

Plasma Thyroid Hormone Kinetics Are Altered in Iron-Deficient Rats¹

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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 (DeMaeyer 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)³ 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'-deiodinase, the enzyme that converts plasma-derived thyroxine (T₄) to T₃ (Raasmaja and Larsen 1989). Increased 5'-deiodinase synthesis and the subsequent increased activity of the enzyme result in an increase in BAT intracellu-

lar 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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³ Abbreviations used: BAT, brown adipose tissue; DR, disposal rate; FCR_p, fractional catabolic rate; Hb, hemoglobin; Hct, hematocrit; IBAT, intrascapular brown adipose tissue; ID, iron deficient; rT₃-deiodinase, reverse T₃ deiodinase; T₂, 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 (Lukaski 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 follows: 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_4 in the form of constant-release pellets sufficient to normalize plasma T_4 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 $30 \pm 1^\circ\text{C}$ or $15 \pm 1^\circ\text{C}$ (mean $\pm$ 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). Cornstarch 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 anes-

thetized (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 an 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 [$\sim 7.5 \mu\text{g}/(\text{kg} \cdot \text{d})$], 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 ($3',5'-^{125}\text{I}-\text{T}_4$, specific activity $\sim 210.9 \text{ GBq}/\mu\text{g}$ and $3',5'-^{125}\text{I}-\text{T}_3$, specific activity $\sim 122.1 \text{ GBq}/\mu\text{g}$; DuPont 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 $^{125}\text{I}-\text{T}_3$ in the injection solution, an aliquot of prepared $^{125}\text{I}-\text{T}_3$ 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_2O 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 μL . $^{125}\text{I}-\text{T}_4$ (1480 MBq) or $^{125}\text{I}-\text{T}_3$ (1480 MBq) was injected into the carotid artery catheters of recipient rats. The catheters were then flushed with sterile physiological saline (500 μL). 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 μL) of the injection solution were reserved for later gamma counting so 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_4 plasma pool size and 40% of the T_3 plasma pool size.

Blood samples (250 μL) 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 $3000 \times g$ 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_4 , the assay underestimated the actual T_4 concentration by 50%. The intra-assay CV was 5.8% for T_3 and 4.8% for T_4 ; the interassay CV was 6.1% for T_3 and 4.8% for T_4 .

At the end of the sampling period, anesthetized rats (50 mg/kg

TABLE 1

Body weight, blood hemoglobin (Hb), plasma thyroid hormone concentrations, resting metabolic rate (RMR), and liver nonheme iron (Fe) concentrations in iron-deficient and control rats housed at 30 and 15°C and in iron-deficient rats receiving exogenous T_4 while living at 15°C¹

Group	Body weight	Hb	T_4 ²	T_3 ²	RMR	Liver Fe
	g	g/L	nmol/L	nmol/L	kJ/(kg ^{0.75} · h)	μmol/g
ID-30°C	168 ± 5 ^d	38 ± 2 ^c	62.5 ± 1.7 ^b	0.58 ± 0.07 ^b	15.2 ± 0.6 ^c	0.56 ± 0.08 ^b
CN-30°C	295 ± 9 ^b	150 ± 3 ^a	66.4 ± 5.9 ^b	0.73 ± 0.06 ^b	14.4 ± 0.4 ^c	2.18 ± 0.01 ^a
ID-15°C	234 ± 8 ^c	48 ± 3 ^c	60.5 ± 3.8 ^b	1.92 ± 0.10 ^a	22.2 ± 0.5 ^a	0.45 ± 0.02 ^b
CN-15°C	376 ± 7 ^a	158 ± 3 ^a	90.3 ± 3.9 ^a	2.15 ± 0.17 ^a	19.2 ± 0.6 ^b	2.47 ± 0.10 ^a
ID- T_4 -15°C	268 ± 7 ^c	61 ± 2 ^b	86.2 ± 4.9 ^a	2.43 ± 0.98 ^a	22.3 ± 0.5 ^a	0.45 ± 0.02 ^b

¹ Values are means ± SEM, $n = 12-18$. Groups are iron deficient (ID), control (CN) and thyroxine-treated iron-deficient (ID- T_4) rats living at 30 or 15°C for 7 wk. Within a column, means with differing superscripts are significantly different at $P < 0.05$ by Tukey's test.

² T_4 and T_3 are plasma thyroxine and triiodothyronine.

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 $100,000 \times g$ 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 colorimetric 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 (glucuronide and sulfoconjugated) and nonconjugated iodothyronines [T_4 , T_3 , reverse T_3 and di-iodothyronine (T_2)] by Sephadex LH-20 chromatography using sequential elution with 100 mmol/L HCl, ethanol/water (1:5) and 100 mmol/L methanol/ NH_3 (99:1), respectively (Sorimachi 1979). The nonconjugated iodothyronine fractions were separated into T_3 and T_4 fractions by published methods (Bianco and Silva 1987). Fractions corresponding to T_3 and T_4 peaks were collected and their radioactivity determined by gamma counting to a 1σ error of 1%.

Liver reverse T_3 deiodinase (r T_3 -deiodinase) assay. Liver r T_3 -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 estimated by measuring the release of ^{125}I from ^{125}I -labeled reverse triiodothyronine (3, 5', 3'-triiodothyronine, r T_3 , 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 ^{125}I . Sample counts were corrected by subtracting the blank counts, and iodide production [pmol I/(mg microsomal protein · 20 min)] at each of the six substrate concentrations was calculated. The inverse of iodide production (1/V) was regressed against the inverse of T_4 concentration (1/S) to yield Michaelis-Menten constants (K_m and V_{max}). The interassay CV for V_{max} was 6.2%; for K_m , it was 9.4%. The R^2 values for the regression equation for the Lineweaver-Burke plots were >0.95 .

IBAT reverse T_3 deiodinase (r T_3 -deiodinase) assay. The IBAT r T_3 -deiodinase assay procedure was done as described previously (Smith et al. 1992, Smith and Lukaski 1992). Enzyme activity [fmol I/(mg microsomal protein · h)] was determined in previous studies in our laboratory to be maximal at the substrate concentration used. Activity of IBAT r T_3 -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_p) of T_4 and T_3 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- T_4 -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 concen-

tration at time 0. The plasma volume (V_p) was estimated as $V_p = [g \text{ body weight} \times (0.066)] \times (1 - \text{Hct})$, with Hct expressed as fractional packed cell volume (Tobin and Beard 1990). The plasma pool (M_p) of T_3 and T_4 was calculated as a mathematical product of the estimated plasma volume and the plasma T_3 or T_4 concentration. The T_3 and T_4 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 $FD_t = \sum [I_i \exp(-g_i t)]$ where FD_t is the fraction of tracer dose in the plasma at time t , I_i are the exponential constants and the g_i 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_i) were calculated as $H_i = I_i / \sum I_i$. The fractional catabolic rate (FCR_p) was calculated as the inverse of the area under the normalized plasma tracer response curve (A), where $A = \sum H_i / g_i$ (Shipley and Clark 1972). Thyroid hormone disposal rate (DR) was calculated as the product of the hormone FCR_p times the plasma pool of T_4 or T_3 (M_p). Other kinetic parameters calculated (Shipley and Clark 1972) were: the plasma thyroid hormone fractional transfer coefficient (the fraction of plasma T_4 or T_3 mass leaving the plasma per hour), the plasma mean transit time (the time an average T_4 or T_3 molecule spends in the plasma during a single transit), the plasma mean residence time (the total time an average T_4 or T_3 molecule spends in the plasma before irreversible loss), the plasma recycling number (the total number of times an average T_4 or T_3 molecule recycles through the plasma before irreversible loss), the plasma mean sojourn time (the total time an average T_4 or T_3 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 T_4 or T_3 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 T_3 and T_4 concentrations, resting metabolic rate, liver nonheme iron concentration, IBAT and liver r T_3 -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

TABLE 2

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°C¹

Group	n	Liver weight	Microsomal protein	K _m rT ₃	V _{max}	V _{max}
		g	mg/g liver	mmol/L	pmol I ⁻ /(mg protein · 20 min)	nmol I ⁻ /(kg body wt ^{0.75} · 20 min)
ID-30°C	11	4.65 ± 0.17 ^d	73.5 ± 6.1 ^b	2.75 ± 0.31	309 ± 35 ^c	2.91 ± 0.36 ^{cd}
CN-30°C	13	9.10 ± 0.39 ^b	71.1 ± 5.7 ^b	2.77 ± 0.28	331 ± 50 ^c	1.87 ± 0.37 ^d
ID-15°C	10	6.82 ± 0.28 ^c	90.1 ± 4.4 ^a	3.16 ± 0.44	792 ± 75 ^b	7.46 ± 1.07 ^b
CN-15°C	15	11.94 ± 0.25 ^a	94.0 ± 3.8 ^a	2.71 ± 0.26	782 ± 91 ^b	5.01 ± 0.58 ^{bc}
ID-T ₄ -15°C	13	7.89 ± 0.28 ^c	97.1 ± 1.2 ^a	3.60 ± 0.39	1553 ± 280 ^a	13.71 ± 2.44 ^a

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.

than controls at both environmental temperatures (30 and 15°C, Table 1). At 30°C, there was no effect of iron deficiency on plasma T₄ and T₃ concentrations. At 15°C, iron-deficient rats had lower plasma T₄ but not T₃ concentrations than controls. Providing an exogenous source of T₄ to the ID rats at 15°C (ID-T₄-15°C) counteracted the effects of iron deficiency on circulating T₄ levels, but had no significant effect on body weight and liver Fe concentration. Hb concentration was slightly elevated in the thyroid hormone-replaced rats. Resting metabolic rate was significantly higher in iron-deficient rats at 15°C compared with control rats. Providing an exogenous source of T₄ to the ID rats at 15°C (ID-T₄-15°C) had no effect on resting metabolic rate.

Liver weight was lower in iron-deficient rats (Table 2) but liver weight:body weight ratios did not differ among the groups (data not shown). The concentration of microsomal protein in the liver was higher in rats housed at 15°C than at 30°C, but did not differ significantly between ID and CN rats (Table 2). The estimated K_m for the liver rT₃-deiodinase did not differ among the groups. The estimated activity (V_{max}) for rT₃-deiodinase was more than doubled at 15°C compared with 30°C for both ID and CN rats. Providing an exogenous source of T₄ to the ID rats at 15°C (ID-T₄-15°C) was associated with a higher rT₃-deiodinase activity compared with that of the ID rats at 15°C that did not receive exogenous T₄ (ID-15°C).

IBAT weight was lower in ID rats than CN rats at all temperatures, and higher at 15°C than at 30°C (Table 3). IBAT weight:body weight ratios were higher at 15°C than at 30°C, but they did not differ significantly between ID and

CN rats (data not shown). The concentration of microsomal protein in the IBAT did not differ significantly between ID and CN rats (Table 3). When expressed on a per milligram microsomal protein basis, IBAT rT₃-deiodinase activity did not differ significantly among the groups.

A summary of the T₄ and T₃ pool sizes, plasma fractional catabolic rates (FCR_p) and disposal rates (DR) are shown in Tables 4 and 5. Iron deficiency was associated with a significantly lower plasma T₄ pool (41% at 30°C and 45% at 15°C). Iron-deficient rats also had a lower T₄ FCR_p (42%) at 30°C, but did not differ from controls at 15°C. Iron deficiency was therefore associated with a lower T₄ DR (64% lower at 30°C and 48% lower at 15°C). Comparing the cool (15°C) to the warm (30°C) environment, T₄ DR was higher by 110% in ID rats and by 45% in CN rats. Iron-deficient rats, supplemented with exogenous T₄ and living at 15°C (ID-T₄-15°C), had a larger plasma T₄ pool and a greater T₄ DR compared with ID rats at 15°C that did not receive exogenous T₄. Normalization of pool size and T₄ DR to metabolic body size was performed to account for the differences in body size of iron-deficient and control rats. In this context, iron deficiency had no effect on T₄ pool size, although the normalized T₄ DR in iron deficiency was still significantly lower than in controls at both environmental temperatures. Exogenous thyroid hormone led to a higher T₄ DR in the ID rats at this cooler temperature.

Plasma T₃ pool size was not lower in iron-deficient rats compared with CN rats (Table 5). Comparing the cool (15°C) to the warm (30°C) environment, T₃ plasma pool size was about sixfold higher in ID rats and threefold higher in CN

TABLE 3

Interscapular brown adipose tissue (IBAT) 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°C¹

Group ¹	n	IBAT weight	Microsomal protein	V _{max}	V _{max}
		g	mg/g IBAT	fmol I ⁻ /(mg protein · h)	pmol I ⁻ /(kg body wt ^{0.75} · h)
ID-30°C	11	0.21 ± 0.02 ^d	4.82 ± 0.48 ^b	164 ± 18	0.14 ± 0.02 ^b
CN-30°C	13	0.42 ± 0.03 ^c	3.51 ± 0.18 ^c	144 ± 11	0.25 ± 0.04 ^b
ID-15°C	10	0.51 ± 0.02 ^c	7.50 ± 0.35 ^a	289 ± 70	1.84 ± 0.46 ^{ab}
CN-15°C	15	0.86 ± 0.04 ^a	7.61 ± 0.30 ^a	184 ± 53	2.31 ± 0.62 ^a
ID-T ₄ -15°C	13	0.66 ± 0.03 ^b	7.42 ± 0.37 ^a	167 ± 84	1.29 ± 0.67 ^{ab}

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.

TABLE 4

Plasma thyroxine (T_4) pool (POOL), fractional catabolic rate (FCR_p), and disposal rate (DR) in iron-deficient and control rats housed at 30 and 15°C and in iron-deficient rats receiving exogenous T_4 while living at 15°C¹

Group	n	T_4 POOL	T_4 POOL	T_4 FCR_p	T_4 DR	T_4 DR
		pmol	nmol/kg ^{0.75}	h ⁻¹	pmol/h	pmol/(h · kg)
ID-30°C	6	555 ± 51 ^c	2.11 ± 0.14	0.170 ± 0.025 ^c	89 ± 6 ^c	345 ± 28 ^c
CN-30°C	6	946 ± 212 ^b	2.24 ± 0.47	0.294 ± 0.053 ^{ab}	256 ± 53 ^b	607 ± 100 ^b
ID-15°C	6	666 ± 51 ^c	2.11 ± 0.21	0.283 ± 0.019 ^b	192 ± 27 ^b	619 ± 103 ^b
CN-15°C	10	1208 ± 91 ^a	2.45 ± 0.17	0.311 ± 0.011 ^b	372 ± 25 ^a	763 ± 44 ^b
ID- T_4 -15°C	8	1053 ± 36 ^a	2.91 ± 0.18	0.360 ± 0.035 ^a	381 ± 42 ^a	1078 ± 167 ^a

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.

rats. Iron deficiency was associated with an 82% higher T_3 FCR_p at 30°C, but was without effect on T_3 FCR_p at 15°C. The disposal rate for T_3 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 T_3 FCR_p in CN rats, the FCR_p of ID rats increased by only 12% compared with their FCR_p at thermoneutrality. There was a sevenfold greater T_3 DR in ID rats and an eightfold greater T_3 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 T_4 to the ID rats at 15°C (ID- T_4 -15°C) had a significant effect on T_3 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 T_4 fractional transfer coefficient, a 500% higher plasma T_4 mean transit time, and a 63% higher T_4 mean residence time. When the rats lived at 15°C, none of these parameters were significantly different from controls. Provision of exogenous T_4 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 T_4 fractional transfer coefficient was sixfold higher at the cooler temperature, T_4 plasma recycling number was fourfold higher and T_4 plasma residence time was cut nearly in half. The mean T_4 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 compar-

isons revealed no significant kinetic adjustments to living at the cooler temperature. Provision of exogenous T_4 had little effect on these kinetic parameters.

Iron deficiency had no significant effect on T_3 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 T_3 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 T_4 had no effect on T_3 kinetic parameters in iron deficiency.

Our thyroid kinetics results allow the calculation of the estimated fraction of T_4 converted to T_3 via deiodination. In this study, the estimated T_4 to T_3 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. T_4 -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 T_3 and T_4 disposal rates compared with controls. T_4 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 T_4 concentration and pool size were lower in iron deficiency, metabolic rate was higher and T_4 disposal rate was still lower than controls. Iron deficiency also was associated

TABLE 5

Plasma triiodothyronine (T_3) pool (POOL), fractional catabolic rate (FCR_p) and disposal rate (DR) in iron-deficient and control rats housed at 30 and 15°C and in iron-deficient rats receiving exogenous T_4 while living at 15°C¹

Group	n	T_3 POOL	T_3 POOL	T_3 FCR_p	T_3 DR	T_3 DR
		pmol	pmol/kg ^{0.75}	h ⁻¹	pmol/h	pmol/(h · kg ^{0.75})
ID-30°C	6	4.2 ± 0.9 ^b	16.0 ± 4.4 ^c	2.96 ± 0.68 ^b	11 ± 2 ^c	41 ± 8 ^b
CN-30°C	6	8.1 ± 1.3 ^b	19.9 ± 4.7 ^c	1.68 ± 0.18 ^c	13 ± 3 ^c	31 ± 5 ^b
ID-15°C	6	28.6 ± 2.9 ^a	81.1 ± 8.0 ^a	3.33 ± 0.41 ^{a,b}	92 ± 11 ^b	262 ± 28 ^a
CN-15°C	10	28.7 ± 2.6 ^a	60.7 ± 4.1 ^b	4.57 ± 0.33 ^a	129 ± 11 ^a	276 ± 27 ^a
ID- T_4 -15°C	8	29.0 ± 2.7 ^a	76.6 ± 7.0 ^a	4.27 ± 0.16 ^a	126 ± 12 ^a	333 ± 30 ^a

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.

TABLE 6

Thyroxine (*T*₄) kinetic parameters in iron-deficient and control rats housed at 30 and 15°C and in iron-deficient rats receiving exogenous *T*₄ while living at 15°C¹

Group	L(1,1)	t _p	T _p	V _p	MST	tt _p
	<i>h</i> ⁻¹	<i>h</i>		cycles		<i>h</i>
ID-30°C	1.70 ± 0.81 ^b	0.711 ± 0.109 ^a	6.56 ± 0.85 ^a	9.46 ± 1.66 ^b	19.7 ± 1.8	1.73 ± 0.27 ^a
CN-30°C	9.73 ± 5.32 ^a	0.143 ± 0.062 ^b	4.02 ± 0.68 ^b	32.2 ± 4.71 ^a	23.0 ± 3.4	0.63 ± 0.08 ^b
ID-15°C	12.20 ± 1.91 ^a	0.086 ± 0.009 ^b	3.60 ± 0.18 ^b	42.1 ± 3.1 ^a	17.1 ± 1.3	0.33 ± 0.03 ^b
CN-15°C	13.12 ± 2.92 ^a	0.095 ± 0.011 ^b	3.26 ± 0.11 ^b	40.3 ± 8.1 ^a	17.1 ± 1.0	0.42 ± 0.06 ^b
ID-T ₄ -15°C	12.70 ± 1.95 ^a	0.095 ± 0.015 ^b	2.93 ± 0.23 ^b	33.4 ± 3.2 ^a	16.8 ± 1.3	0.46 ± 0.08 ^b

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; T_p, plasma mean residence time; V_p, plasma recycling number; MST, mean sojourn time; tt_p, plasma recycling time.

with T₃ 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 T₄ and T₃ 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 FCR_p as 0.221 ± 0.023/h and T₄ DR as 150 ± 18 pmol/h, results that are similar to those we found (T₄ FCR_p ranged from 0.173 to 0.360/h and T₄ 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 FCR_p (3.44 ± 0.55/h) and DR (36.4 ± 8.9 pmol/h) were not very different from those in the current

TABLE 7

Triiodothyronine (*T*₃) kinetic parameters in iron-deficient and control rats housed at 30 and 15°C and in iron-deficient rats receiving exogenous T₄ while living at 15°C^{1,2}

Group	L(1,1)	t _p	T _p	V _p	MST	tt _p
	<i>h</i> ⁻¹	<i>h</i>		cycles		<i>h</i>
ID-30°C	29.9 ± 3.9 ^a	0.058 ± 0.021 ^b	0.532 ± 0.156 ^a	9.80 ± 1.23	11.5 ± 2.1 ^{a,b}	1.19 ± 0.16 ^b
CN-30°C	13.6 ± 3.3 ^b	0.155 ± 0.033 ^a	0.675 ± 0.095 ^a	6.16 ± 1.55	13.0 ± 1.7 ^a	2.82 ± 0.91 ^a
ID-15°C	25.1 ± 3.9 ^a	0.062 ± 0.020 ^b	0.367 ± 0.092 ^b	6.31 ± 0.91	8.4 ± 0.4 ^{a,b}	1.43 ± 0.21 ^{a,b}
CN-15°C	28.3 ± 2.7 ^a	0.038 ± 0.004 ^c	0.229 ± 0.026 ^b	5.16 ± 0.28	8.0 ± 0.5 ^b	1.52 ± 0.09 ^{a,b}
ID-T ₄ -15°C	31.1 ± 1.9 ^a	0.033 ± 0.001 ^c	0.237 ± 0.013 ^b	6.31 ± 0.28	7.4 ± 1.1 ^b	1.13 ± 0.12 ^b

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; T_p, plasma mean residence time; V_p, plasma recycling number; MST, mean sojourn time; tt_p, plasma recycling time.

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 T_4 to T_3 conversion, tissue concentrations and plasma concentrations (Yamada et al. 1996). Plasma appearance rates for T_3 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-Uaxilko 1977). In this study, both the ID and CN rats made adaptive changes in thyroid hormone metabolism (i.e., increased production and utilization of T_4 and T_3), 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 Koehle 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 T_4 concentration, T_3 concentration, hepatic 5'-deiodinase V_{max} and serum T_3 DR but no changes in metabolic clearance rate or hepatic 5'-deiodinase K_m (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 T_4 to T_3 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 T_4 to T_3 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 T_3 production is generated from the fast turning over extravascular pool and postulated that this included the liver and kidney conversion of T_4 to T_3 (DiStefano et al. 1982b). The majority of the T_3 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 T_3 and T_4 disposal rates.

Normalizing plasma T_4 concentrations in ID rats at 15°C resulted in several notable effects. Although iron indices, resting metabolic rate and IBAT rT_3 deiodination were unaffected by T_4 replacement, liver rT_3 deiodination, T_4 FCR_p and T_4 DR were higher than those for ID rats not treated with T_4 and equal to or greater than those for CN rats. This apparent normalization of plasma T_4 kinetic parameters in ID rats pro-

vided with exogenous T_4 suggests that low plasma T_4 concentrations contribute to the altered thyroid hormone kinetics associated with iron deficiency. Presumably, a smaller proportion of T_4 was converted to T_3 and a larger proportion was converted to rT_3 , a physiologically inactive thyroxine metabolite, in ID rats. In a previous study in which ID rats were supplied with exogenous T_4 , we found that resting metabolic rate and plasma T_3 concentration were unchanged unless the dose of T_4 given was excessive (Brigham and Beard 1995). A recent paper notes that provision of T_4 alone is insufficient for maintaining tissue T_4 and T_3 levels; provision of at least 0.15 μ g T_3 /100 g body weight per day in addition to T_4 is necessary for normalization 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 T_4 to T_3 conversion ratio beyond its current level, and the availability of T_3 for heat production would become inadequate.

It was beyond the objectives of this study to discern the mechanism by which T_4 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 T_4 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 (Beard et al. 1989). 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

- American Institute of Nutrition. (1980) Second report of the ad hoc committee on standards for nutritional studies. *J. Nutr.* 110: 1726.
- Beard, J., Borel, M. J. & Derr, J. (1990) Impaired thermoregulation and thyroid function in iron deficiency anemia. *Am. J. Clin. Nutr.* 52: 813–819.
- Beard, J. L., Green, W. & Finch, C. A. (1982) Interactions of iron deficiency anemia and thyroid hormone levels. *Life Sci.* 30: 691–697.
- Beard, J., Green, W. & Finch, C. A. (1984) Effect of anemia and iron deficiency on thyroid hormone levels and thermoregulation during acute cold exposure. *Am. J. Physiol.* 247: R114–R119.
- Beard, J., Tobin, B. & Green, W. (1989) Evidence for thyroid hormone deficiency in iron-deficient rats. *J. Nutr.* 119: 772–778.
- Beard, J. L., Tobin, B. & Smith, S. M. (1988) Norepinephrine turnover in iron deficiency at three environmental temperatures. *Am. J. Physiol.* 24: R90–R96.
- Berman, M., Beltz, W. F., Greif, P. C., Chabay, R. & Boston, R. C. (1983) CON-SAM User's Guide, PHS Publication no. 1983–421–132:3279. U.S. Government Printing Office, Washington, DC.
- Berman, M. & Weiss, M. F. (1978) SAAM Manual, DHEW Publication No. (NIH) 78–180. U.S. Government Printing Office, Washington, DC.
- Bianco, A. C. & Silva, J. E. (1987) Intracellular conversion of thyroxine to trii-

- dothyronine is required for the optimal thermogenic function of brown adipose tissue. *J. Clin. Invest.* 79: 295–300.
- Brigham, D. E. & Beard, J. L. (1995) Effect of thyroid hormone replacement in iron-deficient rats. *Am. J. Physiol.* 38: R1140–R1147.
- DeMaeyer, E. & Adiels-Tegman, M. (1985) The prevalence of anemia in the world. *World Health Stat. Q.* 38: 302–306.
- Dillman, E., Gale, C., Green, W., Johnson, D. G., Mackler, B. & Finch, C. (1980) Hypothermia in iron deficiency due to altered triiodothyronine metabolism. *Am. J. Physiol.* 239: R377–R381.
- DiStefano, J. J., Jang, M., Malone, K. & Broutman, M. (1982a) Comprehensive kinetics of triiodothyronine production, distribution, and metabolism in blood and tissue pools of the rat using optimized blood-sampling protocols. *Endocrinology* 110: 198–213.
- DiStefano, J. J., Malone, T. K. & Jang, M. (1982b) Comprehensive kinetics of thyroxine distribution and metabolism in blood and tissue pools of the rat from only six blood samples: dominance of large, slowly exchanging tissue pools. *Endocrinology* 111: 108–117.
- DiStefano, J. J., Nguyen, T. T. & Yen, Y. M. (1993) Transfer kinetics of 3,5,3'-triiodothyronine and thyroxine from rat blood to large and small intestines, liver, and kidneys in vivo. *Endocrinology* 132: 1735–1744.
- Escobar-Morreale, H. F., del Rey, F. E., Obregon, M. J. & de Escobar, G. M. (1996) Only the combined treatment with thyroxine and triiodothyronine ensures euthyroidism in all tissues of the thyroidectomized rat. *Endocrinology* 137: 2490–2500.
- Fregley M. J. (1989) Activity of the hypothalamic-pituitary thyroid axis during exposure to cold. *Pharmacol. Ther.* 41: 85–142.
- Guernsey, D. L. & Edelman, I. S. (1983) Regulation of thermogenesis by thyroid hormones. In: *Molecular Basis of Thyroid Hormone Action* (Oppenheimer, J. H. & Samuels, H. H., eds.), pp. 293–324. Academic Press, New York, NY.
- Hesch, R. & Koehle, J. (1986) Intracellular pathways of iodothyronine metabolism. In: *Werner's The Thyroid: A Fundamental and Clinical Text*, 5th ed. (Ingbar, S. H. & Braverman, L. E., eds.), pp. 154–200. Lippincott, Philadelphia, PA.
- Himms-Hagen, J. (1990) Brown adipose tissue thermogenesis: interdisciplinary studies. *FASEB J.* 4: 2890–2898.
- Ingram, D. L. & Kaciuba-Uaxilko, H. (1977) The influence of food intake and ambient temperature on the rate of thyroxine utilization. *J. Physiol. (Lond.)* 270: 431–438.
- Kopecky, J., Sigurdson, L., Park, I. R. A. & Himms-Hagen, J. (1986) Thyroxine 5'-deiodinase in hamster and rat brown adipose tissue: effect of cold and diet. *Am. J. Physiol.* 251: E1–E7.
- Lee, T. F., Mora, F. & Myers, R. D. (1985) Dopamine and thermoregulation: an evaluation with special reference to dopaminergic pathways. *Pharmacol. Biochem. Behav.* 9: 589–598.
- Lukaski, H. C., Hall, C. B. & Nielsen, F. H. (1990) Thermogenesis and thermoregulatory function of iron-deficient women without anemia. *Aviat. Space Environ. Med.* 61: 913–920.
- Nelson, C., Erikson, K., Pinero, D. J. & Beard, J. L. (1997) In vivo dopamine metabolism is altered in iron-deficient anemic rats. *J. Nutr.* 127: 2282–2288.
- Pazos-Moura, C. C., Moura, E. G., Dorris, M. L., Rehnmark, S., Melendez L., Silva, J. E. & Taurog, A. (1991) Effect of iodine deficiency and cold exposure on thyroxine 5'-deiodinase activity in various rat tissues. *Am. J. Physiol.* 260: E175–E182.
- Raasmaja, A. & Larsen, P. R. (1989) α_1 - and β -adrenergic agents cause synergistic stimulation of the iodothyronine deiodinase in rat brown adipocytes. *Endocrinology* 125: 2502–2509.
- Reed, H. L., D'Alessandro, M. M., Kowalski, K. R. & Homer, L. D. (1992) Multiple cold air exposures change oral triiodothyronine kinetics in normal men. *Am. J. Physiol.* 263: E85–E93.
- Reed, H. L., Quesada, M., Hesslink, R. L., D'Alessandro, M. M., Hays, M. T., Christopherson, R. J., Turner, B. V. & Young, B. A. (1994) Changes in serum triiodothyronine kinetics and hepatic type I deiodinase activity of cold-exposed swine. *Am. J. Physiol.* 266: E786–E795.
- Shipley, R. A. & Clark, R. E. (1972) *Tracer Methods for In Vivo Kinetics*, Academic Press, New York, NY.
- Silva, J. E. (1988) Full expression of uncoupling protein gene requires the concurrence of norepinephrine and triiodothyronine. *Mol. Endocrinol.* 2: 706–713.
- Silva, J. E. (1995) Thyroid hormone control of thermogenesis and energy balance. *Thyroid* 5: 481–492.
- Silva, J. E., Gordon, M. B., Crantz, F. R., Leonard, J. L., Larsen, P. R. (1985) Qualitative and quantitative differences in pathways of extrathyroidal T_3 generation between euthyroid and hypothyroid rats. *J. Clin. Invest.* 73: 898–907.
- Silva, J. E. & Larsen, P. R. (1985) Potential of brown adipose tissue type II thyroxine 5'-deiodinase as a local and systemic source of triiodothyronine in rats. *J. Clin. Invest.* 76: 2296–2305.
- Smith, S. M. & Lukaski, H. C. (1992) Type of dietary carbohydrate affects thyroid hormone deiodination in iron-deficient rats. *J. Nutr.* 122: 1174–1181.
- Smith, S. M., Deaver, D. R. & Beard, J. L. (1992) Metabolic rate and thyroxine monodeiodinase activity in iron-deficient female Sprague-Dawley rats: effects of the ovarian steroids. *J. Nutr. Biochem.* 3: 461–466.
- Sorimachi K. (1979) Improved chromatographic methods for the separation of thyroid hormones and their metabolites. *Anal Biochem.* 93: 31–36.
- Suda, A. K., Pittman, C. S., Shimizu, T. & Chambers, J. B. (1978) The production and metabolism of 3,5,3'-triiodothyronine and 3,3',5'-triiodothyronine in normal and fasting subjects. *J. Clin. Endocrinol. Metab.* 47: 1311–1319.
- Tang, F., Wong, T. M. & Loh, T. T. (1988) Effects of cold exposure or TRH on the serum TSH levels in the iron-deficient rat. *Horm. Metab. Res.* 20: 616–619.
- Tobin, B. W. & Beard, J. L. (1990) Interactions of iron deficiency and exercise training relative to tissue norepinephrine turnover, triiodothyronine production and metabolic rate in rats. *J. Nutr.* 120: 900–980.
- Torrance, J. D. & Bothwell, T. H. (1980) Tissue iron stores. In: *Methods in Hematology: Iron* (Cook, J. D., ed.), pp. 90–115. Churchill Livingstone, New York, NY.
- Veronikis, I. E., Braverman, L. E., Alex, S., Fang, S. L., Norvell, B. & Emerson, C. H. (1996) Comparison of the effects of propylthiouracil and selenium deficiency on T_3 production in the rat. *Endocrinology* 137: 2580–2585.
- Yamada, H., DiStefano, J. J., Yen, Y. & Nguyen, T. T. (1996) Steady-state regulation of whole-body thyroid hormone pool sizes and interconversion rates in hypothyroid and moderately T_3 -stimulated rats. *Endocrinology* 137: 5624–5633.