# Metabolic rate and thyroxine monodeiodinase activity in iron-deficient female Sprague-Dawley rats: Effects of the ovarian steroids

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*Iron deficiency results in altered thyroid hormone metabolism throughout the estrous cycle. To assess the interactions of the ovarian steroids, thyroid hormones, and iron deficiency, we examined indices of thyroid function in ovariectomized iron-deficient animals and control animals treated with estradiol (mean plasma concentration, 260 pmol/L), progesterone (mean plasma concentration, 45 nmol/L), estradiol and progesterone, or no steroid (sham-treated). Neither steroid affected plasma triiodothyronine ($T_3$) concentrations relative to sham-treated animals, but estradiol treatment (either alone or with progesterone) elevated plasma thyroxine ($T_4$) concentrations ($P \leq 0.001$). Plasma $T_3$ and $T_4$ concentrations were lower in iron-deficient ovariectomized rats ($T_3$ = 0.38 ± 0.08 nmol/L; $T_4$ = 22.0 ± 9.7 nmol/L) relative to ovariectomized controls ($T_3$ = 0.44 ± 0.11 nmol/L; $T_4$ = 27.4 ± 8.4 nmol/L; $P \leq 0.05$ and 0.01, respectively). Hepatic thyroxine monodeiodinase activity was lower in iron-deficient rats (422 ± 241 versus 565 ± 251 pmoles $I \cdot mg\ protein^{-1} \cdot 20\ minutes^{-1}$; $P \leq 0.03$). Brown adipose tissue deiodinase activity decreased in estradiol-treated groups relative to sham-treated animals ($P \leq 0.03$); however, the brown adipose tissue thyroxine monodeiodinase was unaffected by iron deficiency. Metabolic rates were elevated in iron-deficient rats ($P \leq 0.003$) and were lower in estradiol-treated rats. Iron-deficient rats exhibit an altered thyroid response to exogenous ovarian steroids. In conclusion, plasma parameters of thyroid function are altered in iron-deficient rats, and these appear to be affected by the ovarian steroids. Tissue deiodinase activities are lower and metabolic rates are higher in iron-deficient rats.*

**Keywords:** thyroid hormones; thermogenesis; estradiol; progesterone; indirect calorimetry

## Introduction

Nutritional iron deficiency results in a defect in thermoregulatory capacity in cold-stressed humans[1,2] as well as in animal models.[3–5] When iron-deficient animals are cold stressed they fail to maintain core temperature.[3–5] This may be explained by altered thyroid hormone system function[6,7] because iron-deficient animals exhibit symptoms of both peripheral and pituitary hypothyroidism.[3,4,8] Iron-deficient animals have a decreased thyroid stimulating hormone and thyroid hormone response to thyrotropin releasing hormone;[8] also, the in vitro rate of production of triiodothyronine ($T_3$) from thyroxine ($T_4$) is depressed in iron-deficient male rats.[8] Iron-deficient anemic humans and rats both have significantly lower plasma triiodothyronine concentrations than their iron-sufficient counterparts.[1,3,4,8]

Findings from our laboratory (Smith, Deaver, and Beard, unpublished observations) indicate decreased hepatic monodeiodinase activity in female iron-deficient rats as compared to female controls only on the day of estrus. The reproductive cycle and reproductive hormones affect deiodinase activity,[9–11] thyroid hormone metabolism,[10,12] and thermogenesis.[13] Thyroxine monodeiodinase activity is increased in livers and kid-

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neys of pregnant rats[11,12] and is decreased during lactation[11,12] relative to virgin controls. These changes in peripheral production of $T_3$ are presumably related to changes in metabolic rate. Brown adipose tissue thermogenesis is reduced in ovariectomized rats,[14] and likewise is stimulated by treatment with estradiol.[14,15]

Based on our initial observations of changes in deiodinase activity during the estrous cycle, we examined metabolic rate and thyroid hormone interrelationships in iron-deficient female rats that had ovarian steroid replacement. The objective of this study was to examine the effect of ovarian steroids on thyroid hormone metabolism in iron deficiency. The primary dependent variables measured in these studies were thyroxine monodeiodinase and oxygen consumption. These were chosen to provide information about both $T_4$ to $T_3$ conversion and whole body effects of thyroid hormones as influenced by iron deficiency. Monodeiodinase activity was measured in two tissues, liver and brown adipose, which have different deiodinase isozymes.[16] The liver contains primarily type I deiodinase, which is responsible for production of $T_3$ for export to the circulation.[16] The brown adipose tissue type II deiodinase produces $T_3$ primarily for intracellular utilization and, in the brown adipose, stimulation of thermogenesis.[16]

## Materials and methods

### *Design*

Weanling female Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) were housed individually in stainless-steel cages, maintained on a 12-hour light:dark (07:00–19:00 hr) in a temperature controlled room (24 ± 1.0° C, mean ± SD) and were provided with distilled deionized water ad libitum. Rats were assigned to one of two dietary treatments. All rats were fed a modified AIN-76 diet[17] ad libitum either without added iron (ID, ≈2.5 ppm Fe, $n$ = 25) or with iron added as ferrous sulfate (CN, ≈60 ppm Fe, $n$ = 25). The dietary carbohydrate source was corn starch, and fiber was omitted from the diet to minimize iron contamination.[18] The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University, and all procedures were in accordance with National Institutes of Health guidelines.

After 5 weeks of dietary treatment, ID and CN animals were ovariectomized under ether anesthesia. Four days following surgery, at 1200 hrs, animals received subcutaneous pellets (17β-estradiol, $n$ = 6/group; progesterone, $n$ = 6/group; 17β-estradiol and progesterone, $n$ = 7/group; Innovative Research of America, Toledo, OH, USA). Pellets were designed to provide physiological plasma concentrations[19] of estradiol ($E_2$, 25–50 pg/mL) and progesterone ($P_4$, 25–50 ng/mL). Groups not receiving steroids were sham-treated (SH, $n$ = 6/group). The subcutaneous placement of these pellets was performed under light ether anesthesia and required less than 5 minutes to complete. A 24-hour treatment period was chosen to estimate the time frame of steroid changes in intact animals and to avoid chronic effects of steroids on oxygen consumption and thyroid hormones.

Animals were fasted from 08:00 hr the morning after pellet implantation. Resting oxygen consumption ($\dot{V}O_2$) measurements were carried out between 13:00 and 15:00 hr (see description below). Immediately following each period (15:00–15:30 hr), animals were killed by decapitation and tissues were rapidly removed and frozen at −70° C for later analysis. Trunk blood was collected in heparinized tubes, stored on ice, and plasma obtained following centrifugation.

### *Hepatic thyroxine monodeiodinase*

Thyroxine monodeiodinase was measured in the liver based on the methods of Leonard and Rosenberg[20] and as described previously.[21] Livers were homogenized in 10 mL buffer (0.25 M sucrose-0.1 M phosphate-1 mmol/L EDTA buffer; pH = 7.0) using a Polytron homogenizer (Brinkmann Instruments Inc., Westbury, NY, USA). Crude microsomal fractions were obtained by centrifugation of the homogenate for 15 minutes at 180$g$ at 4° C, the supernatant was then centrifuged at 105,000$g$ for 65 minutes at 4° C. The microsomal pellet was then re-suspended in 0.1 M phosphate buffer (pH = 7.0).

High specific activity (>1200 μCi/μg) labeled [$^{125}$I-]-thyroxine (Amersham Corp., Arlington Heights, IL, USA) was used and was purified prior to each assay using a Sephadex column (LH-20). Samples were incubated in triplicate (total volume = 200 μL) for 20 minutes at 37° C under $N_2$ gas. Incubations included dithiothreitol (3 mmol/L), phosphate buffer (0.1 M, pH 7.0), microsomal protein (approximately 100 μg/tube), and substrate (both labeled and unlabeled $T_4$, approximately 80,000 cpm/tube). Substrate concentrations ranged from 1.2–12 μmol/L. Following the incubation period, samples were placed on ice, 50 μL of a 7% bovine serum albumin solution and 300 μL of a 12% trichloroacetic acid (TCA) solution were added. The suspension was vortexed and then centrifuged for 2 minutes in an Eppendorf microcentrifuge. The supernatant was added to a Dowex column (AG 50 W-X2, suspended in acetic acid:$H_2O$, 1:10). The column was washed three times with 1 mL of acetic acid:$H_2O$ to separate the free $^{125}$I$^-$ from any Dowex-bound $^{125}$I-thyroxine.[20] The Dowex eluent was collected and counted in a Packard gamma counter. A blank (homogenization buffer added instead of protein) was run to determine non-enzymatic production of $^{125}$I$^-$ and linearity of activity to protein concentration determined between 50 and 150 μg of microsomal protein.

### *Brown adipose tissue monodeiodinase assay*

The brown adipose tissue deiodinase assay was adapted from the methods of Kates and Himms-Hagen[22] as described previously.[21] Interscapular brown adipose tissue (IBAT) was homogenized in 2 mL of buffer (0.25 M sucrose, 1 mmol/L HEPES, 0.1 mmol/L EDTA, pH 7.2) using a teflon homogenizer. Crude microsomal fractions were obtained as in the liver assay.

Samples were incubated for 60 minutes at 37° C under $N_2$ gas. Incubations included ≈100 μg microsomal protein, dithiothreitol (10 mmol/L), propylthiouracil (1 mmol/L), 0.1 M phosphate buffer, pH 7.0, and thyroxine (≈13.3 nmol/L). Following the incubation period, samples were processed exactly as in the liver assay. Eight replicates of all samples were used, and a blank was also used to determine non-enzymatic production of $^{125}$I$^-$. This concentration of substrate was determined in preliminary studies to be a saturating concentration and to allow an accurate estimation of $V_{max}$.

### *Enzyme calculations*

Kinetic parameters of the hepatic deiodinase were estimated using double reciprocal plots. Slope and intercepts for double reciprocal plots were determined using unweighted least squares regression calculated using the Lotus 1-2-3 software

package for the personal computer (Lotus 1-2-3, Release 2.0, Lotus Development Corp., Cambridge, MA, USA). $V_{max}$ was estimated by using the inverse of the Y intercept, and apparent $K_m$ by using the inverse of the X intercept.

Thyroxine monodeiodinase activity in IBAT was estimated using the average of the assay replicates.

### *Indirect calorimetry*

Resting oxygen consumption ($\dot{V}O_2$) was measured using an open circuit indirect calorimeter.[23] Briefly, animals were placed in the calorimeter 45–60 minutes prior to the collection of data. Expired gases were measured every 15 seconds for 3 minutes (the first four were deleted to account for instrument delay), with eight such collections throughout a 2-hour period. The median of the resulting 64 data points was then calculated and was used as the estimate of $\dot{V}O_2$. The median was used to eliminate the effect of transient increases in oxygen consumption due to animal activity, which would falsely elevate the arithmetic mean.

### *Hormones*

Plasma was analyzed using previously described radioimmunoassays for estradiol[24] and progesterone.[25] Reagents were obtained from Ciba-Corning Diagnostics (Norwell, MA, USA progesterone, catalogue #472421000; estradiol, catalogue #472417000). Inter- and intra-assay coefficients of variation were 7% and 6%, respectively, for both estradiol and progesterone assays.

Plasma concentrations of thyroid hormones were determined by radioimmunoassay (ICN Diagnostics, Costa Mesa, CA, USA). Inter- and intra-assay coefficients of variation were ≤10% and 6%, respectively, for both $T_3$ and $T_4$.

### *Statistics*

Data were subjected to a three-way analysis of variance (ANOVA), with class variables iron status (ID or CN), estradiol status (+ or −), progesterone status (+ or −), and all possible interaction permutations. Dependent variables were the $V_{max}$ and apparent $K_m$ for monodeiodinase activity in the liver, IBAT thyroxine monodeiodinase activity, and $\dot{V}O_2$. Unless noted in tables or figure legends, interaction terms were not significant. Due to unequal cell sizes, type III sums of squares were used for the calculation of the *F* statistic. Three-way ANOVA was calculated using the GLM procedure of SAS (Statistical Analysis Systems Inc., Cary, NC, USA).

All data, unless otherwise noted, represent arithmetic mean ± sample standard deviation.

### *Materials*

All chemicals unless otherwise noted were obtained from Sigma Chemical Company (St. Louis, MO, USA). Vitamin-free casein and AIN-76 Vitamin mix were obtained from ICN Biochemicals (Cleveland, OH, USA).

## Results

### *Growth and hematology*

At the time of ovariectomy, ID animals weighed less than controls ($P \leq 0.001$, *Table 1*). Four days following surgery, 42 of 49 animals had regained their pre-operative body weight. The relative change in body weight during the 24 hours post steroid implantation was affected by both iron status ($P \leq 0.001$) and estradiol treatment ($P \leq 0.001$), with ID animals losing relatively more weight than controls, and $E_2$-treated animals losing relatively more weight than other groups *(Table 1)*.

**Table 1** Body weight at time of surgery and relative change in body weight (BW) following 24 hours of steroid treatment ($E_2$, estradiol; $P_4$, progesterone) in iron-deficient and control rats

| Group | n | Body weight (g)[a] | Relative change in body weight following steroid treatment (% of BW at implantation) |
|---|---|---|---|
| CN-SH | 6 | 157 ± 18 | 100.8 ± 1.0 |
| CN-$E_2$ | 6 | 160 ± 8 | 98.7 ± 0.5 |
| CN-$P_4$ | 7 | 161 ± 17 | 100.3 ± 1.3 |
| CN-$E_2$+$P_4$ | 6 | 170 ± 14 | 97.8 ± 2.2 |
| ID-SH | 6 | 131 ± 11 | 98.8 ± 1.7 |
| ID-$E_2$ | 6 | 136 ± 13 | 96.0 ± 1.4 |
| ID-$P_4$ | 6 | 127 ± 16 | 98.5 ± 1.9 |
| ID-$E_2$+$P_4$ | 6 | 131 ± 9 | 95.0 ± 2.4 |
| AOV Table | | | |
| Fe Status | | 0.001 | 0.001 |
| Estradiol | | NS | 0.001 |
| Progesterone | | NS | NS |

[a]Data presented are mean ± sample standard deviation. NS, not significant.

ID animals had reduced body weights and hemoglobin concentrations compared with controls ($P \leq 0.001$, *Table 2*). ID animals also had increased liver:body weight ratios ($P \leq 0.001$) and interscapular brown adipose tissue weights ($P \leq 0.001$) than CN animals. There was no effect of steroid treatment on relative or absolute tissue weights ($P \geq 0.05$, *Table 2*).

### *Hormones*

**Thyroid hormones.** The mean plasma $T_3$ and $T_4$ concentrations were reduced in ID animals compared with controls ($P \leq 0.05$, *Table 3*); however, there was no effect of either steroid on plasma $T_3$. Estradiol treatment increased plasma $T_4$ concentration in both dietary treatment groups ($P \leq 0.001$). Progesterone had no effect on plasma thyroid hormone concentrations.

**Ovarian steroids.** Plasma progesterone concentrations in groups treated with $P_4$ averaged approximately 45 nmol/L *(Table 3)*. One animal in the CN $E_2$ + $P_4$ group had a much higher plasma progesterone concentration than the rest of the group (100.8 nmol/L versus an average of 58.8 nmol/L for the rest of the group); this point was removed from the analysis of plasma progesterone concentration, which negated the significant interaction term noted in the primary analysis.

**Oxygen consumption.** $\dot{V}O_2$ was increased in ID animals compared with controls ($P \leq 0.003$) and there was an

**Table 2** Body weights (BW), hematologic parameters, and absolute and relative tissue weights in iron-deficient and control animals treated with ovarian steroids[a]

| Group | n | Body weight (g) | HCT (% PCV) | Hb (g/L) | Liver weight (g/kg BW) | IBAT weight (g/kg BW) |
|---|---|---|---|---|---|---|
| CN-SH | 6 | 162 ± 21 | 43 ± 2 | 142 ± 12 | 34.1 ± 3.0 | 1.50 ± 0.22 |
| CN-$E_2$ | 6 | 160 ± 12 | 44 ± 2 | 155 ± 20 | 36.3 ± 2.2 | 1.53 ± 0.21 |
| CN-$P_4$ | 7 | 164 ± 14 | 44 ± 3 | 147 ± 13 | 34.5 ± 2.5 | 1.67 ± 0.45 |
| CN-$E_2$+$P_4$ | 6 | 174 ± 16 | 42 ± 1 | 151 ± 11 | 36.5 ± 1.3 | 1.50 ± 0.20 |
| ID-SH | 6 | 131 ± 15 | 21 ± 2 | 56 ± 12 | 38.7 ± 2.8 | 1.29 ± 0.19 |
| ID-$E_2$ | 6 | 126 ± 15 | 21 ± 3 | 53 ± 10 | 38.5 ± 1.2 | 1.47 ± 0.15 |
| ID-$P_4$ | 6 | 132 ± 15 | 21 ± 2 | 52 ± 8 | 38.0 ± 1.5 | 1.45 ± 0.29 |
| ID-$E_2$+$P_4$ | 6 | 124 ± 9 | 22 ± 3 | 58 ± 13 | 39.0 ± 4.7 | 1.38 ± 0.24 |
| AOV Table | | | | | | |
| Fe Status | | 0.001 | 0.001 | 0.001 | 0.001 | 0.04 |
| Estradiol | | NS | NS | NS | NS | NS |
| Progesterone | | NS | NS | NS | NS | NS |

[a]Data presented are mean ± sample standard deviation; NS, not significant. HCT, hematocrit; Hb, hemoglobin; PCV, packed cell volume; $E_2$, estradiol; $P_4$, progesterone.

**Table 3** Plasma thyroid hormone and ovarian steroid data in iron-deficient (ID) and control (CN) animals treated with ovarian steroids[a]

| Group | n | Plasma $T_3$ (nmol/L) | Plasma $T_4$ (nmol/L) | Plasma $E_2$ (pmol/L) | Plasma[b] $P_4$ (nmol/L) |
|---|---|---|---|---|---|
| CN-SH | 6 | 0.50 ± 0.17 | 24.3 ± 4.0 | 14.7 ± 16.2 | 5.1 ± 2.9 |
| CN-$E_2$ | 6 | 0.44 ± 0.10 | 32.1 ± 6.3 | 286.3 ± 46.6 | 8.3 ± 2.9 |
| CN-$P_4$ | 7 | 0.41 ± 0.13 | 20.5 ± 6.8 | 14.7 ± 8.1 | 45.8 ± 8.0 |
| CN-$E_2$+$P_4$ | 6 | 0.41 ± 0.09 | 34.1 ± 8.2 | 266.9 ± 28.6 | 58.8 ± 16.9 |
| ID-SH | 6 | 0.38 ± 0.07 | 12.5 ± 3.1 | 16.2 ± 16.9 | 4.8 ± 3.5 |
| ID-$E_2$ | 6 | 0.41 ± 0.11 | 27.7 ± 8.8 | 253.7 ± 16.9 | 6.7 ± 4.5 |
| ID-$P_4$ | 6 | 0.40 ± 0.08 | 19.9 ± 11.1 | 13.9 ± 5.1 | 48.7 ± 14.0 |
| ID-$E_2$+$P_4$ | 6 | 0.32 ± 0.07 | 27.5 ± 5.9 | 256.6 ± 16.9 | 43.9 ± 8.0 |
| AOV Table | | | | | |
| Fe Status | | 0.05 | 0.01 | NS | NS |
| Estradiol | | NS | 0.001 | 0.001 | 0.001 |
| Progesterone | | NS | NS | NS | NS |

[a]Data presented are mean ± sample standard deviation; NS, not significant. $T_3$, triiodothyronine; $T_4$, thyroxine; $E_2$, estradiol; $P_4$, progesterone.
[b]Data analyzed with one point from the CN-$E_2$+$P_4$ group removed (see text for discussion)

effect of estradiol status on $\dot{V}O_2$, with $E_2$ tending to lower oxygen consumption rate ($P \leq 0.05$, *Figure 1*). The effect of iron deficiency or estradiol status on $\dot{V}O_2$ was not affected by calculation using the median or arithmetic mean of the data points (see Materials and methods section).*

**Liver thyroxine monodeiodinase.** The hepatic thyroxine monodeiodinase $V_{max}$ was reduced in ID animals relative to CN ($P \leq 0.03$, *Figure 2*). There was a significant interaction between iron status and estradiol status ($P \leq 0.01$) on $V_{max}$. Apparent $K_m$ was not affected by any treatment (data not presented).

**IBAT thyroxine monodeiodinase.** The brown adipose tissue thyroxine monodeiodinase activity was affected by estradiol treatment ($P \leq 0.03$) in the ID rats; an almost 50% decrease in activity was found when either $E_2$, $P_4$, or $E_2$ + $P_4$ was given *(Figure 3)*. This effect was not observed in the CN animals and there was no effect of iron status on IBAT monodeiodinase activity.

## Discussion

Iron deficiency in female rats resulted in a significant decrease in plasma concentrations of $T_3$ and $T_4$ as was

*$\dot{V}O_2$ for CN-SH calculated with the median was 25.0 ± 1.6 and with the mean was 26.1 ± 2.2 mL•min$^{-1}$•kg$^{-1}$, for ID-SH calculated with the median was 26.1 ± 2.3 and calculated with the mean was 27.9 ± 2.6 mL•min$^{-1}$•kg$^{-1}$.

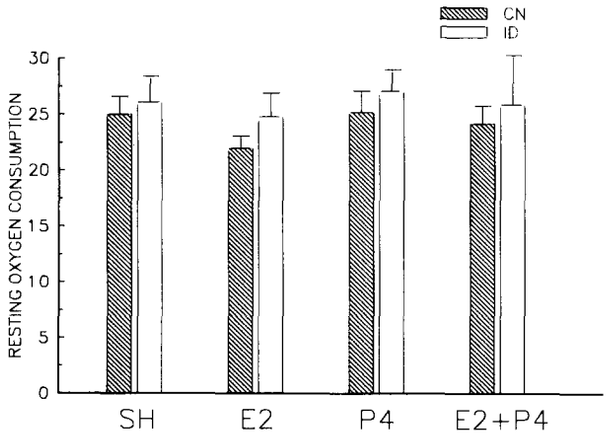


**Figure 1** Resting oxygen consumption in ovariectomized iron-deficient (ID) and control (CN) rats treated with ovarian steroids. SH, sham treated; $E_2$, estradiol treated; $P_4$, progesterone treated; $E_2+P_4$, estradiol and progesterone treated. Units are mL $O_2$ · $min^{-1}$ · kg body $weight^{-1}$. Data are mean ± SD. ANOVA results indicated significant effects of iron status ($P \leq 0.003$) and estradiol status ($P \leq 0.05$).

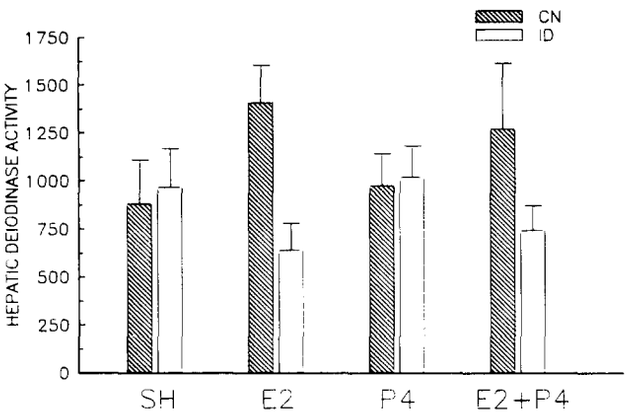


**Figure 2** Hepatic monodeiodinase activity in ovariectomized iron-deficient (ID) and control (CN) rats treated with ovarian steroids. SH, sham treated; $E_2$, estradiol treated; $P_4$, progesterone treated; $E_2+P_4$, estradiol and progesterone treated. Monodeiodinase units are pmol $I^-$ produced · mg $protein^{-1}$ · 20 $min^{-1}$. Data are mean ± SEM. ANOVA results indicated significant effects of iron status ($P \leq 0.03$) and a significant interaction between iron status and estradiol status ($P \leq 0.01$).

expected from previous studies of iron deficiency in male rats.[3,4] Reproductive steroid replacement in ovariectomized rats had no effect on the plasma $T_3$ concentration, while estradiol treatment caused a significant increase in plasma $T_4$ concentration. These data are in contrast to previous reports[26,27] in which plasma $T_4$ concentrations were reported to decrease in estradiol-treated rats in comparison with ovariectomized and oil (sham)-treated rats. However, these earlier studies used large, single daily doses of steroid (2 μg estradiol benzoate/day;[27] and 2.5 or 25 μg/day[26]), which may confound comparisons. D'Angelo and Fisher[28] showed that treatment with physiologic amounts of estradiol (0.004–0.246 μg estradiol benzoate/day for 9–11 days) increased thyroid-stimulating hormone (TSH) release in vivo and that pharmacological doses of the steroid depress TSH release. These results support the present study, which also used physiologic concentrations of steroid, and help to resolve some of the conflict with previous findings.[26,27] Estradiol elevated plasma $T_4$ concentrations in both control and iron-deficient rats in the present study. This effect of estradiol might be due to a decrease in pituitary type II thyroxine monodeiodinase activity. This would serve to decrease the availability of $T_3$ for nuclear receptors in the pituicyte[29] with subsequent up-regulation of thyroxine release from the thyroid.[30,31]

Estradiol regulation of deiodinase activity is altered by iron deficiency; specifically, estradiol stimulates enzyme activity in iron-sufficient but not iron-deficient rats. Progesterone treatment alone had no effect on the deiodinase, and attenuated the $E_2$ response in the combined treatment group. These effects of the ovarian steroids on hepatic deiodinase are similar to those found by other investigators,[10,12] i.e., estradiol increases activity of the enzyme.

The type II thyroxine monodeiodinase is affected by physiological status, such as pregnancy[32] and lactation,[9,10] while the ovarian steroids are probable regulators of metabolism. In a recent article, Viñas et al.[10] referred to unpublished data that showed a decrease in IBAT deiodinase activity following chronic progesterone treatment.

Iron-deficient animals lost relatively more weight than controls, both after surgery and after steroid treatment, an effect similar to one reported in male iron-deficient animals.[33] Ovarian steroid treatment resulted in similar changes in body weight in both ID and CN rats, suggesting that the steroids have similar metabolic effects in ID animals as in controls, although effects of the steroids on food and water intake cannot be discounted. The ability of estradiol to attenuate weight

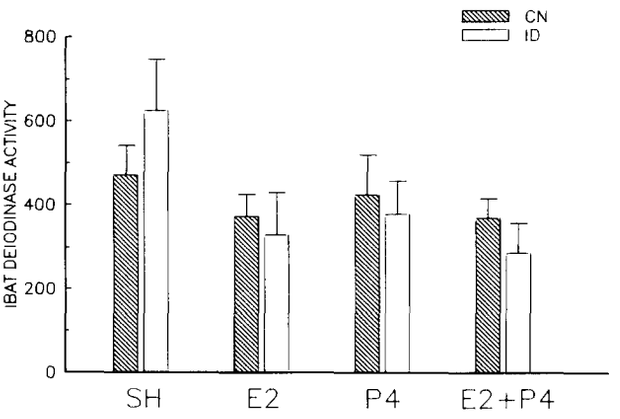


**Figure 3** IBAT monodeiodinase activity in ovariectomized iron-deficient (ID) and control (CN) rats treated with ovarian steroids. SH, sham treated; $E_2$, estradiol treated; $P_4$, progesterone treated; $E_2+P_4$, estradiol and progesterone treated. Monodeiodinase units are fmol $I^-$ produced · mg $protein^{-1}$ · $h^{-1}$. Data are mean ± SEM. ANOVA results indicated a significant effect of estradiol status ($P \leq 0.03$).

gain is in agreement with other reports.[34] However, in most of these studies steroid-induced changes were examined over a longer period of time or with higher doses of steroid given in daily injections. There are published data to support an effect of estradiol and progesterone on indices of brown adipose tissue thermogenesis. Estradiol stimulates brown adipose tissue norepinephrine turnover[14] and oxygen consumption.[15] Progesterone treatment has no effect on indices of brown adipose thermogenesis, and combined with estradiol, progesterone attenuates the estradiol-induced stimulation of brown adipose tissue thermogenesis.[15]

In summary, the elevation in metabolic rate in iron deficiency and the depression in plasma thyroid hormone concentrations are generally unaffected by the ovarian steroids. While estradiol is a potent stimulus for hepatic $T_3$ production in controls, this effect is not observed in iron-deficient animals, suggesting that iron deficiency may specifically alter regulatory control points for this important site of $T_3$ production.

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