



## Marginal iodide deficiency and thyroid function: Dose–response analysis for quantitative pharmacokinetic modeling

M.E. Gilbert<sup>a,\*</sup>, E.D. McLanahan<sup>b</sup>, J. Hedge<sup>c</sup>, K.M. Crofton<sup>c</sup>, J.W. Fisher<sup>d</sup>, L. Valentín-Blasini<sup>e</sup>, B.C. Blount<sup>e</sup>

<sup>a</sup> Toxicity Assessment Division (MD-B105-05), National Health and Environmental Effects Research Laboratory, 109 TW Alexander Drive, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, USA

<sup>b</sup> National Center for Environmental Assessment, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

<sup>c</sup> Integrated Systems Biology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

<sup>d</sup> National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, USA

<sup>e</sup> Centers for Disease Control and Prevention, Atlanta, GA, USA

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### ABSTRACT

Severe iodine deficiency (ID) results in adverse health outcomes and remains a benchmark for understanding the effects of developmental hypothyroidism. The implications of marginal ID, however, remain less well known. The current study examined the relationship between graded levels of ID in rats and serum thyroid hormones, thyroid iodine content, and urinary iodide excretion. The goals of this study were to provide parametric and dose–response information for development of a quantitative model of the thyroid axis. Female Long Evans rats were fed casein-based diets containing varying iodine (I) concentrations for 8 weeks. Diets were created by adding 975, 200, 125, 25, or 0  $\mu\text{g}/\text{kg}$  I to the base diet ( $\sim 25 \mu\text{g}$  I/kg chow) to produce 5 nominal I levels, ranging from excess (basal + added I, Treatment 1: 1000  $\mu\text{g}$  I/kg chow) to deficient (Treatment 5: 25  $\mu\text{g}$  I/kg chow). Food intake and body weight were monitored throughout and on 2 consecutive days each week over the 8-week exposure period, animals were placed in metabolism cages to capture urine. Food, water intake, and body weight gain did not differ among treatment groups. Serum T4 was dose-dependently reduced relative to Treatment 1 with significant declines (19 and 48%) at the two lowest I groups, and no significant changes in serum T3 or TSH were detected. Increases in thyroid weight and decreases in thyroidal and urinary iodide content were observed as a function of decreasing I in the diet. Data were compared with predictions from a recently published biologically based dose–response (BBDR) model for ID. Relative to model predictions, female Long Evans rats under the conditions of this study appeared more resilient to low I intake. These results challenge existing models and provide essential information for development of quantitative BBDR models for ID during pregnancy and lactation.

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### 1. Introduction

Iodine is a micronutrient essential for the production of thyroid hormone. Adequate levels of iodine are critical for normal functionality of the thyroid, an important regulator of energy metabolism and crucial for the development of different tissues, particularly the brain (Delange, 1994). Vertebrates have developed a number of specialized adaptive responses to conditions of temporary iodine deficiency. Under conditions of prolonged iodine deficiency, however, these mechanisms can fail, leading to detrimental but presumably recoverable physiological effects in adults (Pedraza et al., 2006). Insufficient levels of iodine during fetal and early neonatal life can have devastating effects on neurological func-

tion that persist throughout life. Iodine deficiency remains today the single most important and preventable cause of mental defects (Delange, 1994; Zimmermann, 2008) and insufficient iodine intake is still prevalent in almost one third of the world population (Hamann et al., 2006). Marginal iodine insufficiency has been identified in more than 15% of women of child bearing age in the United States (Hollowell and Haddow, 2007).

A number of thyroid-active environmental contaminants have been identified that can interfere with the highly regulated hypothalamic–pituitary–thyroid axis and alter thyroid homeostasis (Brucker-Davis, 1998). There is limited animal data on graded levels of iodine deficiency and even less on the interaction of these xenobiotics in an already iodine-compromised individual. A recent report from the Centre for Disease Control indicates an increased sensitivity of thyroid hormone disruption induced by the environmental toxicant, perchlorate, in women with marginal iodine deficiency (Blount et al., 2006). As such, the goals of this initial

\* Corresponding author. Tel.: +1 919 541 4394; fax: +1 919 541 4849.

E-mail address: [gilbert.mary@epa.gov](mailto:gilbert.mary@epa.gov) (M.E. Gilbert).

**Table 1**  
Potassium iodate was added to a base diet in varying quantities. Iodide (I) content was assessed in chow samples and daily iodine intake determined based on mean daily food intake calculated for each animal (~20 gm/day).

Treatment group	µg iodide (I) added/kg chow	Nominal I/g of chow	Measured ng of I/g of chow	µg I/day based on daily food intake
1 (Control)	975	1000	1300 ± 121 (n = 18)	26.02 ± 1.92
2	200	225	205 ± 8.2 (n = 6)	4.15 ± 0.08*
3	125	150	135 ± 29.9 (n = 3)	2.52 ± 0.04*
4	25	50	36 ± 2.02 (n = 16)	0.65 ± 0.02*
5	0	25	10.7 ± 1.18 (n = 27)	0.21 ± 0.01*

\* Significantly different from Treatment 1,  $p < 0.05$ .

project were twofold: (1) to provide parametric and dose–response information for purposes of quantitative modeling of the thyroid axis and (2) to establish an animal model for marginal iodine deficiency upon which impact of low level thyroid hormone disruption from xenobiotics can be assessed.

Although a number of reports have been published on experimental models of iodine deficiency in laboratory animals, their utility is limited because only severe depletions were evaluated, comparable nutritional composition of various diets was not ensured, and few have examined graded degrees of iodine deficiency. Comparison across studies is difficult due to inadequate documentation of the nutritional composition and iodine content of various diets, and duration of iodine deficiency. Differences also exist in the sex, age, and strain of the animals used in various studies, and often only a limited characterization of thyroid function was provided (see Pedraza et al., 2006). To address some of these issues, the present study examined a number of parameters (e.g., food intake, body weight, serum hormones, thyroid weight, urinary and thyroid iodine content) in young, nonpregnant female rats to establish a range of dietary I concentrations. This information is essential to establish baseline data for designing studies of marginal iodine deficiency the pregnant and lactating animal. Results were compared with model estimates derived from a biologically based dose–response (BBDR) model of the hypothalamic pituitary thyroid axis of the adult rat recently published by McLanahan et al. (2008). This BBDR model is unique as it is the only available report that characterizes the effects of altered dietary iodide on the thyroid axis. Other published reports describe radioactive iodide distribution and metabolism in adult rats (Merrill et al., 2003) and developmental life stages (Clewel et al., 2003a,b), but these models are not well suited to predict the effects of various concentrations of dietary iodide on thyroid axis homeostasis. The McLanahan et al. (2008) BBDR model forms the basis of ongoing efforts to generate a mathematical model of thyroid hormone insufficiency induced from iodine insufficiency and xenobiotic exposure during critical periods of development in the rat (see Fisher et al., 2010; Li et al., 2010).

## 2. Methods

### 2.1. Animals

Young adult female Long Evans rats ( $n = 25$ ) were obtained from Charles River Breeding Laboratory (Raleigh, NC) on postnatal day (PN) 40. Animals were housed in an AAALAC-certified (Association for Assessment and Accreditation of Laboratory Animal Care) facility and experiments were conducted under an approved IACUC (Institutional Animal Care and Use Committee) protocol. Animal rooms were maintained at 22 °C, with relative humidity of 40–60%, and a 12 h light:dark (7:00 am:7:00 pm) photoperiod. Upon arrival, animals were housed individually in polycarbonate cages on pine shaving bedding and permitted free access to food (Purina 5001 chow) and water.

### 2.2. Iodide deficient diets

Beginning approximately two weeks after arrival to the facility, animals were transferred to individual metabolism cages at 8:00 am on Tuesday mornings and maintained there until 8:00 am Thursday mornings when they were returned to their home cage. Two metabolism sessions were conducted under normal Purina

5001 chow (750 ng/g iodide) to acclimate animals to the cages. Animals were then assigned to 1 of 5 treatment groups, counterbalanced for weight, and provided diets containing one of five iodine concentrations ( $n = 5$ /group, Research Diets, Newark, NJ). The stock casein diet was based on nutritional standards for laboratory animal diets established by the American Institute of Nutrition (1977). With the exception of iodine content, this diet was nutritionally complete and animals did not differ in body weight gain than similarly aged female Long Evans rats maintained on Purina 5001 chow (data not shown). The iodine content of the five casein-based diets was varied by adjusting the quantity of iodine added to the base AIN-76A stock diet, nominally ranging from ample (1.0 µg/g, Treatment 1) to deficient (0.025 µg/g, Treatment 5) as summarized in Table 1. Treatment 2 contains the recommended iodine for AIN-76A diet, Treatment 5 represents the stock AIN diet with no iodine added. Iodine content was also measured in samples from each diet as described below.

### 2.3. Metabolism cages

Animals were acclimated to Nalgene metabolism cages for 4 days during the two weeks preceding introduction of dietary manipulation. Once feeding with the iodine diets commenced, weekly return to the metabolism cages (Tuesday 8:00 am, removed Thursday 8:00 am) resumed for the next 8 weeks. While in the metabolism cages, two day-time (8 h) and two overnight (16 h) urine samples were collected. Water was freely available but food was removed between 8:00 am and 4:00 pm after the second week to allow urine collection uncontaminated by food droppings. Urine volumes were recorded and samples collected each morning and afternoon, and stored at –80 °C for later analysis. Food consumption, water consumption, and body weight were recorded 5 days/week throughout the dosing period and average daily consumptions and body weight gains calculated. After 8 weeks on the diet, animals were sacrificed by decapitation and trunk blood collected for serum hormone analysis (see below). Thyroid glands were collected for iodine analysis (see Fig. 1).

### 2.4. Analysis of anions in urine and food

A subset of 9 urine samples per animal, collected between 13 and 56 days on the diet, were selected for analysis of iodide and three other anions known to disrupt iodine uptake into the thyroid gland (Tonacchera et al., 2004). Urinalysis was conducted on samples collected during the daytime period to reduce contamination by food droppings. Samples were analyzed using ion chromatography/tandem mass spectroscopy (IC/MS/MS). Iodide, perchlorate, nitrate, and thiocyanate levels were measured using an isotope dilution IC/MS/MS method as described previously (Valentín-Blasini et al., 2007). Slight modifications to these methods were made to ascertain levels of these same anions in rat chow (Valentín-Blasini et al., 2005, 2007). Perchlorate, thiocyanate, and nitrate were assayed in the chow of only Treatment 2 (the standard AIN-76A diet) and Treatment 5 (most deficient iodine diet). Iodide was measured in sample pellets from all dietary treatment groups. For each evaluation, four rat chow pellets were ground to a fine powder with a mortar and pestle and a 1.0 g sample was dispersed in 15 mL of 0.1 N HCl. Iodine fortification of the rat chow was accomplished using iodate as the source of iodide. To assure quantitative measurement of total iodide in the diet, samples were incubated with HCl. Separate experiments were performed to confirm quantitative conversion of iodate to iodide under these conditions. The mixture was sonicated at room temperature for 60 min, with vortexing every 15 min. After overnight incubation at 4 °C, samples were centrifuged (3270 × g, 25 °C) for 30 min. An aliquot of the supernatant (800 µL) was placed into a centrifuge filter tube (Costar Spin-X, 0.22 µm Nylon) and centrifuged at 16,060 × g. A 500 µL aliquot of the filtrate was transferred to an autosampler vial with 500 µL of internal standard solution and vortex mixed. Quantification was based on the peak area ratio of analyte to stable isotope-labeled internal standard. Two quality control pools were analyzed in each analytical batch with unknown samples. Reported results met the accuracy and precision specifications of the quality control/quality assurance program of the Division of Laboratory Sciences, National Center of Environmental Health, CDC (Caudill et al., 2008).



**Fig. 1.** Experimental time line. Animals arrived on postnatal day 45 and were maintained on Purina 5001 chow for 2 weeks prior to switching to one of 5 casein-based diets with varying levels of iodine. They were maintained on this diet and sacrificed on the morning of the 57th day. Beginning on week 2, animals were placed in metabolism cages for Tuesday through Thursday morning. Food was removed from the cage during the 8 h urine collection period.

### 2.5. Animal sacrifice

Animals were sacrificed on the morning of the final day of the 8 weeks of dietary exposure. Trunk blood was collected following decapitation and allowed to clot on ice for a minimum of 30 min. Serum was separated via centrifugation of clotted samples and stored at  $-80^{\circ}\text{C}$  for later analyses. Thyroid glands were exposed on the trachea, removed and trimmed under a dissecting scope, weighed and stored at  $-80^{\circ}\text{C}$  for iodide content analysis using the methods of Benotti et al. (1965).

### 2.6. Thyroid hormone analysis

All animals were sacrificed between 8:00 am and 12:00 pm. To avoid a temporal bias in sample collection, each treatment group was sampled consecutively until all animals were sacrificed. Serum concentrations of total thyroxine (T4) and total triiodothyronine (T3) were analyzed by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Thyroid stimulating hormone (TSH) was measured using double antibody assay as described by Thibodeaux et al. (2003). All samples were run in duplicate and the intra- and inter-assay variations ranged from 1 to 10%. The lowest calibrator was 5 ng/mL for the T4 and 10 ng/mL for the T3 assays. The limits of detection for each assay were 4.64 ng/mL for T4, 9.88 ng/dL for T3, and 0.39 ng/mL for TSH. All samples evaluated in this study were well above these detectable limits.

### 2.7. Statistical analysis

All data were subjected to analysis of variance (ANOVA) using Statistical Analysis Software (SAS, Cary, NC). Repeated measures ANOVAs were utilized for body weight, food and water consumption, and urinary output measures. One-way ANOVAs were used to evaluate thyroid hormones, thyroid weights, thyroidal and urinary iodine content. When significant main effects or interactions were achieved, group differences were evaluated using Dunnett's *t*-test with *p*-value set at 0.05.

### 2.8. Mathematical modeling

Data from the current study using young female Long Evans rats were compared to predictions generated using the HPT axis model of McLanahan et al. (2008). Briefly, the model, derived from literature reports on adult male rats of albino strains, was run in acsIX (version 2.5.0.6, Aegis Technologies, Huntsville, AL) to estimate serum T4, T3, TSH, and total thyroid iodide. To examine the data from this study, we used study-specific daily iodine intakes, as well as measured body and thyroid weights

(assuming a linear increase in thyroid weight during the course of the study). For initial model simulations, all other model parameters for the female Long Evans rat were assumed to be the same as those developed by McLanahan et al. (2008) for male albino rats. Additional model simulations were performed as a hypothesis generation mechanism to evaluate potential differences between the strains.

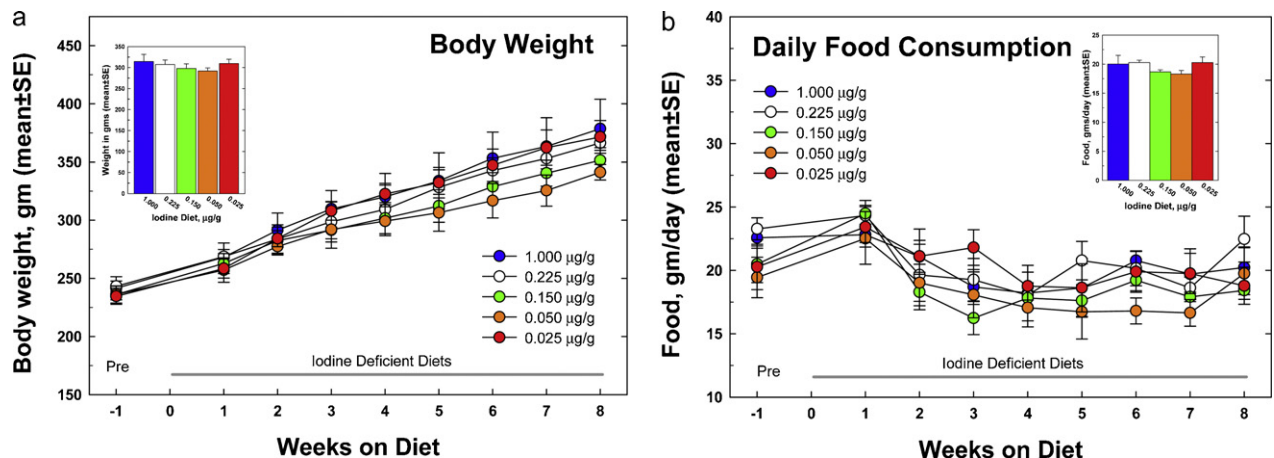
## 3. Results

### 3.1. Food, body weight and urinalysis

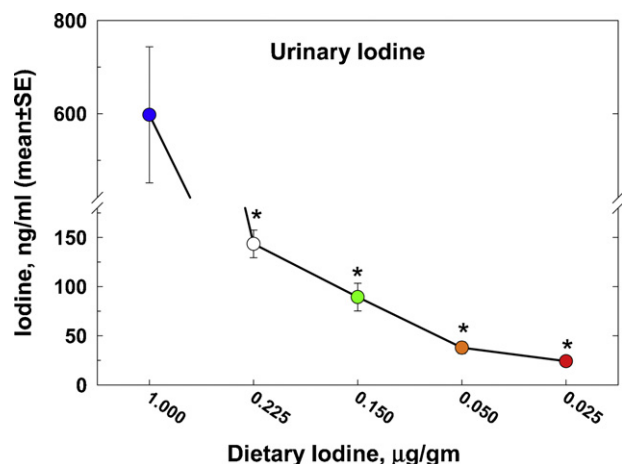
Mass spectrometric analysis of samples from each diet revealed slightly lower concentrations of iodine than targeted levels and ranged from 10 to 1300 ng/g of chow (summarized in Table 1). By comparison, a sample of the standard Purina 5001 laboratory chow animals were fed prior to commencement of the study contained 754 ng iodine/g.

Food consumption was recorded on a weekly basis beginning 1 week prior to introduction of the specialized diets. Food consumption did not vary among the treatment groups with an average of 19.8 g/day (Fig. 2). A significant main effect of Time [ $F(8,152)=7.67$ ,  $p<0.001$ ] was detected in the absence of a main effect of Treatment [ $p>0.16$ ] or Treatment  $\times$  Time interaction [ $p>0.21$ ]. The main effect of Time appears to be driven by a slight reduction in weekly food consumption between the 2nd and 4th week on the diet. However, mean contrast tests failed to reveal any Treatment-related differences in food consumption over time (all *p*-values  $>0.08$ ).

Mean body weight and food consumption averaged across the 8-weeks of dietary exposure are summarized in the insets of Fig. 2, with no significant differences evident as a function of treatment (both *p* values  $>0.30$ ). Based on measured iodine content in the chow (Table 1) and mean daily food consumption (Fig. 2B, inset), the average daily iodine intake was calculated and is summarized



**Fig. 2.** Body weight and food consumption. (A) All animals gained weight (mean  $\pm$  SEM) over the course of the experiment and no differences were detected as a function of diet. Inset shows the mean body weight averaged across the 8-weeks of the dietary exposure. (B) Average ( $\pm$ SEM) daily food consumption over each week of the 8-week dietary exposure period was similar. Inset shows average daily food consumption.



**Fig. 3.** Dose-dependent reductions in urinary iodine were evident with significant differences from the control diet produced at the lowest iodine diet. No treatment-related differences in urinary content of the other anions were seen. \* $p < 0.05$  compared to Treatment 1 (1.0 µg/gm).

in Table 1. Mean daily iodine intake varied as a function of diet [ $F(4,20) = 159.5$ ,  $p < 0.0001$ ].

Body weight increased over the course of the 8-weeks of the study with minimal group differences detected (Fig. 2A). Repeated measures ANOVA for mean weekly body weights revealed no main effect of Treatment [ $F(4,20) = 1.22$ ,  $p > 0.33$ ], but a significant effect of Time [ $F(8,160) = 226.3$ ,  $p < 0.0001$ ] and a Treatment  $\times$  Time interaction [ $F(32, 160) = 2.50$ ,  $p < 0.0001$ ] were revealed. Step-down contrast ANOVAs indicated that the significant interaction was driven by a slightly lower body weight in Treatment 4 animals relative to Treatment 2 (~12%) that emerged during weeks 4 and 5 of treatment. However, none of the mean contrasts achieved statistically significant levels.

Based on an average daily food consumption of 20 g/day and measured iodine levels in the chow, the daily iodine intake through food was reduced in a predictable fashion from 26 µg/day at the highest iodine content to 0.21 µg/day in the most deficient diet (Table 1). Thiocyanate, perchlorate, and nitrate were also assessed in chow samples from two of the five treatment groups, the moderate (Treatment 2, 0.225 µg/g) and the most deficient (Treatment 5, 0.025 µg/g) iodine diet. Neither thiocyanate ( $1149 \pm 17$  ng/g vs.  $1063 \pm 17$  ng/g,  $p > 0.05$ ), nor nitrate ( $10,699 \pm 178$  ng/g vs.  $10,443 \pm 146$  ng/g,  $p > 0.05$ ) concentration differed between these two diets ( $n = 12$  samples/diet group). Perchlorate concentrations in the moderate diet were slightly higher ( $1.92 \pm 0.74$  ng/g) than in the most deficient diet ( $1.10 \pm 0.05$  ng/g,  $p < 0.0001$ ). These concentrations of perchlorate correspond to daily intakes of 0.13 and 0.07 µg/kg/day, respectively, based on mean body weights and daily food intake.

The daily mean water intake averaged  $24.4 \pm 1.1$  ml and did not differ as a function of treatment group. Urine volumes ranged from 4 to 6 ml per 8 h period and did not differ among treatment groups or over days consuming the diet ( $p > 0.25$ ). The mean urinary anions were calculated per animal from 8 daytime collection periods (no food in the cage) that spanned the 56 days of dietary exposure. Levels of thiocyanate (mean range 5351–7088 ng/ml), perchlorate (0.843–0.97 ng/ml), and nitrate (23,170–27,000 ng/ml) in the urine did not differ as a function of treatment group (all  $p$ 's  $> 0.76$ ). Dose-dependent reductions in urinary iodine were observed as a function of decreasing iodine in the diet (Fig. 3), [ $F(4,20) = 13.13$ ,  $p < 0.0001$ ] and no Treatment  $\times$  Day interaction was evident. As expected, very low levels of iodine were present in the urine of the two lowest iodine diets.

### 3.2. Thyroid weight and iodine content

Dose-dependent increases in thyroid gland weight were observed after animals had consumed the iodine deficient diet for 56 days [ $F(4,20) = 5.07$ ,  $p < 0.0055$ ], with significant thyroid weight increases evident in the most iodine deficient diet group (Fig. 4A). Thyroidal iodine content was also dose-dependently reduced [ $F(4,20) = 3.87$ ,  $p < 0.0173$ ], with significant reductions in the two lowest dietary iodine groups (Fig. 4B). As a result of declining thyroid iodine content commensurate with increasing thyroid weight, thyroidal iodine content based on thyroid weight presented in Fig. 4C reveals a stronger dose-dependent reduction in iodine content based on dietary iodine [ $F(4,20) = 18.17$ ,  $p < 0.0001$ ]. All dietary groups had significantly lower iodine content relative to Treatment 1 (1.0 µg l/gm of chow).

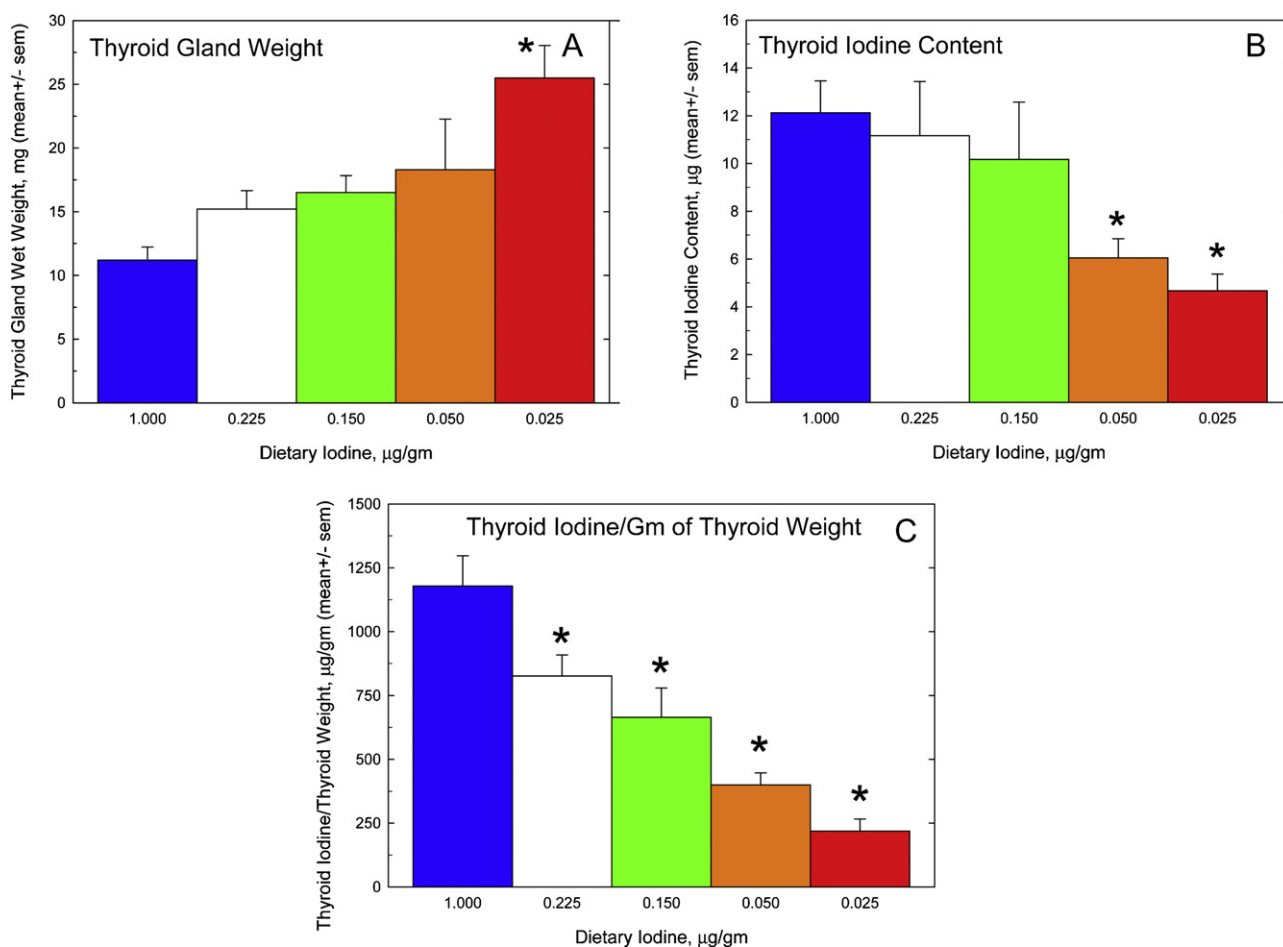
### 3.3. Serum hormone measures

Serum levels of total T4 varied as a function of dietary iodine content [ $F(4,20) = 5.85$ ,  $p < 0.0028$ ]. The two lowest dietary levels resulted in a 19% and 48% reduction in circulating levels of T4 compared to Treatment 1, respectively, and were significantly lower than the other three treatment groups (Dunnett's,  $t$ -test,  $p < 0.05$ ) (Fig. 5A). No statistical differences in serum total T3 were detected [Fig. 5B,  $p > 0.42$ ] and although TSH displayed a modest upward trend as a function of reduced dietary iodine, the increase was not statistically significant (Fig. 5C,  $p > 0.41$ ).

### 3.4. Mathematical modeling

The BBDR model of the HPT axis that was calibrated for the adult male albino rat (McLanahan et al., 2008) was tested to compare model predictions to the Long Evans adult female rat data obtained in this study. To test the ability of the BBDR model to predict our data, we used body and thyroid weights specific to this study, no other changes were made to the model for initial testing purposes. Total thyroid iodide for treatment groups 1 and 2 (26 and 4.2 µg iodine/day) and serum T4 for treatment group 1 were similar to model predictions. As daily iodide intake decreased from 2.5 to 0.7 µg l/day, however, the model predicted a more precipitous drop in total thyroid iodide stores (Fig. 6A) and serum T4 (Fig. 6B) than was observed. In contrast, the BBDR model under-predicted serum T3 levels across all iodide diets (Fig. 6C) by 50%, but did, however, predict the minimal influence of dietary iodide on serum T3 levels. TSH was over-predicted across all iodide diets (6D). Stimulation of TSH production was predicted by the BBDR model for iodide deficient conditions (0.7 and 0.2 µg iodine/day, but was not observed. We observed modest and nonsignificant increases in TSH, which contrasted dramatically to the sharp rise in TSH, predicted by the model, as a function of decreasing iodine intake (Fig. 6D).

The failure of the albino strain calibrated BBDR model prompted hypotheses about HPT axis strain differences. We hypothesized that differences in thyroid hormone turnover may exist between strains and adjusted the appropriate parameters available in the existing albino model accordingly. We reduced the value of a few of the model parameters that describe thyroid hormone metabolism (e.g.,  $V_{max}^{UGT_{T4}} - V_{max}$  for T4-G formation in the liver was reduced from 3435.89 to 50 nmol/h/kg<sup>0.75</sup>;  $k_{el_{T4}} - 1$ st order metabolic rate constant for T4 in the body was reduced from 0.05 to 0.01 1/h/kg<sup>0.25</sup>;  $k_{met_{L_{T3}}} - 1$ st order metabolic rate constant for liver metabolism of T3 was reduced from 3.65 to 0.6 1/h/kg<sup>0.25</sup>). In addition, since changes in serum T4 levels in Long Evans rats do not result in the same magnitude of changes in serum TSH as they do in albino rats, we hypothesized that the set-point or feedback of T4 on TSH production is different between strains. Thus, we reduced the model



**Fig. 4.** (A) Dose-dependent increments in thyroid gland weight were detected at all levels of iodine deficiency, with significant increases in the lowest iodine diet. (B) Thyroidal iodine content was significantly reduced in the two lowest iodine dietary groups. \* $p < 0.05$ . (C) Thyroid iodine content expressed as a function of thyroid weight showed significant reductions at all levels of dietary iodine relative to Treatment 1 (1.0 µg I/gm of chow). \* $p < 0.05$ .

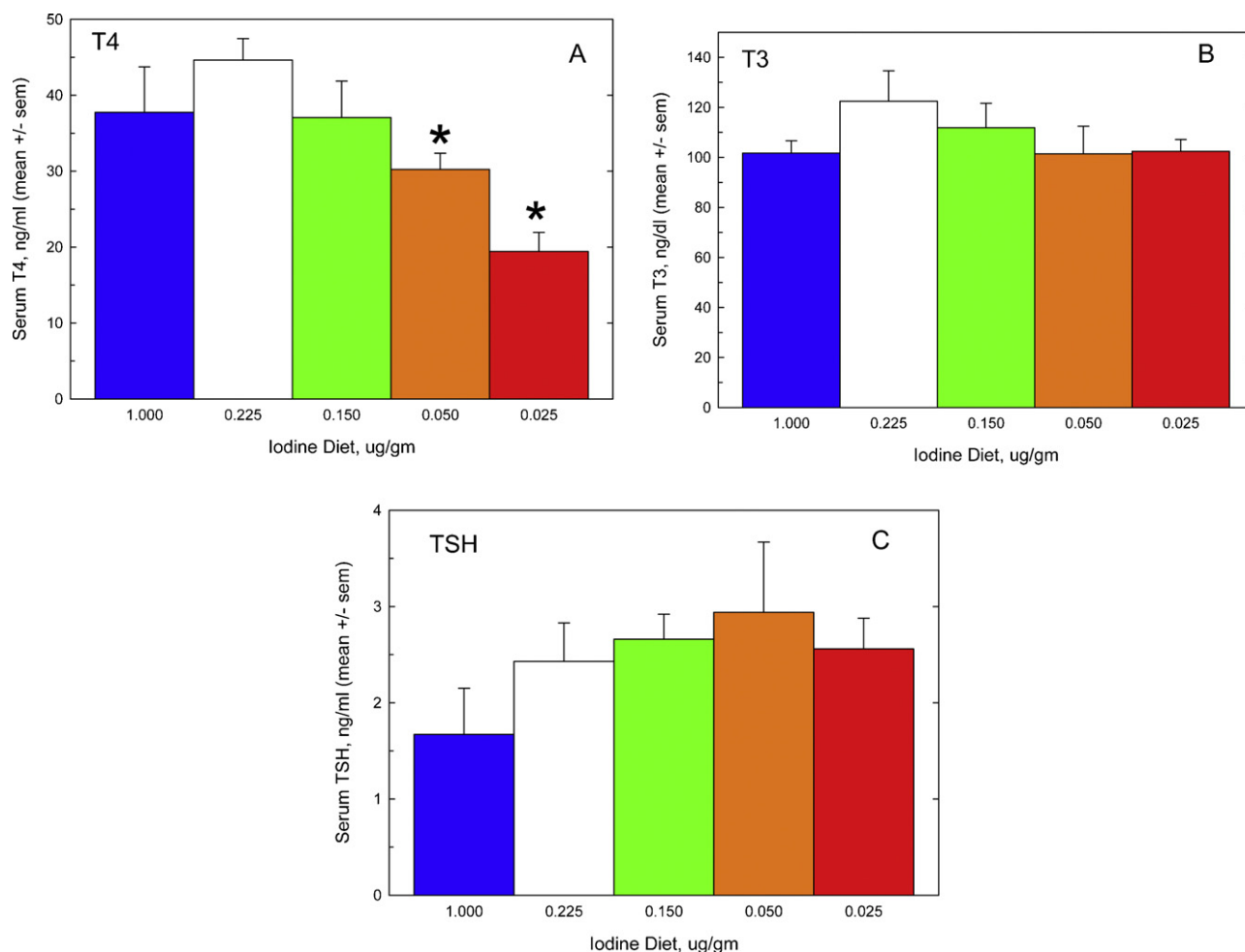
parameter  $K_{inh,T4}$  – T4 concentration that produces half-maximal TSH production, from 0.2 nmol/L to 0.055 nmol/L. Collectively, the results of these changes in model parameters provided a much better fit of the observed data and are depicted in dotted lines in Fig. 6.

#### 4. Discussion

The results of this study provide a characterization of dose-responses for key parameters (e.g., T3, T4, TSH, and thyroid iodide content for known rates of iodide intake) essential for quantitative modeling of the thyroid axis in Long Evans female rats under conditions of marginal iodine deficiency. The level of iodide in these casein-based diets did not affect food or water consumption, or body weight gain. Serum T4 levels decreased in a dose-dependent manner, without a significant change in serum T3 or TSH. Thyroid weights increased and thyroidal iodide content declined with decreasing iodine content in the diet. These results were only partially predicted by the albino strain calibrated HPT axis BBDR model of McLanahan et al. (2008).

The present findings are significant as they include information on a number of variables that were either not well controlled or not reported in previous studies (see Pedraza et al., 2006). Other than iodine, no obvious nutritional deficiencies that have plagued earlier studies (Riesco et al., 1976; Okamura et al., 1981) were evident in the present study as food consumption and body weight gain were comparable across groups. Direct measurement of iodide in the various diets, in the urine, and in the thyroid gland provides critical

information for the quantitative dose–response modeling. These measurements, not previously reported together, provide a more complete picture of iodide economy and utilization. Other common anions present in food with the potential to inhibit iodine uptake, i.e., perchlorate, nitrate, thiocyanate (Tonacchera et al., 2004), were examined and while present, levels of nitrate and thiocyanate were constant between the two diets examined (Treatments 2 and 5). As such, these anions are not likely to have significantly impacted the thyroid-related measures. Somewhat lower levels of perchlorate were observed in Treatment 5, the lowest iodine diet, relative to Treatment 2. Based on mean daily food consumption and body weight in this cohort of animals, perchlorate intake is estimated to be  $\sim 0.073$  µg/kg/day for Treatment 5, the most deficient diet, compared to 0.12 µg/kg/day in Treatment 2. Although the difference in chow perchlorate is statistically significant, the concentrations in both diets are very low, with higher perchlorate in the diet with higher iodide content. Thus, the difference between the two may not be of physiological significance. By comparison, Pedraza et al. (2006) supplemented their iodide deficient chow with “very low amounts” of perchlorate (0.005%  $\sim 6667$  µg/kg/day) to decrease the availability to the thyroid of the small amounts of iodine present in the chow and generated during intra-thyroid iodine recycling. As such, the endogenous perchlorate concentrations of our low iodide chow are about four orders of magnitude less than the supplements added by Pedraza et al. (2006). Nonetheless, it is possible that contaminants such as perchlorate, nitrate and thiocyanate have a greater impact on the thyroid axis in the face of lower iodine intake. Studies designed to examine the interactions of these anions *in vivo*



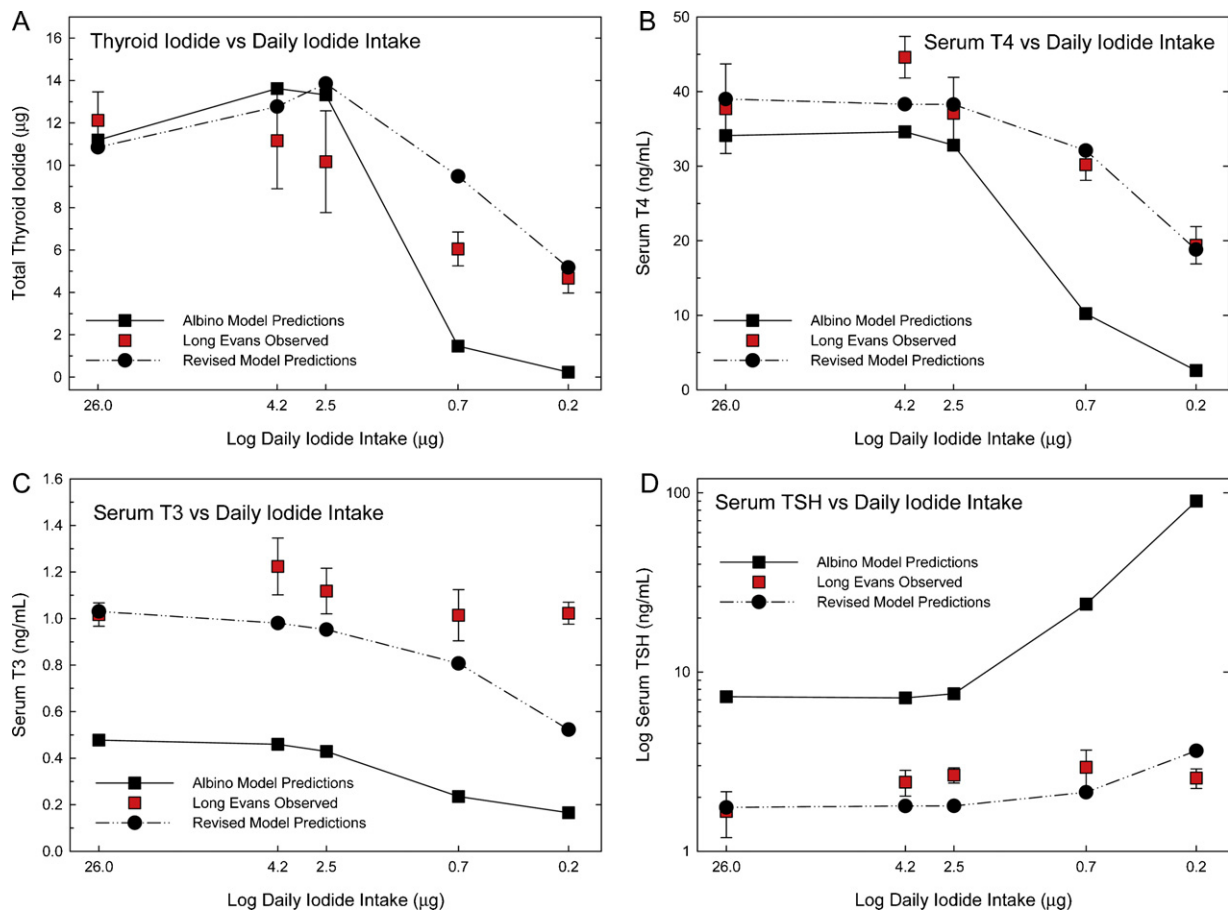
**Fig. 5.** (A) Serum T4 was dose-dependently reduced at the two lowest iodine levels. (B) Serum T3 was not changed by decreasing dietary iodine. (C) TSH was marginally increased in a dose-dependent manner but differences failed to reach statistically significant levels. \* $p < 0.05$  compared to Treatment 1 (1.0  $\mu\text{g}$  l/gm of chow).

have not been undertaken. Background levels of perchlorate and other potential goitrogens in chow formulations are not available from previous studies and thus their contribution to the reported effects on the thyroid system is difficult to evaluate.

Lowering dietary iodine surprisingly did not induce severe reductions in circulating levels of thyroid hormone, the most deficient diet reducing T4 by only 50%, with no significant changes in T3 or TSH. These observations stand in contrast to other reports where severe iodine depletion produced much greater T4 reductions and complementary increases in TSH (e.g., Pedraza et al., 2006; Fukuda et al., 1975). In a recent paper, Pedraza et al. (2006) induced graded levels of dietary iodine deficiency in female Wistar rats and the effects of a level of iodine intake (0.50  $\mu\text{g}/\text{day}$  for 3 months) similar to Treatment 4 of the present study (0.65  $\mu\text{g}/\text{day}$  for 2 months) were reported. As in our study, three months of this degree of iodine deficiency did not impact body weight gain. However, in contrast to our findings, Pedraza et al. (2006) reported greater increases in thyroid weight (96% vs. 63%), more severe reductions in thyroidal iodine content (77% vs. 50%) and serum T4 (49% vs. 19%), and a dramatic increase in serum TSH (500% vs. 50%) relative to our manipulations. Any one of a number of variables that differ among the various studies may contribute to the relatively mild impact of our dietary iodine manipulation of the thyroid axis compared to previous reports. Certainly the practice of pretreatment with high doses of perchlorate as described by Pedraza et al. (2006) to reduce resident iodine stores would alter the baseline from which moderate degrees of iodine deficiency could continue to impair thyroid

function. Others studies also varied widely in the duration of exposure, age, strain, and sex of the animal used (e.g., Heninger and Albright, 1975; Versloot et al., 1997; Riesco et al., 1976; Okamura et al., 1981). As described above, the presence of other contaminating goitrogens in the chow that were not monitored may have influenced the level of hormone disruption in previous studies. Sensitivity and variability of different analytical methods to determine serum TSH and thyroid hormones may also contribute to variability among studies (see Zoeller et al., 2007; Nelson and Wilcox, 1996; McLanahan et al., 2008; Liewendahl, 1990).

Thyroid hormone disruption induced by low dietary iodide also led to patterns in serum hormones distinct from those observed with other thyroid hormone disruptors such as propylthiouracil (PTU) or perchlorate. Significant reductions in serum T4 were not accompanied by dramatic increases in TSH in this study. Females of this strain exposed to PTU or perchlorate in our laboratory at doses producing equivalent reductions in serum T4 to those observed here with iodine deficiency, resulted in 3–4 fold increases in TSH. (Gilbert and Sui, 2006, 2008; Royland et al., 2008). It has been suggested that more dramatic drops in T4 over a long duration may be necessary before increases in TSH are induced (Elcombe et al., 2002). Relatively stable serum TSH and T3 concentrations, even with reductions in T4, may also relate to the observations that increased TSH in the rat appear to relate to increased T3 turnover or the maintenance of stable levels of serum and tissue T3 due to activation of deiodinases (Klaassen and Hood, 2001; Vansell and Klaassen, 2002). It remains to be determined if this mech-



**Fig. 6.** Initial (albino strain calibrated) BBDR model prediction (solid line) and revised model prediction (dotted line) of (A) total thyroid iodide ( $\mu\text{g}$ ) (B) serum T4; (C) serum T3 (ng/mL); and (D) serum TSH (ng/mL) compared with data (individual squares, mean  $\pm$  SEM) from female Long Evans rats determined in this study. Overall the revised model, with alterations in parameters controlling hormone turnover and set point (see text), provided better predictions of the observed changes in thyroid hormones and iodide compared to the albino strain-calibrated model.

anism, based on data from xenobiotics that act via upregulation of hepatic catabolism, is also engaged in response to treatments with different modes and sites of action within the thyroid axis.

Despite a few study-specific modifications (e.g., body and thyroid weight), a rat BBDR model of iodine deficiency for adult albino rats (McLanahan et al., 2008) did not adequately predict all the thyroid axis responses observed in the present study. Compared to the model predictions, the female Long Evans rat appeared relatively insensitive to low-iodide intake. Thyroid iodide stores and serum T4 did not decrease as much as was predicted. It does appear that the baseline for serum T3 and TSH levels may be different between the two strains.

The inability of the albino strain BBDR model to predict effects of iodine deficiency in LE rats was not completely unexpected. Strain and sex differences in circulating levels of thyroid hormones, TSH, thyroid releasing hormone, and pituitary deiodinase activity have been described (Correa da Costa et al., 2001; Gayo et al., 1986; Fukuda et al., 1975; Köhrle et al., 1995; Segal et al., 1982). Differences in T4 half-life and clearance rates may also contribute to strain sensitivities. Earlier reports by Okamura et al. (1981) suggested that the Long Evans rat in particular, controlling for age and sex, responded to a lesser degree to the same iodine deficient diet administered for 28 days compared to the Wistar, Simonsen Albino, and Holtzman and Charles River Sprague–Dawley rats. Follow-up experiments performed in the same laboratory indicated there were no sex differences in these measures within the Long Evans strain (Okamura et al., 1981).

Using the existing model, we explored hypotheses about strain differences by adjusting model parameters that control homeostatic set points for TSH, thyroid hormone metabolism and utilization rates. These parametric changes resulted in better fits of model predictions to observed data and suggest that Long Evans and albino rats may differ in thyroid hormone turnover rates as well as TSH/T4 negative feedback. The existing BBDR model (McLanahan et al., 2008) assumed T4 is the predominant signal for TSH production under conditions of iodine deficiency (but see Klaassen and Hood, 2002; Vansell and Klaassen, 2002). Experimental studies are required to test hypotheses about strain differences in the HPT axis and iodide economy.

Our ultimate goal is to provide sufficient biological data for refining a BBDR model of the hypothalamic–pituitary–thyroid axis in the female rodent for quantitative dose–response modeling of marginal iodine deficiency during gestation and lactation. Hormonal changes and metabolic demands during pregnancy result in profound alterations in the biochemical parameters of thyroid function (e.g., decreases in free hormone concentrations, increases followed by decreases in basal TSH, modifications of peripheral metabolism of maternal thyroid hormones (Glinioer, 1999)). There is insufficient information available to model changes in the pregnant rat. Incorporation of data from this study into a quantitative dose–response model of the thyroid axis for iodine deficiency in the female rat forms the basis for generating a BBDR model capable of quantitatively describing changes that occur in the thyroid axis during pregnancy and lactation (see Fisher et al., 2010; Li et al., 2010; Gilbert et al., 2009). Furthermore, a number of environmental con-

taminants are known to disrupt the thyroid axis (Brucker-Davis, 1998). It is important to determine the impact of environmental toxicants with thyroid disrupting action on neurodevelopment under conditions of maternal iodine insufficiency. Data from this initial study will be applied to recalibrate, refine, and modify the first generation BBDR-Hypothalamic Pituitary Thyroid axis model evaluated here and to develop models for the female pregnant and lactating rat (Fisher et al., 2010; Li et al., 2010). The present findings also formed the basis for ongoing studies on the neurological sequelae associated with developmental hypothyroxenemia (Gilbert et al., 2009). Once established, this model will be useful in predicting outcomes from exposures to xenobiotics that alter thyroid allostasis during critical periods of brain development.

### Conflict of interest

The authors declare there are no conflicts of interest.

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