection.

Purified tracer was diluted in sterile 150 mmol/L NaCl/rat plasma (9:1) containing 10 g/L NaI in a final volume of 100–200 $\mu$L. $^{125}$I-$T_4$ (1480 MBq) or $^{125}$I-$T_3$ (1480 MBq) was injected into the carotid artery catheters of recipient rats. The catheters were then flushed with sterile physiological saline (500 $\mu$L). Injection syringes were weighed to 0.1 mg accuracy both before and after injection; the difference in weight was used to calculate the volume of tracer solution that was injected into each rat. Several aliquots (25 $\mu$L) of the injection solution were collected to confirm that, in combination with the known injection volume, the quantity of radioactivity injected into the rats could be determined. The injected tracer constituted an average of 1% of the $T_3$ plasma pool size and 40% of the $T_4$ plasma pool size.

Blood samples (250 $\mu$L) were drawn via the carotid artery catheters at 3, 6, 12, 18, 24, 36 and 48 min, and 1, 2, 3, 4, 6, 8, 10, 20, 26, 32 and 44 h postinjection. These sampling intervals were determined from the analysis of preliminary data. The rats had free access to food and water throughout the kinetic sampling period. Immediately after sampling, blood was centrifuged at 3000 × g in a microfuge, and the resulting plasma divided into aliquots and frozen at −70°C.

Tissue collection and analysis. Blood Hb and hematocrit (Hct) were determined on a portion of each blood sample using standard cyanomethemoglobin and microcapillary methods, respectively. For Hb, the intra-assay coefficient of variation (CV) was 1.1% and the interassay CV was 3.0%; for Hct, the CV were 0.6 and 1.3%, respectively. A portion of the plasma was reserved for the determination of plasma $T_3$ and $T_4$ concentrations using commercial RIA kits (Incstar, Minneapolis, MN and Monobind, Costa Mesa, CA, respectively). The assays were validated for rat plasma by using chemically pure thyroid hormones (Sigma Chemical, St. Louis, MO) added to hormone-stripped rat plasma. If human standards were used to generate the standard curve, $T_3$ concentrations were unaffected. In contrast, when human standards were used in the rodent assay for $T_3$, the assay underestimated the actual $T_3$ concentration by 50%. The intra-assay CV was 5.8% for $T_4$ and 4.8% for $T_4$; the interassay CV was 6.1% for $T_4$ and 4.8% for $T_4$.

At the end of the sampling period, anesthetized rats (50 mg/kg
pentobarbital) were exsanguinated from the abdominal aorta and blood was collected into a heparinized syringe. Liver and interscapular brown adipose tissue (IBAT) were rapidly dissected from the carcass, and a portion was homogenized and centrifuged at 100,000 × g to obtain the microsomal fraction for later determination of 5′-deiodinase activity (Kopecky et al. 1986). These aliquots were frozen at −70°C. A portion of liver from all rats was frozen at −20°C and reserved for the determination of liver nonheme iron by the colormetric method of Torrance and Bothwell (1980).

Within 24 h, thawed plasma samples (100 μL) obtained at each time point were separated into free iodide, conjugated iodothyronines (glucuronides and sulfocoujugated) and nonconjugated iodothyronines (T4, T3, reverse T3 and di-iodothyronine (T2)) by Sephadex LH-20 chromatography using sequential elution with 100 mmol/L HCl, ethanol/water (1:5) and 100 mmol/L methanol/NH3 (99:1), respectively (Sorimachi 1979). The nonconjugated iodothyronine fractions were separated into T3 and T4 fractions by published methods (Bianco and Silva 1987). Fractions corresponding to T3 and T4 peaks were collected and their radioactivity determined by gamma counting to a 1σ error of 1%.

**Liver reverse T3 deiodinase (rT3-deiodinase) assay.** Liver rT3-deiodinase (EC 3.8.1.4) was assayed using a modified version of previously described methods (Smith and Lukaski 1992, Smith et al. 1992) in which 5′-deiodinase activity was measured by estimating the release of 125I from 127I-labeled reverse triiodothyronine (3, 5′, 3′-triiodothyronine, rT3, Du Pont NEN). All samples were assayed in triplicate at each of the six substrate concentrations used, including a blank (buffer instead of microsomal protein) to account for nonenzymatic production of 125I. Sample counts were corrected by subtracting the blank counts, and iodide production (μmol I/(mg microsomal protein × h)) was determined. The inverse of iodide production ([I]−1) was regressed against the inverse of T3 concentration ([T3]−1) to yield Michaelis-Menten constants (Km and Vmax). The intersassay CV for Vmax was 6.2%; for Km, it was 9.4%. The R² values for the regression equation for the Lineweaver-Burke plots were >0.95.

**IBAT reverse T3 deiodinase (rT3-deiodinase) assay.** The IBAT rT3-deiodinase assay procedure was done as described previously (Smith et al. 1992, Smith and Lukaski 1992). Enzyme activity [μmol I/(mg microsomal protein × h)] was determined in previous studies in our laboratory to be maximal at the substrate concentration used. Activity of IBAT rT3-deiodinase was estimated using the mean of the eight assay replicates. The intra-assay CV was <5%, and the interassay CV was 10.5%.

**Thyroid kinetic parameter determination.** The fractional catabolic rates (FCR) of T4 and T3 and the disposal rates (DR) of each thyroid hormone were the key dependent variables calculated in CN and ID rats housed at 15 and 30°C and in ID-T4-replaced rats living at 15°C. To analyze plasma tracer disappearance curves and determine kinetic parameters, individual animal data for plasma tracer concentration vs. time were expressed relative to estimated plasma tracer concentration at time 0. The plasma volume (Vs) was estimated as Vs = [g body weight × (0.0066) × (1 − Hct)] with Hct expressed as fractional packed cell volume (Tobin and Beard 1990). The plasma pool (M) of T1 and T2 was calculated as a mathematical product of the estimated plasma volume and the plasma T3 or T4 concentration. The T1 and T3 kinetic data were analyzed by a weighted nonlinear regression equation using the Simulation, Analysis, and Modeling computer program (SAAM31, Berman and Weiss 1978) and its conversational version, CONSAM (Berman et al. 1983). It was determined that the data best fit a three-component exponential equation of the form FD = Σ[Ii exp (−gti)] where FD is the fraction of tracer dose in the plasma at time t, Ii are the exponential constants and the g are the exponential coefficients. The data for each rat were individually modeled. A fractional standard deviation of 0.05 was the weighting factor for all data. After estimating the parameters (slope and intercept) for each of the three exponential components, the normalized exponential constants (H) were calculated as H = 1/2 Ii. The fractional catabolic rate (FCR) was calculated as the inverse of the area under the normalized plasma tracer response curve (A) i, where A = Σ H[i](t) (Shipley and Clark 1972). Thyroid hormone disposal rate (DR) was calculated as the product of the hormone FCR times the plasma pool of T3 or T1 (M.). Other kinetic parameters calculated (Shipley and Clark 1972) were: the plasma thyroid hormone fractional transfer coefficient (the fraction of plasma T1 or T3 mass leaving the plasma per hour), the plasma mean transit time (the time an average T3 or T4 molecule spends in the plasma during a single transit), the plasma mean residence time (the total time an average T3 or T4 molecule spends in the plasma before irreversible loss), the plasma recycling number (the total number of times an average T3 or T4 molecule recycles through the plasma before irreversible loss), the plasma mean sojourn time (the total time an average T3 or T4 molecule spends in the body after introduction into the plasma but before irreversible loss) and the plasma recycling time (the time it takes for an average T3 or T4 molecule leaving the plasma to cycle back to the plasma).

**Statistics.** Descriptive and analytical data are presented as means ± SEM. Data were analyzed by one-way ANOVA across diet and environmental temperature groups. An F value was significant if P < 0.05. Pairwise post-hoc comparisons were performed by Tukey’s test for multiple pairwise comparisons to detect significant (P < 0.05) differences among the means. End-point variables that were analyzed included blood Hb, plasma T3 and T4 concentrations, resting metabolic rate, liver nonheme iron concentration, IBAT and liver rT3-deiodinase activities, and thyroid hormone kinetic parameters. Data were examined for normality of distribution. Outliers were determined by boxplots and removed if necessary. For the kinetic parameters, the group means ± SEM were calculated from parameter estimates for individual animals and ANOVA performed.

**RESULTS**

Rats fed the iron-deficient diet had a lower body weight, liver nonheme Fe concentration and blood Hb concentration
Liver reverse triiodothyronine (rT₃) deiodinase activity in iron-deficient and control rats housed at 30 and 15°C and in iron-deficient rats receiving exogenous thyroxine (T₄) while living at 15°C.

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver weight mg/g liver</th>
<th>Microsomal protein (mmol/L)</th>
<th>V₉₀₀ pmol l⁻¹/(mg protein·20 min)</th>
<th>V₉₀₀ pmol l⁻¹/(kg body wt 0.75·20 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID-30°C</td>
<td>4.65 ± 0.17d</td>
<td>73.5 ± 6.1b</td>
<td>2.75 ± 0.31</td>
<td>309 ± 36c</td>
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<td>CN-30°C</td>
<td>7.81 ± 0.28c</td>
<td>90.1 ± 4.4a</td>
<td>3.16 ± 0.44</td>
<td>792 ± 75b</td>
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<td>ID-15°C</td>
<td>11.94 ± 0.25a</td>
<td>94.0 ± 3.8a</td>
<td>2.71 ± 0.26</td>
<td>782 ± 91b</td>
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<tr>
<td>CN-15°C</td>
<td>7.89 ± 0.28c</td>
<td>97.1 ± 1.2a</td>
<td>3.60 ± 0.39</td>
<td>1533 ± 280a</td>
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</tbody>
</table>

1 Values are means ± SEM. See Table 1 for group designations. Within a column, means with differing superscripts are significantly different at P < 0.05 by Tukey's test.

Liver reverse triiodothyronine (rT₃) deiodinase activity in iron-deficient and control rats housed at 30 and 15°C and in iron-deficient rats receiving exogenous thyroxine (T₄) while living at 15°C.

<table>
<thead>
<tr>
<th>Group</th>
<th>IBAT weight mg/g IBAT</th>
<th>Microsomal protein (nmol I⁻¹/(mg protein·h))</th>
<th>V₉₀₀ pmol l⁻¹/(kg body wt 0.75·h⁻¹)</th>
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<tr>
<td>ID-30°C</td>
<td>0.21 ± 0.02d</td>
<td>4.82 ± 0.48b</td>
<td>0.14 ± 0.02b</td>
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<td>CN-30°C</td>
<td>0.42 ± 0.03c</td>
<td>3.51 ± 0.18c</td>
<td>0.25 ± 0.04b</td>
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<tr>
<td>ID-15°C</td>
<td>0.51 ± 0.02c</td>
<td>7.50 ± 0.35a</td>
<td>1.84 ± 0.46ab</td>
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<tr>
<td>CN-15°C</td>
<td>0.86 ± 0.04a</td>
<td>7.61 ± 0.30a</td>
<td>2.31 ± 0.62a</td>
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<tr>
<td>ID-T₄-15°C</td>
<td>0.66 ± 0.03b</td>
<td>7.42 ± 0.37a</td>
<td>1.29 ± 0.67ab</td>
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</table>

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rats. Iron deficiency was associated with an 82% higher T4 FCRp at 30°C, but was without effect on T3 FCRp at 15°C. The disposal rate for T3 was thus unaffected in ID rats at 30°C compared with CN rats, and was 28% lower than controls at 15°C. Although growing in a cool environment was associated with a 170% increase in T4 FCRp in CN rats, the FCRp of ID rats increased by only 12% compared with their FCRp at thermoneutrality. There was a sevenfold greater T3 DR in ID rats and an eightfold greater T3 DR in CN rats living at the cooler temperature compared with those living at the warmer temperature. This indicates little effect of iron deficiency on limiting this aspect of adaptation. Providing an exogenous source of T4 to the ID rats at 15°C (ID-T4-15°C) had a significant effect on T3 DR. Normalization of these kinetic measures to metabolic body size, however, eliminated this effect.

Iron deficiency significantly affected kinetic parameters at both thermoneutrality and at the cooler environmental temperature (Table 6). At thermoneutrality, iron deficiency was associated with a lower T4 fractional transfer coefficient, a 500% higher plasma T4 mean transit time, and a 63% higher T4 mean residence time. When the rats lived at 15°C, none of these parameters were significantly different from controls. Provision of exogenous T4 at this temperature, however, was associated with a lower recycling number and higher plasma recycling time. Comparing ID rats in the cool (15°C) with ID rats in the warm (30°C) environment, the T4 fractional transfer coefficient was sixfold higher at the cooler temperature, T4 plasma recycling number was fourfold higher and T4 plasma residence time was cut nearly in half. The mean T4 transit time was reduced to 12% of what it was at 30°C, and the plasma recycling time was 24% of what it was at the warmer temperature (Table 6). In CN rats, the same kinds of comparisons revealed no significant kinetic adjustments to living at the cooler temperature. Provision of exogenous T4 had little effect on these kinetic parameters.

Iron deficiency had no significant effect on T4 kinetic parameters at 30°C except for the fractional transfer coefficient, mean transit time and mean plasma recycling time (Table 7). These differences between ID and CN kinetic parameters were not apparent at the cooler environmental temperature. Control rats had a significantly higher T4 transfer coefficient, a shorter mean transit time and residence time and a shorter mean sojourn time at 15°C compared with CN rats at 30°C. These kinetic adaptations to a cooler environment did not occur in iron-deficient rats. Provision of exogenous T4 had no effect on T4 kinetic parameters in iron deficiency.

Our thyroid kinetics results allow the calculation of the estimated fraction of T4 converted to T3 via deiodination. In iron deficiency significantly affected kinetic parameters at both thermoneutrality and at the cooler environmental temperature (Table 6). At thermoneutrality, iron deficiency was associated with a lower T4 fractional transfer coefficient, a 500% higher plasma T4 mean transit time, and a 63% higher T4 mean residence time. When the rats lived at 15°C, none of these parameters were significantly different from controls. Provision of exogenous T4 at this temperature, however, was associated with a lower recycling number and higher plasma recycling time. Comparing ID rats in the cool (15°C) with ID rats in the warm (30°C) environment, the T4 fractional transfer coefficient was sixfold higher at the cooler temperature, T4 plasma recycling number was fourfold higher and T4 plasma residence time was cut nearly in half. The mean T4 transit time was reduced to 12% of what it was at 30°C, and the plasma recycling time was 24% of what it was at the warmer temperature (Table 6). In CN rats, the same kinds of comparisons revealed no significant kinetic adjustments to living at the cooler temperature. Provision of exogenous T4 had little effect on these kinetic parameters.

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Our thyroid kinetics results allow the calculation of the estimated fraction of T4 converted to T3 via deiodination. In this study, the estimated T4 to T3 conversion ratios were ~12 and ~5% for ID and CN rats housed at the warmer (30°C) temperature, and ~33 and 31%, respectively, for rats housed at the cooler (15°C) temperature. T4-treated ID rats had a similar conversion ratio at the cooler temperature, i.e., 31%.

To summarize the results, iron deficiency in an environment with minimal demands on the rat to produce heat was associated with normal plasma thyroid hormone concentrations, metabolic rate, deiodinase activity in liver and IBAT, but lower T4 and T3 disposal rates compared with controls. T4 also spent more time in the plasma pool in the ID animals (increased mean residence time and transit time) and was recycled fewer times through the plasma pool. In a cool environment, plasma T3 concentration and pool size were lower in iron deficiency, metabolic rate was higher and T4 disposal rate was still lower than controls. Iron deficiency also was associated

### Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>T4 POOL</th>
<th>T4 POOL</th>
<th>T4 FCRp</th>
<th>T4 DR</th>
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<td></td>
<td></td>
<td>pmol</td>
<td>pmol/kg^{0.75}</td>
<td>h^{-1}</td>
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<td>ID-30°C</td>
<td>6</td>
<td>555 ± 51c</td>
<td>2.11 ± 0.14</td>
<td>0.170 ± 0.025c</td>
<td>89 ± 6c</td>
<td>345 ± 28c</td>
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<td>CN-30°C</td>
<td>6</td>
<td>946 ± 212b</td>
<td>2.24 ± 0.47</td>
<td>0.294 ± 0.053ab</td>
<td>256 ± 59b</td>
<td>607 ± 100b</td>
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<tr>
<td>ID-15°C</td>
<td>6</td>
<td>666 ± 51c</td>
<td>2.11 ± 0.21</td>
<td>0.283 ± 0.019b</td>
<td>192 ± 27b</td>
<td>619 ± 103b</td>
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<td>CN-15°C</td>
<td>10</td>
<td>1208 ± 31a</td>
<td>2.45 ± 0.17</td>
<td>0.311 ± 0.011b</td>
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<td>ID-T4-15°C</td>
<td>8</td>
<td>1053 ± 36a</td>
<td>2.91 ± 0.18</td>
<td>0.360 ± 0.033a</td>
<td>381 ± 42a</td>
<td>1078 ± 167a</td>
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### Table 5

<table>
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<th>Group</th>
<th>n</th>
<th>T3 POOL</th>
<th>T3 POOL</th>
<th>T3 FCRp</th>
<th>T3 DR</th>
<th>T3 DR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pmol</td>
<td>pmol/kg^{0.75}</td>
<td>h^{-1}</td>
<td>pmol/h</td>
<td>pmol/(h·kg^{0.75})</td>
</tr>
<tr>
<td>ID-30°C</td>
<td>6</td>
<td>4.2 ± 0.9b</td>
<td>16.0 ± 4.4c</td>
<td>2.96 ± 0.68b</td>
<td>11 ± 2c</td>
<td>41 ± 8b</td>
</tr>
<tr>
<td>CN-30°C</td>
<td>6</td>
<td>8.1 ± 1.3b</td>
<td>19.9 ± 4.7c</td>
<td>1.68 ± 0.18c</td>
<td>13 ± 3c</td>
<td>31 ± 5b</td>
</tr>
<tr>
<td>ID-15°C</td>
<td>6</td>
<td>28.6 ± 2.9a</td>
<td>81.1 ± 8.0a</td>
<td>3.33 ± 0.41ab</td>
<td>92 ± 11b</td>
<td>262 ± 28a</td>
</tr>
<tr>
<td>CN-15°C</td>
<td>10</td>
<td>28.7 ± 2.6a</td>
<td>60.7 ± 4.1b</td>
<td>4.57 ± 0.33a</td>
<td>129 ± 11a</td>
<td>276 ± 27a</td>
</tr>
<tr>
<td>ID-T4-15°C</td>
<td>8</td>
<td>29.0 ± 2.7a</td>
<td>76.6 ± 7.0a</td>
<td>4.27 ± 0.16a</td>
<td>126 ± 12a</td>
<td>333 ± 30a</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. See Table 1 for group designations. Within a column, means with differing superscripts are significantly different at P < 0.05 by Tukey's test.
with T₄ spending a longer time in the extravascular pools before returning to the plasma or being irreversibly lost. T₄ replacement with a dose sufficient to normalize plasma T₄ concentration at 15°C had normalized plasma kinetics and metabolic rate.

**DISCUSSION**

Our research hypothesis was that iron deficiency leads to lower T₄ and T₃ production and utilization rates, and that this effect would be more pronounced at lower environmental temperatures where the demand for thyroid hormone production and utilization would be much greater. A significantly lower thyroid hormone utilization rate would then suggest limiting thyroid hormone metabolism as the primary cause of impaired thermoregulation in ID rats. We did find that environmental temperature interacts with iron status in affecting thyroid hormone kinetics. In a warm environment (30°C), iron deficiency was associated with lower T₄ and T₃ disposal and utilization. In a cool environment (15°C), both CN and ID rats had higher rates of T₄ disposal, higher rates of conversion of T₄ to T₃ via the 5'-deiodinase enzymes in liver and IBAT, higher rates of T₃ utilization and, of course, higher resting metabolic rate. These latter observations confirm previous studies from our laboratory (Beard et al. 1982, 1984 and 1989, Tobin and Beard 1990).

Iron-deficient rats had attenuated responses compared with control rats at a lower environmental temperature with respect to T₃ and T₄ kinetic parameters. T₄ and T₃ DR remained lower in ID rats than in CN rats (by 48 and 28%, respectively) in the cool (15°C) environment. These results show that, although ID rats were able to increase thyroid hormone production and utilization when challenged with a cool environment, iron deficiency limited their ability to fully upregulate thyroid hormone metabolism to the degree observed in iron-replete (CN) rats.

Other studies have examined thyroid metabolism in iron deficiency, but only one other study directly examined thyroid kinetics in iron deficiency (Beard et al. 1989). In that study, we reported lower plasma T₃ concentrations in iron deficiency (ID, 0.49 ± 0.11 pmol/L; CN, 0.66 ± 0.14 pmol/L), a smaller T₃ plasma pool in iron deficiency (ID, 6.4 ± 1.5 pmol; CN, 9.4 ± 1.8 pmol) and a T₃ DR at an ambient temperature of 24°C. In this study, we found that iron deficiency lowers T₃ DR by 28% at 15°C, compared with CN rats, and was without effect at a thermoneutral temperature.

DiStefano and co-workers (1982a) estimated plasma T₄ FCR₄, as 0.221 ± 0.023/h and T₃ DR as 150 ± 18 pmol/h, results that are similar to those we found (T₄ FCR₄ ranged from 0.173 to 0.360/h and T₃ DR ranged from 89 to 381 pmol/h). Although DiStefano et al. did not report the environmental temperature at which their rats were housed, it is reasonable to assume that they were maintained in an environment that was in the 20–25°C temperature range. Their reported values for plasma T₃ FCR₃ (3.44 ± 5.55/h) and DR (36.4 ± 8.9 pmol/h) were not very different from those in the current

**TABLE 6**

<table>
<thead>
<tr>
<th>Group</th>
<th>L(1,1)</th>
<th>t_p</th>
<th>T₃p</th>
<th>V_p</th>
<th>MST</th>
<th>ttp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h⁻¹</td>
<td>h</td>
<td>cycles</td>
<td>h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ID-30°C</td>
<td>1.70 ± 0.81b</td>
<td>0.711 ± 0.109a</td>
<td>6.56 ± 0.85a</td>
<td>9.46 ± 1.66b</td>
<td>19.7 ± 1.8</td>
<td>1.73 ± 0.27a</td>
</tr>
<tr>
<td>CN-30°C</td>
<td>9.73 ± 5.32a</td>
<td>0.143 ± 0.062b</td>
<td>4.02 ± 0.68b</td>
<td>32.2 ± 4.71a</td>
<td>23.0 ± 3.4</td>
<td>0.63 ± 0.08b</td>
</tr>
<tr>
<td>ID-15°C</td>
<td>12.20 ± 1.91a</td>
<td>0.086 ± 0.099b</td>
<td>3.60 ± 0.19b</td>
<td>42.1 ± 3.18b</td>
<td>17.1 ± 1.3</td>
<td>0.33 ± 0.09b</td>
</tr>
<tr>
<td>CN-15°C</td>
<td>13.12 ± 2.92a</td>
<td>0.095 ± 0.011b</td>
<td>3.26 ± 0.11b</td>
<td>40.3 ± 8.1a</td>
<td>17.1 ± 1.0</td>
<td>0.42 ± 0.06b</td>
</tr>
<tr>
<td>ID-T₄-15°C</td>
<td>12.70 ± 1.95a</td>
<td>0.095 ± 0.015b</td>
<td>2.93 ± 0.23b</td>
<td>33.4 ± 3.2a</td>
<td>16.8 ± 1.3</td>
<td>0.46 ± 0.08b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. See Table 1 for group designations. Means with differing superscripts within a column are considered significantly different at P < 0.05 by Tukey's test.
2 The parameter abbreviations are as follows: L(1,1), fractional transfer coefficient; t_p, plasma mean transit time; T₃p, plasma mean residence time; V_p, plasma recycling number; MST, mean sojourn time; ttp, plasma recycling time.

**TABLE 7**

<table>
<thead>
<tr>
<th>Group</th>
<th>L(1,1)</th>
<th>t_p</th>
<th>T₃p</th>
<th>V_p</th>
<th>MST</th>
<th>ttp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h⁻¹</td>
<td>h</td>
<td>cycles</td>
<td>h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ID-30°C</td>
<td>29.9 ± 3.9a</td>
<td>0.058 ± 0.021b</td>
<td>0.532 ± 0.156a</td>
<td>9.80 ± 1.23</td>
<td>11.5 ± 2.14b</td>
<td>1.19 ± 0.16b</td>
</tr>
<tr>
<td>CN-30°C</td>
<td>13.6 ± 3.3b</td>
<td>0.155 ± 0.033a</td>
<td>0.675 ± 0.95a</td>
<td>6.16 ± 1.55</td>
<td>13.0 ± 1.7a</td>
<td>2.82 ± 0.91a</td>
</tr>
<tr>
<td>ID-15°C</td>
<td>25.1 ± 3.9a</td>
<td>0.062 ± 0.020b</td>
<td>0.367 ± 0.092b</td>
<td>6.31 ± 0.91</td>
<td>8.4 ± 0.4a</td>
<td>1.43 ± 0.21a</td>
</tr>
<tr>
<td>CN-15°C</td>
<td>28.3 ± 2.7a</td>
<td>0.038 ± 0.004c</td>
<td>0.229 ± 0.026b</td>
<td>5.16 ± 0.28</td>
<td>8.0 ± 0.5b</td>
<td>1.52 ± 0.09a</td>
</tr>
<tr>
<td>ID-T₄-15°C</td>
<td>31.1 ± 1.9a</td>
<td>0.033 ± 0.001c</td>
<td>0.237 ± 0.013b</td>
<td>6.31 ± 0.28</td>
<td>7.4 ± 1.1b</td>
<td>1.13 ± 0.12b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 6–10 rats per group. See Table 1 for group designations. Means with differing superscripts within a column are considered significantly different at P < 0.05 by Tukey's test.
2 The parameter abbreviations are as follows: L(1,1), fractional transfer coefficient; t_p, plasma mean transit time; T₃p, plasma mean residence time; V_p, plasma recycling number; MST, mean sojourn time; ttp, plasma recycling time.
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study. The DR data in the current study bracket those reported by DiStefano et al. (1982b). More recently, this research group used a novel whole-body kinetic approach to further their understanding of T4 to T3 conversion, tissue concentrations and plasma concentrations (Yamada et al. 1996). Plasma appearance rates for T3 were consistent with previously published studies that used methods similar to those employed in this project.

The observed physiologic and kinetic responses are consistent with those observed previously when rats were placed in colder environments (Ingram and Kaciuba-Uaxilkó 1977). In this study, both the ID and CN rats made adaptive changes in thyroid hormone metabolism (i.e., increased production and utilization of T3 and T4), but the ID rats did not fully attain the increased level of thyroid hormone metabolism observed in CN rats and thus may not be capable of sufficient thermogenesis to thrive in this marginal environment (Fregly 1989).

In other studies, exposure to cool environmental temperatures has been associated with dramatic increases in the 5'-deiodinase-II activity but not consistently with hepatic 5'-deiodinase (Hesch and Koehrle 1986, Pazos-Moura et al. 1991, Silva and Larsen 1985). Plasma kinetic studies in pigs exposed to 4°C versus 22°C showed dramatic increases in serum T4 concentration, T3 concentration, hepatic 5'-deiodinase Vmax and serum T4, DR but no changes in metabolic clearance rate or hepatic 5'-deiodinase Km (Reed et al. 1994). Pertinent to the current study is the argument that sufficient calories must be provided to animals during these cold exposures to accommodate the increase in metabolic rate. As in the pig study, our own study cannot separate the effects of increased food intake on thyroid hormone kinetics from the effect of cooler ambient temperature (Suda et al. 1978).

In this study, our estimates of T4 to T3 conversion ranged from 5 to 30%, with higher values in the cool environment (15°C) than in the warm environment (30°C). The apparent higher fractional conversion of T4 to T3 that we observed in the rats in the cooler environment compared with the warm environment is consistent with the higher amount of liver and IBAT deiodination. DiStefano et al. (1982a and 1982b) estimated 14–24% conversion efficiency from plasma kinetic data. Silva and co-workers reported 26% in hypothyroid rats and 22% in euthyroid rats (Silva et al. 1984). More recently, whole-body kinetic studies now place this conversion at ~45% in hypothyroid rats and 21% in euthyroid animals (Yamada et al. 1996). DiStefano and colleagues showed that only about 35% of the total T3 production is generated from the fast turning over extravascular pool and postulated that this included the liver and kidney conversion of T4 to T3 (DiStefano et al. 1982b). The majority of the T3 production comes from the slowly turning over pool, and constitutes primarily muscle and other tissue that is resistant to the effects of known inhibitors of 5'-deiodinase-like propylthiouracil and selenium deficiency (Veronikis et al. 1996). The minimal effects of iron deficiency on either the hepatic 5'-deiodinase or the brown fat 5'-deiodinase II observed in this study demonstrate that a more detailed examination of specific tissue pools and kinetics of thyroid movement into and out of those pools is necessary to explain the mechanism of the effect of iron deficiency on T3 and T4 disposal rates.

Normalizing plasma T4 concentrations in ID rats at 15°C resulted in several notable effects. Although iron indices, resting metabolic rate and IBAT rT3 deiodination were unaffected by T4 replacement, liver rT3 deiodination, T4 FCR and T4 DR were higher than those for ID rats not treated with T4 and equal to or greater than those for CN rats. This apparent normalization of plasma T4 kinetic parameters in ID rats provided with exogenous T4 suggests that low plasma T4 concentrations contribute to the altered thyroid hormone kinetics associated with iron deficiency. Presumably, a smaller proportion of T4 was converted to T3 and a larger proportion was converted to rT3, a physiologically inactive thyroxine metabolite, in ID rats. In a previous study in which ID rats were supplied with exogenous T4, we found that resting metabolic rate and plasma T3 concentration were unchanged unless the dose of T4 given was excessive (Brigham and Beard 1995). A recent paper notes that provision of T4 alone is insufficient for maintaining tissue T3 and T1 levels; provision of at least 0.15 µg T4/100 g body weight per day in addition to T4 is necessary for normalizing of almost all parameters of thyroid metabolism (Escobar-Morreale et al. 1996). Tissue thyroid hormone regulation and plasma hormone levels are at times independently regulated events (Yamada et al. 1996).

The question remains whether the alterations we observed in thyroid hormone kinetics in ID rats in this study are related to the inability of ID rats and humans to maintain a normal body temperature in a cold environment. In this study, both ID and CN rats remained euthermic throughout the study, and the temperature exposure was chronic rather than acute as in an earlier study (see Brigham and Beard 1995). Because thyroid hormone plays such a key role in heat production in mammals in cold environments (Guernsey and Edelman 1983), we believe that iron deficiency limits the adaptability of mammals living at a cool temperature. It may be the case that, if the environmental temperature would be further lowered toward 10°C, these ID rats could not increase their T3 to T4 conversion ratio beyond its current level, and the availability of T3 for heat production would become inadequate.

It was beyond the objectives of this study to discern the mechanism by which T4 production is lowered in iron deficiency, but several previous studies provide grounds for speculation. When exposed acutely to the cold (<10°C), ID rats do not have increased plasma T4 and thyroid-stimulating hormone (TSH) concentrations (Beard et al. 1982, Tang et al. 1988). They also have a blunted and delayed TSH response to injected thyrotropin-releasing hormone (TRH) compared with CN rats (Bear et al. 1983). Direct measurements of TRH in iron deficiency have not been made, but suppression of TRH could occur via the elevation in dopamine in dopaminergic tracts in ventral midbrain (Nelson et al. 1997, Lee et al. 1985). Ultimately, it may be that iron deficiency-induced alterations in central nervous system control of thermoregulatory responses are responsible for the lower thyroid hormone response and the overall failure to properly thermoregulate that characterize iron-deficient rats.

LITERATURE CITED


