Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in electronic equipment, home furnishings, textiles, and construction materials. They are similar to polychlorinated biphenyls (PCBs) in structure and in their persistence and bioaccumulative properties (Birnbaum and Staskal 2004). Over the last 20 years, PBDE levels have increased in human samples, whereas PCBs have declined (Schecter et al. 2005).

Because PBDEs are similar in structure to thyroxine (T4) and triiodothyronine (T3) (Hamers et al. 2006), concerns have been raised regarding their effect on thyroid function, which is regulated by the hypothalamo–pituitary–thyroid axis and influences development and gene expression in vertebrates (Zoeller et al. 2007). Reduction of circulating thyroid hormone is compensated for by release of thyroid-releasing hormone from the hypothalamus, which in turn increases thyroid-stimulating hormone (TSH) release from the pituitary, ultimately stimulating thyroid hormone production. T4 and T3 are transported to peripheral tissues bound to proteins, primarily T4-binding globulin (TBG), but also to albumin and transthyretin (TTR). TBG production is stimulated by estrogen and inhibited by testosterone. T3 is the major hormone produced by the thyroid. Some T3 is produced directly by the thyroid, but most is derived from peripheral deiodination of T4. T3 and T4 are primarily metabolized by deiodination to diiodothyronine and reverse T3 (rT3), with some metabolism through glucuronidation, sulfonation, and other pathways. This complex system is vulnerable to disruption by a variety of chemicals through changes in hormone production, transport, and/or metabolism (Zoeller et al. 2007).

Biologic effects of PBDEs in rodents are similar to those of PCBs, with increased risks for reproductive and endocrine disruption (Ellis-Hutchings et al. 2006; Lilienthal et al. 2006; Stoker et al. 2004; Zhou et al. 2002), and neurodevelopmental problems (Kuriyama et al. 2005). In humans, PCBs have been associated with disruption of thyroid hormone homeostasis (Langer et al. 2007; Persky et al. 2001; Turyk et al. 2007), but the effects of PBDEs on thyroid hormones have been investigated only in a few smaller studies (Bloom et al. 2008; Hagem et al. 2001; Julander et al. 2005; Yuan et al. 2006).

In 2001, we reported that PCBs were associated with lower levels of T4 and free T4 index in women and T4 and sex-hormone–binding globulin (SHBG)-bound testosterone in men from a cohort of frequent and infrequent Great Lakes fish consumers (Persky et al. 2001). In 2003, we invited participants from the original cohort to participate in a follow-up study to explore potential mechanisms by which PBDEs, PCBs, and p,p’-diphenyl-dichloroethene (DDE) might be affecting thyroid hormone balance. In addition to the standard hormones (free and total T4 and T3, as well as TSH), we explored via additional laboratory parameters specific mechanisms of action suggested by laboratory studies, such as changes in transport by serum-binding proteins (Hallgren et al. 2001; Hamers et al. 2006) and increase in thyroglobulin antibodies (Langer et al. 2007). In this study we explored the relationship of PBDE exposure with hormone homeostasis, thyroglobulin antibodies, and thyroid disease in men. Associations of thyroid hormones with PCB congeners and DDE will be reported separately.
the University of Illinois at Chicago, and all subjects gave written informed consent before participation.

**Exposure analyses.** Serum samples were tested for PBDEs, PCBs, and DDE by the Wisconsin State Laboratory of Hygiene as previously described (Anderson et al. 2008). Briefly, sera were extracted with hexane/ethyl ether, with cleanup and fractionation using Florisil, silica gel, and concentrated sulfuric acid. PBDEs were analyzed by gas chromatography–mass spectrometry (GC-MS), and PCBs and DDE by GC. Quality control was monitored by the use of method blanks, spikes of bovine serum, duplicates of bovine serum spikes or sample duplicates, surrogate spikes, and confirmation of the analytes by second column or GC-MS, as appropriate. Mean recoveries were 76–91% for 24 tri- to decaBDE congeners, 97% for DDE, and 81–94% for di- to hexaPCB congeners.

**Hormone analyses.** Hormone assays were performed on serum and urine samples at Northwestern University in R.C.’s laboratory. Total $T_4$ (serum and urine), total $T_3$, and the free unbound concentrations of these thyroid hormones were measured by radioimmunoassay (Dissociative Products Corporation, Inc., Los Angeles, CA). Specificity was > 99%. Interassay and intraassay coefficient of variations (CVs) were, respectively, 3.0% and 3.3% for total $T_4$, 4.0% and 5.0% for total $T_3$, 6.9% and 4.3% for free $T_3$, 28.8% and 7.9% for free $T_3$, and 5.6% and 14.9% for urinary total $T_4$. We measured $T_3$ and $T_4$ cross-react by < 0.1%. Interassay and intraassay CVs were 13.4% and 5.5%, respectively.

We measured TSH and TBG in the Immulite System (Diagnostic Products). The TSH assay had a sensitivity of 0.002 mU/mL and was highly specific, with < 0.1% cross-reaction with other glycoprotein hormones. The TSH assay also had highly specific, with a sensitivity of 1.1 µg/mL. Interassay CVs were 14.7% for TSH and 9.7% for TBG.

We examined the distribution of $T_4$ binding in plasma by radioelectrophoresis (Borst et al. 1982; Leopold et al. 1987). We separated albumin- and TBG-bound $^{125}$I-$T_4$ on agarose gels after incubation of $^{125}$I-$T_4$ with the serum for 2 hr at 37°C. TTR, which we did not quantify in this analysis, is clearly separated from TBG in this system. The gels were stained with bromothymol blue to identify albumin in the samples. Standards of TBG and TTR were run in parallel to determine the location relative to albumin on the gel. The areas corresponding to TBG and albumin were cut out of the gel and counted in a gamma counter, and the percentage of the total $^{125}$I-$T_4$ in each fraction was determined.

Interassay CVs were 3.4% for TBG-bound $T_4$ and 11.9% for albumin-bound $T_4$.

We measured urine creatinine spectrophotometrically by the Jaffe reaction after ethyl ether extraction. Interassay and intraassay CVs were 9.3% and 5.6%, respectively.

We measured testosterone in serum using a coated tube assay that employs $^{125}$I-labeled testosterone as the tracer (Diagnostic Systems Laboratories, Webster, TX). The antisera cross-reacted < 0.9% with androstenedione and androstenediol and 5.8% with dihydrotestosterone. Interassay and intraassay CVs were 17.0% and 6.6%, respectively. We measured SHBG using a competitive radioimmunoassay with a sensitivity of 5 nmol/L (Diagnostic Systems Laboratories). The interassay and intraassay CVs were 15.7% and 6.6%, respectively.

SHBG-bound testosterone was determined as described by Bonfrer et al. (1989). We equilibrated a 0.2-mL volume of serum diluted 1/8 with buffer with $^3$H-estradiol overnight at 4°C. A 0.1 mL suspension of a conconavalin-A (Con-A) Sepharose conjugate was added to the serum. SHBG was allowed to bind to the Con-A during a 30-min incubation period at room temperature. Testosterone in the serum maintains its equilibrium concentration with SHBG in the presence of endogenous factors such as other androgens, estrogens, and free fatty acids (Bonfrer et al. 1989; Street et al. 1989). Separation of unbound $^3$H-testosterone from that bound to the Sepharose Con-A was achieved by centrifugation at 0°C to minimize dissociation of bound estradiol. The interassay and intraassay CVs were 4.5% and 3.6%, respectively.

Thyroglobulin antibodies and hemoglobin A1c (HA1c) were measured by Quest Diagnostics (Auburn Hills, MI, and Wood Dale, IL). HA1c was measured by affinity chromatography, which measured total glycosylated hemoglobin, from which HA1c is calculated. Thyroglobulin antibodies were detected in an immunochemiluminometric assay that used avidin beads, biotinylated thyroglobulin, and acridinium ester–labeled thyroglobulin. Total cholesterol and triglycerides were measured by Quest Diagnostics for samples collected in 2004–2005 and by Meriter Laboratories (Madison, WI) for samples collected in 2001–2003. Total serum lipids were calculated by the following formula: total cholesterol (mg/dL) $\times 2.27$ + triglycerides (mg/dL) $\times 62.3$.

**Statistical analyses.** For results below the limit of detection (LOD), we imputed BDE and PCB congener concentrations as the LOD for the individual congener divided by 2. We summed BDE congeners 28, 47, 49, 85, 99, 100, 138, and 153 to derive PBDEs. Similarly, PCBs included PCB congeners 66, 74, 99, 118, 128, 146, 167, 172, 177, 178, 180, 183, 193, 194, 201, and 206, as well as coeluting congeners 163/138, 170/190, 203/196, 202/171, 208/195, 187/182, and 132/153/105. We used natural log transformations (ln) of ΣPBDEs, BDE-47, ΣPCBs, DDE, TSH, T3, free $T_3$, $T_4$, urinary $T_4$, and SHBG to approximate a normal distribution.

We explored associations of thyroglobulin antibodies and thyroid disease with ΣPBDEs greater than the 90th or 95th percentiles in the full cohort of 405 men using logistic regression models, with adjustment for age.

Participants were excluded from the hormone analyses if they reported medical conditions or medication use known to affect thyroid hormone levels (Meier and Burger 2005). Complete data for exposure and hormone measures were available for 308 men for the hormone analysis after excluding participants missing data for lipids ($n = 12$); using thyroid hormones or having thyroid disease ($n = 21$); using blood-glucose–regulating medications or having diabetes ($n = 66$); using other hormones ($n = 11$; testosterone, systemic corticosteroids, melatonin, human growth hormone), or using other medications known to affect thyroid hormones ($n = 4$; dilantin, tergtolet, lithium, carbodopa).

Associations of hormones with ΣPBDEs and BDE-47 were modeled using linear regression, and Pearson’s partial correlation coefficients for associations of hormones with exposures were estimated with the same variables used in the linear regression models. We considered age, body mass index (BMI), and serum lipids to be important covariates and included them in all multivariate models. Other potential confounding variables were added individually to these models to determine if their inclusion affected the conclusion about the significance of the PBDE/hormone association ($p < 0.05$ or $p > 0.05$). Factors that were evaluated as potential confounders included smoking, alcohol use, medication use (antilipids, beta blockers, furosemide), Great Lakes sport fish meals in the past year, sport fish meals in the past year, ΣPCBs, DDE, years consuming sport fish meals, years consuming Great Lakes sport fish meals, years consuming Great Lakes sport fish meals, and HA1c level. We also considered levels of testosterone, SHBG, and SHBG-bound testosterone as potential confounders for thyroid hormone analyses.

We examined modification of the effect of ΣPBDEs on hormones by other exposure covariates (all potential confounding variables noted above) in linear regression models that included multiplicative interaction terms for ΣPBDEs and the potential effect modifier, adjusting for age, BMI, and lipids. We did not evaluate covariates identified as effect modifiers ($p < 0.05$ for interaction term) as potential confounders, but we stratified models of the effects of ΣPBDEs on hormones by above and below median levels of the effect modifier.
To determine if results were affected by extreme hormone values, we estimated models after exclusion of participants with values more than three interquartile ranges above the 75th percentile or below the 25th percentile for hormone measures. Models were also reestimated using a variable for ΣPBDEs where congeners below the LOD were imputed as 0, but this did not affect our findings.

We designed this study to explore associations of PBDEs with standard thyroid hormone parameters, free and total T₄ and T₃, as well as TSH, and also with additional laboratory parameters to test specific mechanisms of action. We explored patterns in the associations of PBDEs with thyroid hormones regarding congener-specific associations and independence of associations to examine mechanistic hypotheses.

We estimated dose–response models by linear regression for BDE congeners 47, 99, 100, and 153 using either indicator variables for tertiles 2 and 3, with tertile 1 as the reference category, or the ordinal tertile variable to test for a trend over the categories. For BDEs 99, 100, and 153, the lowest tertile included all participants with measurements < LOD. BDE tertiles (ng/g) were defined as follows: BDE-47, < LOD to 0.06 (n = 106), > 0.06–0.15 (n = 101), > 0.15 (n = 101); BDE-99, < LOD (n = 117), 0.025–0.046 (n = 97), > 0.046 (n = 94); BDE-100, < LOD (n = 205), 0.026–0.05 (n = 52), > 0.05 (n = 51); BDE-153, < LOD (n = 212), 0.05–0.099 (n = 49), > 0.099 (n = 47). We examined similar models for ΣPBDE quartiles.

Because an effect of PBDEs at one point in thyroid homeostasis could potentially change other related thyroid hormone parameters, we examined the independence of significant associations of thyroid hormones with ΣPBDEs regarding other measured thyroid hormones. When we identified a significant association between ΣPBDEs and a thyroid hormone, we further adjusted the linear regression model for other thyroid hormone levels individually. When the β-coefficient for ΣPBDEs changed by > 20% after adjustment for a second hormone, this suggested that the effect of ΣPBDEs on the original hormone may be related to or mediated by the second hormone.

Results

Characteristics of the cohort included in the hormone analysis are shown in Table 1. Most men drank alcohol at least once a month (78%), but few smoked cigarettes (11%), and medication use varied, with 33% using antilipidemics, 17% using beta blockers, and 3% using furosamide (data not shown). Levels of ΣPBDEs in the men were similar to those found for a large sample representative of the U.S. population regarding other age and ethnicity; in this study, geometric mean = 27 ng/g lipid [95% confidence interval (CI), 24–30 ng/g lipid]; in the National Health and Nutrition Examination Survey, geometric mean = 34 ng/g lipid (95% CI, 27–43 ng/g lipid) (Anderson et al. 2008). However, levels of ΣPCBs were somewhat higher in the present study than in the National Health and Nutrition Survey (Anderson et al. 2008). Because we excluded men with thyroid disease, thyroid hormone levels were predominantly within normal ranges (Table 1).

ΣPBDEs was significantly and positively associated with several thyroid hormones, including total T₄, free T₄, urinary T₄, rT₃, and albumin-bound T₄ (only after exclusion of two extreme outliers) and was negatively associated with TSH, but only after control for sport fish consumption (Table 2). We found generally similar associations for these thyroid hormones with BDE-47, the dominant BDE congener (Table 2).

Figure 1 shows dose–response models for quartiles of ΣPBDE. We saw the strongest dose response for urinary T₄, whereas only the highest ΣPBDE quartile was elevated for free T₄ and rT₃. Total T₃, which was not significantly associated in the continuous analysis (Table 2), was significantly negatively associated with ΣPBDE quartiles. On the other hand, total T₄ and TSH were not significantly associated with ΣPBDEs in the ordinal dose–response models. The effect of ΣPBDEs on T₄ binding to serum proteins was limited to the highest exposure quartile.

Urinary T₄ was the only hormone associated with all four BDE congeners (Table 3). rT₃, total T₄, and free T₄ were positively associated with BDE-99 and BDE-153, total T₃ was negatively associated with BDE-47 and BDE-153, and free T₃ was negatively associated with BDE-153. BDE-100 was negatively associated with TBG-bound T₄ and positively associated with albumin-bound T₄, with similar associations for BDE-153, but only in the highest tertile (Table 3).

We found significant associations among many of the thyroid hormone measurements (data not shown). Because an effect of PBDEs at one point in thyroid homeostasis could potentially change other related thyroid hormone parameters, we examined the independence of significant associations of thyroid hormones with ΣPBDEs regarding other measured thyroid hormones (Table 4). The associations of urinary T₄ and albumin-bound T₄ with ΣPBDEs were independent of other thyroid hormones (Table 4). However, associations of ΣPBDEs with rT₃, free T₄, total T₄,

| Table 1. Distribution of covariates, exposure measures, and endogenous hormone levels in 308 men. |
|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Characteristic          | Mean Minimum 25th 50th 75th 95th Maximum |
|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Age (years)              | 59 30 53 59 67 74 82 |
| BMI (kg/m²)              | 29.8 18.4 26.8 29.2 32.0 36.4 40.2 |
| Serum lipids (mg/dL)     | 720.8 570.1 600.1 693.6 810.0 976.6 2459.1 |
| Urinary creatinine (μg/mL)| 1402.4 658.9 843.3 1268.1 1877.3 2753.8 7293.0 |
| HA1c (%)                 | 5.6 4.4 5.4 5.6 5.9 6.5 8.8 |
| ΣPBDE₆₂⁴ng/g lipid       | 69.9 15.8 29.3 38.4 62.4 193.4 1360.2 |
| ΣPBDE₆/ng/g      | 0.47 0.13 0.20 0.26 0.41 1.49 10.15 |
| BDE-47 ng/g             | 0.22 0.01 0.07 0.11 0.18 0.89 5.90 |
| BDE-99 ng/g             | 0.06 0.01 0.01 0.03 0.05 0.16 2.60 |
| rT₃ (ng/mL)              | 7.2 3.3 6.4 7.1 8.0 9.6 20.6 |
| T₃ (ng/mL)               | 1.19 0.62 1.01 1.19 1.35 1.60 1.82 |
| Urinary T₄ (ng/dL)       | 1216.9 686.9 581.5 1049.7 1522.6 3035.2 5677.7 |
| TBG (μg/mL)              | 19.1 1.5 16.4 18.8 21.0 27.0 43.7 |
| TBG-bound T₄ (%)         | 77.3 49.5 74.4 77.7 80.8 84.4 90.9 |
| Albumin-bound T₄ (%)     | 17.7 5.9 14.7 17.2 20.2 25.9 46.2 |
| Testosterone (ng/mL)     | 3.08 0.10 2.28 2.93 3.85 5.24 6.33 |
| SHBG (μmol/L)            | 169.6 0 88.3 142.4 221.2 421.5 630.0 |
| SHBG-bound testosterone (%) | 32.5 0.2 27.5 32.0 37.5 44.7 54.4 |

*For BDE and PCB congeners and DDE, we imputed values < LOD as the LOD for each analyte/2: LOD = 0.025 ng/g for BDES 28, 47, 99, 100, and 100 LOD = 0.05 ng/g for BDES 138 and 153. Proportion of samples > LOD: BDE-47 = 96%, BDE-99 = 62%, BDE-100 =33% and BDE-153 = 31%. Normal reference range TSH = 0.4–4.7 μIU/mL, total T₄ = 7–195 ng/dL, free T₄ = 1.4–2.4 pg/mL, total T₃ = 5–12 μg/dL, free T₃ = 0.8–2.4 ng/mL, TBG = 13–39 μg/mL.*
Variables with hormone levels. All models adjusted for age, BMI, and serum lipids. Urinary total T4 was also adjusted for urinary creatinine.

Table 2. Associations of hormones with ΣPBDEs and BDE-47: Pearson’s correlation coefficients.

| Hormone                        | No. | Measure  | Unadjusted | Adjusted | Unadjusted | Adjusted |
|--------------------------------|-----|----------|------------|----------|------------|----------|
| Ln TSH (μIU/mL)                | 304 | r-Value  | -0.05      | -0.10    | -0.08      | -0.14    |
|                                |     | p-Value  | 0.39       | 0.07     | 0.18       | 0.02     |
| Total T4 (ng/dL)               | 305 | r-Value  | -0.02      | -0.04    | -0.02      | -0.04    |
|                                |     | p-Value  | 0.39       | 0.44     | 0.79       | 0.51     |
| Ln free T3 (pg/mL)             | 306 | r-Value  | -0.05      | -0.06    | -0.03      | -0.01    |
|                                |     | p-Value  | 0.35       | 0.32     | 0.95       | 0.90     |
| Ln T4 (ng/dL)                  | 304 | r-Value  | 0.22       | 0.14     | 0.21       | 0.12     |
|                                |     | p-Value  | <0.0001    | 0.02     | 0.0003     | 0.04     |
| Total T4 (pg/dL)               | 307 | r-Value  | 0.10       | 0.12     | 0.07       | 0.09     |
|                                |     | p-Value  | 0.07       | 0.03     | 0.21       | 0.12     |
| Free T4 (ng/mL)                | 308 | r-Value  | 0.13       | 0.16     | 0.09       | 0.13     |
|                                |     | p-Value  | 0.03       | 0.005    | 0.12       | 0.03     |
| Ln urinary total T4 (pg/mL)    | 268 | r-Value  | 0.20       | 0.25     | 0.19       | 0.25     |
|                                |     | p-Value  | 0.001      | <0.0001  | 0.002      | <0.0001  |
| TBG (μg/mL)                    | 303 | r-Value  | 0.04       | 0.05     | 0.02       | 0.02     |
|                                |     | p-Value  | 0.46       | 0.39     | 0.78       | 0.73     |
| TBG-bound T4 (%)               | 267 | r-Value  | -0.08      | -0.11    | -0.07      | -0.11    |
|                                |     | p-Value  | 0.18       | 0.06     | 0.27       | 0.08     |
| Albumin-bound T4 (%)           | 267 | r-Value  | 0.07       | 0.11     | 0.05       | 0.11     |
|                                |     | p-Value  | 0.26       | 0.06     | 0.37       | 0.08     |
| Testosterone (ng/mL)           | 307 | r-Value  | -0.06      | -0.01    | -0.01      | -0.06    |
|                                |     | p-Value  | 0.26       | 0.06     | 0.81       | 0.29     |
| Ln SHBG (nmol/L)               | 269 | r-Value  | 0.04       | 0.04     | 0.05       | 0.05     |
|                                |     | p-Value  | 0.47       | 0.54     | 0.41       | 0.44     |
| SHBG-bound testosterone (%)    | 269 | r-Value  | -0.02      | -0.04    | -0.03      | -0.05    |
|                                |     | p-Value  | 0.73       | 0.49     | 0.62       | 0.38     |

*Adjusted for age, BMI, and serum lipids. Unless otherwise noted, significance of adjusted models did not change with further adjustment for the following covariates (added individually to model): smoking, alcohol use, antilipid medications, beta blocker medications, furosemide medication, Great Lakes sport fish meals in the past year, urinary creatinine. Adjusted for urinary creatinine.

Discussion

Exposure to PBDEs at levels comparable with those in the general U.S. population was associated with thyroid and steroid hormone levels in adult men without thyroid disease or diabetes. PBDEs were positively related to measures of TBG-bound T4, free T4, and rT3, and inversely related to total T3 and TSH. PBDEs were positively related to the percentage of T4 bound to albumin and inversely related to the percentage of T4 bound to TBG. Associations of BDE congeners with thyroid hormones varied. BDE-47 was positively associated with testosterone levels.

Our finding of increased thyroglobulin antibodies in 31% of participants with the highest PBDE body burdens is potentially of biologic significance because thyroglobulin antibodies are found in 80–90% of patients with chronic autoimmune thyroiditis and 50–60% of patients with Grave’s disease (Marcocci and Marino 2005). The 8% prevalence of antibodies in the entire cohort is similar to rates seen in normal adult male populations (Hollowell et al. 2002). Exposure to PCBs, which are similar in structure to PBDEs, has been associated with increased antithyroxoperoxidase antibodies (Langer et al. 2007). The small number of cases of hypoparathyroidism and hyperthyroid disease limit our ability to draw conclusions on effects of PBDEs on thyroid disease, but the thyroglobulin results may indicate an increased susceptibility to autoimmune thyroiditis in PBDE-exposed persons.
To our knowledge, epidemiologic data on the effects of PBDEs on thyroid hormones in adults is limited to four published studies. First, a longitudinal study of 11 electronic recycling employees found no significant associations of BDE congeners with TSH, total T3, or free T4, but did note nonsignificant trends for increasing free T4 with BDEs 28, 153, and 183 (Julander et al. 2005). Second, free T4 and TSH were not significantly associated with PBDEs in 36 New York anglers, although the associations of BDE congeners with free T4 were consistently positive (Bloom et al. 2008), and the authors estimated that 318 persons would be required to reach significance for the association of 3PBDEs with free T4. Third, Hammar et al. (2001) found a significant negative association of BDE-47 with TSH but no significant association with free and total T3 and T4 in 110 men with high consumption of fish from the Baltic Sea. Our results are consistent with the decreased TSH in Hammar et al.’s study and with the positive direction of the free T4 associations of Bloom et al. (2008) and Julander et al. (2005). Fourth, Yan et al. (2008) found higher TSH levels in electronic waste workers compared with unexposed persons, but PBDE exposures levels were substantially higher in that study than in our fish consumer cohort.

Our findings of a positive association of PBDEs with T3 and free T4 are not, however, consistent with results of laboratory animal studies. In rats and mice, PBDE mixtures and BDE-47 have been shown to decrease T4 and free T4 (Hallgren et al. 2001; Hallgren and Darnauder 2002; Stoker et al. 2004; van der Ven et al. 2008; Zhou et al. 2001, 2002). T3 was also decreased in some studies, but to a lesser extent than total T4 (Zhou et al. 2001), was also decreased in some studies, but to a lesser extent than total T4 (Zhou et al. 2001), and TSH was not affected, except in a 31-day exposure in male rats that had decreased TSH (Stoker et al. 2004). It is not clear why our results are inconsistent with decreased T4 found in PBDE-exposed laboratory animals. Thyroid hormone regulation is similar in vertebrates, but some functions differ by species. For example, more T3 is produced by the thyroid in rats than in that of humans (40% vs. 20%), increasing the importance of deiodinases in controlling T3 levels in humans. In addition, TTR is the dominant binding protein in rats, whereas most thyroid hormone circulating bound to TRG in humans. Rats are more sensitive to effects of PBDEs on thyroid hormones than are mice (Hallgren et al. 2001). Inconsistencies could also be related to generally higher exposure levels in animals, younger life stage at exposure, and congener-specific effects. Mice exposed to BDE-209 had decreased T3, but not T4 (Tseng et al. 2008). Finally, there may be substantial differences in the effects of acute versus chronic exposure.

A major strength of our study is the measurement of specific hormones and BDE congeners, which may offer insights into potential biological pathways. The analysis of the independence of associations between thyroid hormones and PBDEs regarding other measured thyroid hormones suggests independent pathways for PBDE effects on urinary T4 levels and T4 serum protein binding proportions, whereas changes in rT3, total T4, free T4, total T3, and TSH were interrelated. BDE-congener-specific relationships also support different pathways, with associations of BDEs 47, 99, 100, and 153 with urinary T4, BDEs 100 and 153 with T4 serum protein binding proportions, and BDEs 99 and 153 with T4, and free T4.

The association of PBDEs with T1 suggests that PBDEs may affect thyroid hormone deiodinases. Deiodinases play a key role in control of cellular levels of T3 (Blanco and Kim 2006). D3 deiodinase removes iodide from outer ring of thyroid hormones (meta position), converting T4 to T3, whereas D3 deiodinase removes an iodide from the inner ring (ortho position) converting T4 to rT3. D1 can remove iodide from the inner and outer

### Table 3. Associations of hormones with BDE-47, BDE-99, BDE-100, and BDE-153 tertiles.

| Hormone Measure | BDE-47 | BDE-99 | BDE-100 | BDE-153 |
|-----------------|--------|--------|---------|---------|
| Ln TSH (μIU/mL) | | | | |
| β for tertile 2* | -0.14* | -0.14* | -0.07 | -0.05 |
| p-Value for trend | 0.08 | 0.27 | 0.42 | 0.96 |
| Ln T3 (ng/dL) | | | | |
| β for tertile 2* | -2.49 | -2.78 | -5.61** | -3.34 |
| p-Value for trend | 0.02 | 0.84 | 0.18 | 0.04* |
| Ln free T3 (pg/mL) | | | | |
| β for tertile 2* | -0.04 | -0.01 | -0.004 | 0.03 |
| p-Value for trend | 0.16 | 0.56 | 0.47 | 0.04* |
| TSH (μIU/mL) | | | | |
| β for tertile 2* | 0.03 | 0.01 | -0.04 | 0.04 |
| p-Value for trend | 0.09* | 0.13** | 0.11** | 0.12** |

### Legend

*Coefficient estimate from linear regression for association of BDE tertile with hormone level, adjusted for age, BMI, and serum lipids. Unless otherwise noted, significance of adjusted models did not change with further adjustment for the following covariates (added individually to model): smoking, alcohol use, antilipid medications, beta blocker medications, and with the positive direction of the free T4 associations of Bloom et al. (2008) and Julander et al. (2005). Fourth, Yan et al. (2008) found higher TSH levels in electronic waste workers compared with unexposed persons, but PBDE exposures levels were substantially higher in that study than in our fish consumer cohort.

Our findings of a positive association of PBDEs with T3 and free T4 are not, however, consistent with results of laboratory animal studies. In rats and mice, PBDE mixtures and BDE-47 have been shown to decrease T4 and free T4 (Hallgren et al. 2001; Hallgren and Darnauder 2002; Stoker et al. 2004; van der Ven et al. 2008; Zhou et al. 2001, 2002). T3 was also decreased in some studies, but to a lesser extent than total T4 (Zhou et al. 2001), and TSH was not affected, except in a 31-day exposure in male rats that had decreased TSH (Stoker et al. 2004). It is not clear why our results are inconsistent with decreased T4 found in PBDE-exposed laboratory animals. Thyroid hormone regulation is similar in vertebrates, but some functions differ by species. For example, more T3 is produced by the thyroid in rats than in that of humans (40% vs. 20%), increasing the importance of deiodinases in controlling T3 levels in humans. In addition, TTR is the dominant binding protein in rats, whereas most thyroid hormone circulates bound to TRG in humans. Rats are more sensitive to effects of PBDEs on thyroid hormones than are mice (Hallgren et al. 2001). Inconsistencies could also be related to generally higher exposure levels in animals, younger life stage at exposure, and congener-specific effects. Mice exposed to BDE-209 had decreased T3, but not T4 (Tseng et al. 2008). Finally, there may be substantial differences in the effects of acute versus chronic exposure.

A major strength of our study is the measurement of specific hormones and BDE congeners, which may offer insights into potential biological pathways. The analysis of the independence of associations between thyroid hormones and PBDEs regarding other measured thyroid hormones suggests independent pathways for PBDE effects on urinary T4 levels and T4 serum protein binding proportions, whereas changes in rT3, total T4, free T4, total T3, and TSH were interrelated. BDE-congener-specific relationships also support different pathways, with associations of BDEs 47, 99, 100, and 153 with urinary T4, BDEs 100 and 153 with T4 serum protein binding proportions, and BDEs 99 and 153 with T4, and free T4.

The association of PBDEs with T1 suggests that PBDEs may affect thyroid hormone deiodinases. Deiodinases play a key role in control of cellular levels of T3 (Blanco and Kim 2006). D3 deiodinase removes iodide from outer ring of thyroid hormones (meta position), converting T4 to T3, whereas D3 deiodinase removes an iodide from the inner ring (ortho position) converting T4 to rT3. D1 can remove iodide from the inner and outer
rings of thyroid hormones. Changes in deiodinase activity can affect circulating hormone levels, as demonstrated by studies of mice carrying deletion mutations. For example, mice carrying deletion mutations in D2 have elevated T4 and TSH but no changes in T3 (Schneider et al. 2001), those with D3 mutations have elevated T4 and rT3 but no change in T3 and TSH (Schneider et al. 2006), whereas those with D2 mutations are hypothyroid with decreased T4 and T3 but no change in TSH (Hernandez et al. 2006). These studies suggest that inhibition of outer ring deiodinases, most likely D1, by PBDEs could account for the increased T4 and rT3 in our participants with higher exposures. A possible mechanism is competitive inhibition of outer ring deiodinase by BDEs. Evidence that outer ring deiodinases in fish may debranched BDE-99 to BDE-47 by removal of a bromine from the meta position (Benedict et al. 2007), as well as our finding that BDEs 99 and 153, both of which have a bromine in the meta position, were positively associated with rT3, free T4, and total T4, supports this hypothesis. The negative relationship of PBDEs with TSH might be a normal feedback response to elevated T4 levels. Decreased production of total T3 could also be a consequence of decreased outer ring deiodinase activity, although mice with outer ring deiodinase deletion mutations did not have abnormal T3 levels.

The strongest PBDE association we observed was related to urinary total T4 levels. Urinary total T4 levels are not routinely observed in clinical practice. The increase we found, however, is consistent with the noted increases in serum free T4 and albumin-bound T4, although the association of urinary T4 with PBDEs was independent of serum free T4 and albumin-bound T4. Future studies could assess effects of PBDEs on urinary thyroid hormone metabolites as a potential mechanism.

The associations of PBDEs with T4 serum protein binding proportions suggest that PBDEs could be displacing T4 from TBG. Hydroxylated BDE metabolites were able to bind to TTR in vitro (Hamers et al. 2006), and TTR in serum from BDE-47–treated rats showed decreased binding to [125I]T4 serum compared with serum from untreated rats (Hallgren and Darnerud 2002). However, to our knowledge, the potential for BDE congeners and metabolites to compete with T4 binding to TBG has not been tested.

Steroid hormones can affect thyroid hormones through changes in TSH production. We did not find that testosterone levels modified the effects of PBDEs on thyroid hormones, but testosterone and SHBG did confound several associations of PBDEs and thyroid hormones. In addition, we found a positive association of testosterone with BDE-47. Hagmar et al. (2001) did not find associations of BDE-47 with free testosterone, follicle-stimulating hormone, luteinizing hormone, or prolactin in men. In male rats, the onset of prepuberal separation was delayed and ventral prostate and seminal vesicle weights were decreased, but luteinizing hormone and testosterone were not changed by PBDE exposure (Stoker et al. 2004). However, Stoker et al. (2005) found increased luteinizing hormone and a trend for increased steroid concentrations in PBDE-exposed adult male rats, and Lilienthal et al. (2006) observed that testosterone was decreased in male pups prenatally exposed to BDE-99. BDE congeners, in particular BDE-100, are androgen antagonists in vitro (Hamers et al. 2006; Stoker et al. 2005).

Although we excluded persons with diabetes from the hormone analyses, our data suggest that the effects of PBDEs on rT3, free T4, and albumin- and TBG-bound T4 are stronger in persons with higher HAIc levels, which could place persons with moderately increased blood glucose at higher risk of thyroid hormone disruption by PBDEs. rT3 is increased by fasting, malnutrition, and poorly controlled diabetes. Alternatively, changes in thyroid hormones may affect blood glucose (Chidakel et al. 2005).

Our results also suggest that fish consumption may modify the effect of PBDEs on thyroid function. We saw a stronger effect of PBDEs on rT3, and albumin-bound T4 among infrequent consumers and stronger effects on TSH among frequent sport fish consumers. Furthermore, some associations of PBDEs with hormones were modified by consumption of sports fish, but not by PCB or DDE body burdens. These findings are consistent with an interaction between PBDEs and other contaminants in fish on thyroid hormones.

The strengths of the present study include the use of a large, well-defined cohort; assessment of multiple hormones; and consideration of other environmental exposures that can affect thyroid hormones. Our conclusions are limited by those of any cross-sectional investigation. Although our results are inconsistent with animal studies, they are consistent with several human studies. The associations we found were relatively weak, and the highest proportion of hormone variation explained by

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**Table 4.** Associations of thyroid hormones with ΣPBDEs: confounding of significant associations by other thyroid hormones.

| Hormone | Unadjusted | ΣPBDEs with adjustment* |
|---------|------------|-------------------------|
| Ln urinary T4 | 0.21 | 0.21 |
| p-Value | 0.001 | 0.001 |
| Albumin-bound T4 | 0.79 | 0.79 |
| p-Value | 0.04 | 0.04 |
| Ln TSH | -0.10 | -0.10 |
| p-Value | 0.04 | 0.04 |
| Total T4 | 0.21 | 0.21 |
| p-Value | 0.03 | 0.03 |
| Free T4 | 0.054 | 0.054 |
| p-Value | 0.005 | 0.005 |
| Ln rT3 | 0.071 | 0.071 |
| p-Value | 0.02 | 0.02 |
| Total rT3 | -1.50 | -1.50 |
| p-Value | 0.03 | 0.03 |

*All linear regression were adjusted for age, BMI, and serum lipid; urinary T4 was also adjusted for creatinine; and TSH models were also adjusted for Great Lakes fish meals, Extreme outliers for albumin-bound T4, were excluded for albumin-bound T4 models. Ordinal variables for 2PBDE quartiles were used in total T4 models. *-Coefficient and p-value for ΣPBDEs from linear regression model predicting hormone levels. \( ^{\text{β}} \)-Coefficient change of > 20% with control for second hormone.

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**Table 5.** Age-adjusted odds of thyroid disease and thyroglobulin antibodies with high PBDE exposure in full cohort of 405 men.

| Condition | \( \Sigma \text{PBDE > 95th percentile}\) | \( \Sigma \text{PBDE > 90th percentile}\) |
|-----------|--------------------------------------|--------------------------------------|
| Any thyroid disease | 20/405 (5.0) | 4/405 (1.0) |
| Hypothyroid disease | 14/405 (3.5) | 2/405 (0.5) |
| Hyperthyroid disease | 5/405 (1.2) | 2/405 (0.5) |
| Thyroglobulin antibodies | 27/348 (7.8) | 5/36 (1.4) |

*PBDE 95th percentile = 1.47 ng/g. \( \Sigma \text{PBDE 90th percentile} = 0.78 \text{ ng/g. Any thyroid disease includes hypothyroidism, hyperthyroidism, goiter, Graves’ disease, Hashimoto’s disease, and thyroid tumor.}
PBBE was approximately 6% for urinary T$_4$ ($r = 0.25$). There were some inconsistencies between results of models with continuous and ordinal exposure variables, with effects seen only in highest exposure category for some hormones. This pattern might be related to the extremely skewed distribution of PBBEs in the study cohort (Table 1). In addition, hormone parameters show inconsistencies between models of ΣPBBEs and individual BDE congeners, which may be explained by congener-specific effects, as supported by animal and in vitro data.

Although PBBE levels are lower than PCB or DDE levels, PBDE body burdens are increasing (Schechter et al. 2005). Older adults, who have a high risk of thyroid disease, are more likely to have BDE-47 levels above the 95th percentile level of 291 ng/g lipid (Sjodin et al. 2008). In the present study, exposures were similar to those of the U.S. population (Anderson et al. 2008). With increasing PBDE body burdens, we found increases in T$_4$, but decreases in T$_3$, and TSH. In addition, thyroglobulin antibodies were higher in men with the highest PBDE body burdens. This is the first large study to link PBDE exposure with changes in thyroid antibodies and thyroid hormone homeostasis in men.

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