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Prenatal dioxin exposure and thyroid hormone levels in the Seveso Second Generation Study

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Abstract

Background: In animal studies, perinatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters thyroid homoeostasis and thyroid hormone concentrations; epidemiologic evidence is limited.

Objectives: We aimed to determine the association of prenatal exposure to TCDD with thyroid hormone concentrations in the Seveso Second Generation Study, a unique cohort of children born to TCDD-exposed women resulting from a 1976 chemical factory explosion in Seveso, Italy.

Methods: We included 570 children (288 female, 282 male) with complete follow-up data, including a fasting blood draw. Serum levels of total and free thyroxine (T4), free triiodothyronine (T3), and thyroid stimulating hormone (TSH) were measured using immunoassays. We defined prenatal TCDD exposure as: 1) maternal initial TCDD concentration measured in serum collected soon after the explosion and 2) maternal TCDD estimated at pregnancy.

Results: Compared to the lowest quartile (Q1), maternal initial serum TCDD was associated with lower free T3 (Q2: adj-β = −0.13, 95%CI −0.26, 0.00; Q3: adj-β = −0.22, 95%CI −0.35, −0.09; Q4: adj-β = −0.14, 95%CI −0.28, 0.00; *p*-trend = 0.02). In participants with high thyroid antibody

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Brenda Eskenazi. Conceptualization, Funding Acquisition, Writing - Review & Editing

The authors declare they have no actual or potential competing financial interests.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

status, inverse associations between maternal initial serum TCDD and free T3 were significantly stronger than in participants with normal antibody status (*p*-interaction = 0.02). We also observed a positive association between maternal initial serum TCDD and TSH concentrations in participants with high thyroid antibody status (Q2: adj-β = 11.4%, 95%CI −25.2, 66.1; Q3: adj-β = 49.0%, 95%CI 3.0, 115.5; Q4: adj-β = 105.5, 95%CI 36.6, 209.2; *p*-trend < 0.01) but not in those participants with normal antibody status (*p*-interaction < 0.01). Similar results were found for TCDD estimated at pregnancy.

Discussion: Our results suggest prenatal exposure to TCDD, a potent endocrine-disrupting compound, may alter thyroid function later in life. Populations with additional thyroid stress may be particularly susceptible to *in utero* exposure of thyroid disrupting chemicals.

Keywords

dioxins; prenatal exposure; Seveso; thyroid hormones; TCDD

INTRODUCTION

The thyroid-endocrine system plays a key role in regulating energy homeostasis, metabolic pathways, and the growth and differentiation of many tissues and organs (Biondi and Cooper 2008; Cooper and Biondi 2012). The thyroid gland produces two related hormones, thyroxine (T4) and triiodothyronine (T3), which are under the control of the hypothalamus-pituitary-thyroid (HPT) axis. Measurement of total hormone (T4, T3) reflects bound and unbound fractions; however, only unbound or free hormone (<1%) is biologically available. Thyroid-axis function is based on negative feedback control of the HPT to maintain free T4 and T3 levels within a narrow range (Giacomini et al. 2006). A small reduction of thyroid hormone triggers a rapid increase of TRH and TSH secretion by the hypothalamus-pituitary axis, resulting in thyroid gland stimulation and increased thyroid hormone production. TSH, with variations much larger than those of T4 and T3, is considered the most sensitive marker of thyroid hormone action.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic member of a class of planar halogenated aromatic hydrocarbons; it is a ubiquitous environmental contaminant, with potent endocrine disrupting effects including thyroid disruption (Birnbaum and Tuomisto 2000; Casals-Casas and Desvergne 2011; Zook and Rappe 1994). Most of TCDD's effects occur via its sustained binding and activation of the aryl hydrocarbon receptor (AhR) (Mimura and Fujii-Kuriyama 2003), a key transcription factor with diverse biological roles including regulation of genes involved in thyroid function (Nishimura et al. 2005a; Pocar et al. 2006). Given its long half-life (~7–9 years), TCDD can be detected in lipid stores of humans, and fetal exposure has been shown to occur through transplacental transfer (Centers for Disease Control and Prevention 2009; Pirkle et al. 1989; Schecter et al. 1990). In animal studies, perinatal dioxin exposure has been shown to disrupt the HPT axis in offspring (Ahmed 2011; Nishimura et al. 2003; Nishimura et al. 2005b; Seo et al. 1995). Decreased levels of total T4 or T3 along with increased levels of TSH have been reported in offspring of dosed pregnant rats (Ahmed 2011; Nishimura et al. 2003; Nishimura et al. 2005b; Seo et al. 1995). Free T4 concentration was also decreased in the only study that measured it (Nishimura et al. 2005b). Sex-specific effects, albeit inconsistent, have been noted in some

studies (Nishimura et al. 2003; Nishimura et al. 2005b; Seo et al. 1995). For example, significant increases in TSH were noted only in male offspring (Nishimura et al. 2005b), and stronger decreases in total T4 were reported in male (Nishimura et al. 2003) or female (Seo et al. 1995) offspring.

Although several epidemiologic studies have examined associations of *in utero* TCDD exposure with neonatal TSH levels (Baccarelli et al. 2008; Koopman-Esseboom et al. 1994; Wang et al. 2005; Wilhelm et al. 2008), only two longitudinal birth cohort studies have examined the relationship between prenatal dioxin exposure and child thyroid levels beyond the first year of age (Ilsen et al. 1996; Leijs et al. 2012; Pluim et al. 1993; Su et al. 2010; Su et al. 2015; ten Tusscher et al. 2008). In Taiwan, 92 children were followed to age 8 years (Su et al. 2015) and in the Netherlands, 38 children were followed into adolescence (14 to 19 years) (Leijs et al. 2012). Results of these two studies suggest prenatal TCDD and related dioxins exposure may disrupt thyroid function, but associations are not consistent and limited by small sample size. To date, no study has examined the longer-term effect of prenatal TCDD exposure on thyroid hormone levels in adulthood.

On 10 July 1976, an explosion inside the ICMESA chemical factory near Seveso, Italy resulted in a toxic plume that exposed nearby residents to high levels of TCDD (Di Domenico et al. 1980; Mocarelli et al. 1988; Needham et al. 1991), but not to other dioxin-like compounds (Mocarelli et al. 1990). The Seveso Women's Health Study (SWHS) is a cohort of female residents exposed to a high dose of TCDD during or before their childbearing years (Eskenazi et al. 2000; Eskenazi et al. 2004). It is unique in that individual-level TCDD exposure was measured in serum collected soon after the explosion. Almost 40 years after the explosion, we followed up the SWHS cohort and enrolled their post-explosion offspring in the Seveso Second Generation study. Here, we examine the relationship of prenatal TCDD exposure with thyroid hormone concentrations in the Seveso second generation.

METHODS

Study Population

Details of SWHS and the Seveso Second Generation Study have been presented elsewhere (Eskenazi et al. 2000; Eskenazi et al. 2018). Briefly, the SWHS cohort, initiated in 1996, includes 981 women who were newborn to 40 years of age in 1976, resided in the most highly contaminated areas at the time of the explosion, and had adequate stored sera collected soon after the explosion in which to measure individual-level TCDD exposure. Enrollment and data collection in the Seveso Second Generation Study took place from May 2014 to June 2016. Eligible participants included SWHS women and their children who were born after the explosion on July 10, 1976 and were 2 years of age or older. A total of 611 children (66.4% of 920 alive and eligible) born to 402 SWHS mothers participated in the study visit. We excluded 28 who did not provide a blood sample and 13 who reported current or recent treatment for thyroid disease, leaving a final analysis sample of 570 children born to 383 mothers.

Data collection

The study was approved by the Institutional Review Boards of the participating institutions. Before data collection, we obtained written informed consent from children 18 years or older and mothers of children less than 18 years, written assent from children who were 13 to 17 years, and oral assent from children who were 7 to 12 years of age. Data collection for all second generation participants included a fasting blood draw and anthropometric measurements, interview, and medical record abstraction. Children 18 years or older completed a structured personal interview; for children <18 years, the mother completed a structured personal interview which included questions about the health of her children. Information collected during the interview included demographic and lifestyle characteristics as well as medical histories.

Prenatal TCDD exposure

We examined prenatal TCDD exposure in two ways: 1) maternal initial (1976) serum TCDD concentration and 2) maternal TCDD serum concentrations estimated at pregnancy. For all SWHS participants, TCDD was measured in archived sera collected soon after the explosion by high-resolution gas chromatography/ high-resolution mass spectrometry methods (Patterson et al. 1987). Details of serum sample selection and 1976 serum TCDD concentrations are presented elsewhere (Eskenazi et al. 2000; Eskenazi et al. 2004). For a subset of SWHS participants who reported a live birth between 1994 and 2014, TCDD was also measured in archived sera (n=312) collected at the 1996 or 2008 follow-up study by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry methods (Patterson and Turner 2005). Details of TCDD concentrations measured in 1996 or 2008 serum are presented elsewhere (Eskenazi et al. 2018; Warner et al. 2014). All values are reported on a lipid weight basis as picograms-per-gram lipid or parts-per-trillion (ppt) (Akins et al. 1989). Non-detectable values were assigned a value of one-half the detection limit (Hornung and Reed 1990). As previously described, maternal TCDD at pregnancy was estimated by extrapolation from the TCDD level closest to but preceding the pregnancy (1976, 1996, 2008) using a first-order kinetic model with a half-life that varies with initial dose, age, and other covariates (Eskenazi et al. 2018; Warner et al. 2014). Maternal TCDD at pregnancy estimates are based on extrapolation from maternal serum TCDD levels measured in samples collected in 1976 for 417 children, in 1996 for 149 children, and in 2008 for 4 children.

Measurement of thyroid hormone levels

Thyroid hormone concentrations were measured at the Hospital of Desio Clinical Chemistry laboratory in serum samples collected in 2014 to 2016 using sandwich (TSH) or competitive (free T3, free T4, and total T4) electrochemiluminescence immunoassays (Roche Diagnostics, Mannheim, Germany). The limits of detection for TSH, free T3, free T4, and total T4 were 0.014μIU/mL, 0.40 pg/mL, 0.19 ρg/mL, and 0.42 μg/dL, respectively. In addition, antithyroid antibodies including thyroglobulin (TgAb) and thyroid peroxidase (TPOAb) antibodies, and TSH receptor antibody (TRAb), were measured in serum by electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany) with limits of detection of 10 IU/mL, 5 IU/mL, and 0.3 IU/mL, respectively. Participants were

considered to have high thyroid antibodies if they had levels of TgAb >115 IU/mL, TPOAb >34 IU/mL, or TRAb >1.8 IU/mL.

Statistical Analyses

Both TCDD exposure variables were initially $\log_{10}$-transformed to reduce the influence of outliers. Concentrations of TSH were strongly right-skewed, so values were $\log_{10}$-transformed to reduce the influence of outliers. Free T3, free T4, and total T4 values were approximately normally distributed and thus expressed on the arithmetic scale. We used multivariable linear regression to evaluate the relationship between prenatal TCDD exposure and thyroid hormone levels. Generalized additive models (GAM) using 3-degree-of-freedom cubic splines identified departures from linearity ($p<0.1$) in several cases (free T3, total T4). As a result, the primary analysis modeled exposures as categorical (quartile) variables. Regression coefficients represent the mean (free T3, free T4, total T4) change or percent (TSH) change associated with each quartile of exposure relative to the lowest quartile. Percent change in TSH was calculated using the following formula $[(10^{\beta} - 1) \times 100]$.

Based on our review of the literature, we considered the following variables collected from interview as potential confounders: maternal age at explosion, maternal age at pregnancy, maternal smoking at pregnancy, household socioeconomic status including education, occupation, income, and marital status, child age and sex, body mass index, and child tobacco or alcohol use. The final set of covariates was determined using a Directed Acyclic Graph (DAG) (Supplementary Figure 1), and included age at interview (continuous) and primary wage earner's education (categorical, ≤ required, secondary school, > secondary). We considered effect modification by sex and by thyroid antibody status (high versus normal) as those with multiple thyroid stressors may be more susceptible to thyroid disrupting chemicals (Webster et al. 2014) by performing stratified analyses, as well as performing formal tests for interaction using crossproduct terms. Interactions with *p*-values < 0.2 were considered significant.

We performed several sensitivity analyses. First, we repeated final models with exposure variables $\log_{10}$-transformed and analyzed as continuous variables. Second, we reran models limiting the analysis to participants who were 13 years or older at the time of blood draw, and then to 18 years or older at the time of blood draw, to minimize the potential impact of thyroid hormone variability observed at younger ages and prepuberty (Taylor et al. 2017). Finally, we used Poisson regression to evaluate whether exposure was associated with increased risk of hypothyroidism or hyperthyroidism, based on reference ranges provided by the laboratory or physician diagnosis including the 13 participants excluded from the main analysis sample. Participants who had TSH levels greater than 4.5 (μIU/L (n=34), had free T4 levels less than 0.9 pg/mL (n= 9), or were taking thyroid hormone supplements (n=8 of the excluded cases) were classified as hypothyroidic (N=51); no participants had TSH levels less than 0.1 (μIU/L or free T4 levels greater than 1.9 pg/mL but 5 of the excluded cases reported current treatment for hyperthyroidism and therefore were considered as hyperthyroidic. For all models, standard errors were estimated using the robust Huber-White sandwich estimator and a clustered sandwich estimator of variance was used to account for

non-independence of sibling clusters. All statistical analyses were performed using STATA 15.0 (College Station, TX).

RESULTS

Select maternal and child characteristics of the study population are presented in Table 1. At the time of the explosion in 1976, mothers (n=383) were an average age of 15.9 (±7.6) years and about one-third were premenarche. Maternal age at pregnancy averaged of 29.1 (±5.0) years, and about 10% of women reported smoking during the pregnancy. At interview, the 570 children were an average of 24.3 (±8.9) years, with approximately equal numbers of males and females. Nearly three-quarters (73.2%) of children were 18 years or older and of these, about one-third were current smokers and two-thirds regularly consumed alcohol. The majority (70.4%) of their households' primary wage earners had more than the required education (16 years is compulsory). Males were more likely than females to report regular consumption of alcohol, but otherwise characteristics did not differ by child sex (see Supplementary Table 1).

Prenatal TCDD exposure based on maternal initial (1976) serum TCDD level was high [median (IQR) = 60.2 (28.4, 156.0) ppt]. As reported previously, in the full SWHS cohort, initial 1976 serum TCDD levels were higher among those who were youngest or who were still premenarche at the time of explosion (Eskenazi et al. 2004). Consistent with this report, we found maternal initial serum TCDD levels were higher for the children who were youngest (2–17 years) since they were also more likely to be born to mothers who were youngest at the time of the explosion; maternal initial serum TCDD levels did not differ by other child factors. With birth years spanning 1976 to 2014, prenatal TCDD exposure based on maternal TCDD estimated at pregnancy was lower [median (IQR) = 14.4 (6.4, 33.3) ppt], but with a wide range (0.1, 1,786 ppt). Maternal TCDD estimated at pregnancy was higher among the children who were oldest (30+ years) since they were born sooner after the explosion (within one half-life), and among children whose mothers were older and postmenarche at the time of the explosion.

The geometric mean TSH was 2.2 (μIU/ml and the means for free T3, free T4, and total T4 were 3.6 pg/mL, 12.0 pg/mL, and 6.6 ug/dL, respectively (see Table 1). A total of 43 (7.5%) participants had thyroid hormone levels outside the reference range provided by the laboratory; 34 had high TSH (> 4.5 (μIU/L) and 9 had low free T4 (< 0.9 pg/mL). The overall prevalence of high antibody status among participants was 11.2 %, with 7.4% and 6.7% positive for TgAb and TPOAb, respectively. In the high antibody group, TSH levels were higher and free T3 and free T4 levels were lower, but only free T3 was significantly different from the normal antibody group. Thyroid hormone levels differed somewhat by participant sex (see Supplementary Table 1). Free T3 and free T4 levels were higher and total T4 levels were lower among males than females, but TSH levels did not differ by participant sex. The prevalence of high thyroid antibody status as well as TgAb and TPOAb was higher among females. None of the measures of high thyroid antibody status (together or individually) was associated with prenatal TCDD exposure in the full sample or by participant sex.

Adjusted linear regression models of the associations between quartiles of prenatal TCDD exposure with thyroid hormone levels are presented in Table 2, with the lowest quartile (Q1) as the reference exposure category. There was little evidence for an association of either measure of prenatal TCDD exposure with TSH or total T4. Maternal initial serum TCDD above Q1 was associated with lower free T4 (Q2: adj-β = −0.42, 95% CI −0.78, −0.05; Q3: adj-β = −0.08, 95% CI −0.46, 0.30; Q4: adj-β = −0.52, 95% CI −0.92, −0.11; *p*-trend = 0.06) and free T3 (Q2: adj-β = −0.13, 95% CI −0.26, 0.00; Q3: adj-β = −0.22, 95% CI −0.35, −0.09; Q4: adj-β = −0.14, 95% CI −0.28, 0.00; *p*-trend = 0.02). Maternal TCDD estimated at pregnancy showed a similar pattern for free T3 (Q2: adj-β = −0.23, 95% CI −0.37, −0.09; Q3: adj-β = −0.18, 95% CI −0.35, −0.02; Q4: adj-β = −0.22, 95% CI −0.39, −0.06; p-trend = 0.02). We did not observe evidence of interaction by sex for either measure of prenatal TCDD exposure (Supplementary Tables 2 and 3).

The adjusted linear regression models of the association between maternal initial (1976) serum TCDD exposure and thyroid hormones when considering antibody status are presented in Table 3 and Figure 1A, B. Inverse associations between maternal initial TCDD levels and free T3 were significantly stronger in participants with high thyroid antibody status compared to those with normal antibody status (*p*-interaction = 0.02). We also observed a positive association between maternal initial (1976) serum TCDD levels and TSH concentrations in participants with high thyroid antibody status but not in those with normal antibody status (*p*-interaction < 0. 01).

The adjusted linear regression models of the association between maternal TCDD estimated at pregnancy and thyroid hormones when considering antibody status are presented in Table 4 and Figure 1 C, D. In participants with high thyroid antibody status, inverse associations between maternal TCDD estimated at pregnancy and free T3 were significantly stronger than in participants with normal antibody status (*p*-interaction = 0.10). We do not see a consistent pattern between maternal TCDD estimated at pregnancy and TSH concentrations in participants with high versus normal thyroid antibody status (*p*-interaction<0.01).

Adjusted linear regression models for continuous ($\log_{10}$) prenatal TCDD exposure measures results were largely consistent with the quartile models (Supplementary Table 4). Results of sensitivity analyses limiting participants to those 13 years or older (Supplementary Tables 5 and 6) and those 18 years or older (Supplementary Tables 7 and 8) yielded similar although somewhat dampened results with the restricted sample size. When we classified participants in binary hypothyroid and hyperthyroid disease categories, there were too few cases of hyperthyroid (N=5) for further analysis. In Poisson regression models for hypothyroid (n=51 cases), we found some evidence to suggest prenatal TCDD exposure may increase risk of hypothyroid. Maternal TCDD estimated at pregnancy in the highest quartile (Q4) compared to Q1 was associated with significantly increased risk of hypothyroid (Q2: adj-RR = 1.85, 95% CI 0.90, 3.78; Q3: adj-RR = 1.37, 95% CI 0.51, 3.63; Q4: adj-RR = 3.19, 95% CI 1.14, 8.91). Maternal initial serum TCDD above Q1 was not associated with risk of hypothyroid (data not shown).

DISCUSSION

In this study, we found associations of prenatal TCDD exposure with altered serum thyroid hormone levels in children born to Seveso women up to 40 years after the explosion. Specifically, we found that prenatal TCDD exposure, as measured by maternal initial 1976 serum TCDD concentration and maternal TCDD estimated at pregnancy, is associated with lower free T3 levels. Maternal initial TCDD was also associated with lower free T4 levels of offspring. Neither measure of prenatal TCDD exposure was associated with changes in total T4 or TSH. We found no evidence of sex-specific effects; results are similar for female and male children. We found some evidence that those with high thyroid antibody status may represent a potentially sensitive group as prenatal TCDD exposure is associated with an increasing trend in serum TSH levels in the high antibody group. The association of prenatal exposure TCDD with lower free T3 levels was found for all children, but was stronger in those with high antibody status.

Our results are not consistent with previous studies. The only two previous studies to go beyond the neonatal period in examining the relationship of prenatal dioxin exposure and thyroid hormones reported early associations of prenatal TCDD exposure with increased TSH and total T4 or T3 at very young ages, 11 weeks in the Netherlands (Pluim et al. 1993) and 2 years in Taiwan (Su et al. 2010); however, these relationships were not sustained with longer follow-up (8 years in Taiwan (Su et al. 2010; Su et al. 2015) and 14 to 19 years in the Netherlands (Ilsen et al. 1996; Leijs et al. 2012; ten Tusscher et al. 2008). However, not all of our findings are directly comparable as neither previous cohort study measured free T3 levels at any follow-up time point. Both the Dutch and Taiwan cohort studies are also limited by small sample and inadequate control for confounding which may partially explain the difference in our results. Additionally, in the Dutch cohort, prenatal exposure was assessed as dioxin-like compounds in breast milk and all study children were breastfed, limiting generalizability of their results to other cohorts (Pluim et al. 1993).

Our findings that prenatal TCDD exposure can alter thyroid hormone homeostasis are biologically plausible. The primary mechanism proposed for thyroid disruption by TCDD is via upregulating expression of thyroid hormone metabolizing enzyme, UDP-GT, in the liver when TCDD binds to AhR (Miller et al. 2009). The potential impact of this mechanism could be increased clearance of T4 or T3 without an associated increase in TSH. Other postulated mechanisms for thyroid disruption by TCDD include inhibition of deiodinases (Boas et al. 2006; Viluksela et al. 2004) and altered binding to serum transport proteins including thyroid-hormone binding globulin (Miller et al. 2009; Zoeller 2010). Our observation of an increasing trend in TSH levels among those with high antibody status suggests those with additional thyroid stress may be more susceptible to thyroid disrupting effects of TCDD, possibly reflecting differences in underlying genetic susceptibility. Though both genetic and environmental factors shape risk of thyroid autoimmunity (Tomer and Huber 2009) and TCDD has been linked to immunotoxicity (Gogal and Holladay 2008; Marshall and Kerkvliet 2010), we did not find evidence that high thyroid antibody status was associated with prenatal exposure to TCDD. Previous studies of organochlorine exposures and elevated thyroid antibody levels also generally support null relationships (Benson et al.

2018; Gaum et al. 2016; Lignell et al. 2016; Pavuk et al. 2003), with some exceptions (Langer et al. 2008).

For the majority of participants in this study, thyroid hormone measurements were within the normal reference range, nonetheless the observed associations with prenatal TCDD exposure may have potential clinical significance. Variation in thyroid hormone levels within the population reference range is associated in meta-analysis with a wide range of adverse health outcomes (Taylor et al. 2013). Specifically, high-normal serum TSH levels are associated with worse cardiovascular risk factors including an adverse serum lipid profile, high blood pressure, high body mass, and metabolic syndrome. Most of the studied associations are with TSH levels only; data are lacking on the phenotypic consequences of variation in FT3 and FT4 (Taylor et al. 2013). It is noteworthy, prenatal TCDD exposure has been previously associated with several of these cardiometabolic outcomes in this study population (Warner et al. 2019).

This study has several strengths. The Seveso Second Generation study represents a unique cohort with a relatively large study sample, the majority of whom were followed into adulthood. We were able to consider a wide range of potential confounders in our analysis, and the study population is relatively homogeneous, minimizing potential uncontrolled confounding. We measured initial TCDD exposure in maternal serum collected near the time of the explosion, and there was a wide range of exposure in the population. In addition to total T4, we were able to measure free T3 and T4 levels, which may be more biologically relevant as free hormone reflects the physiological effects on thyroid hormones better than total hormone concentrations.

This study has several limitations, including a lower than desired participation rate. However, participants did not differ from non-participants in terms of maternal characteristics at explosion or initial TCDD exposure. Maternal TCDD at pregnancy was estimated from maternal serum measurements, not measured directly, but we expect any exposure misclassification to be non-differential. We were unable to consider maternal background environmental exposure to other dioxin-like compounds or postnatal environmental TCDD exposure, but we expect any exposure misclassification would also be non-differential. The wide age range of the cohort likely increased variability in TSH and thyroid hormone measures, and the number of participants with high thyroid antibody status was relatively small. However, sensitivity analyses excluding the young and prepubertal children yielded similar results.

In summary, we report an inverse association between prenatal TCDD exposure, as measured by maternal initial 1976 serum TCDD concentration and maternal TCDD estimated at pregnancy, with serum free T3 and free T4 (but not total T4 or TSH) levels of children born to Seveso women up to 40 years after the explosion. We found some evidence those with high thyroid antibody status may represent a potentially sensitive group, as prenatal TCDD exposure was associated with an increasing trend in serum TSH levels among the subset of participants with high thyroid antibody status. Taken together, our results suggest prenatal exposure to TCDD, a potent endocrine-disrupting compound, may

alter thyroid function later in life. Populations with additional thyroid stress may be particularly susceptible to *in utero* exposure of thyroid disrupting chemicals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- A 1976 explosion in Seveso, Italy exposed residents to high levels of TCDD.
- The Seveso Women's Health Study has followed the female residents over 40 years.
- Children of female residents were enrolled in the Seveso Second Generation study.
- We examined the relation of prenatal TCDD exposure with child thyroid hormone levels.
- Prenatal TCDD exposure was associated with lower free T3 and free T4 levels.
- Results suggest prenatal TCDD exposure may alter thyroid function later in life.

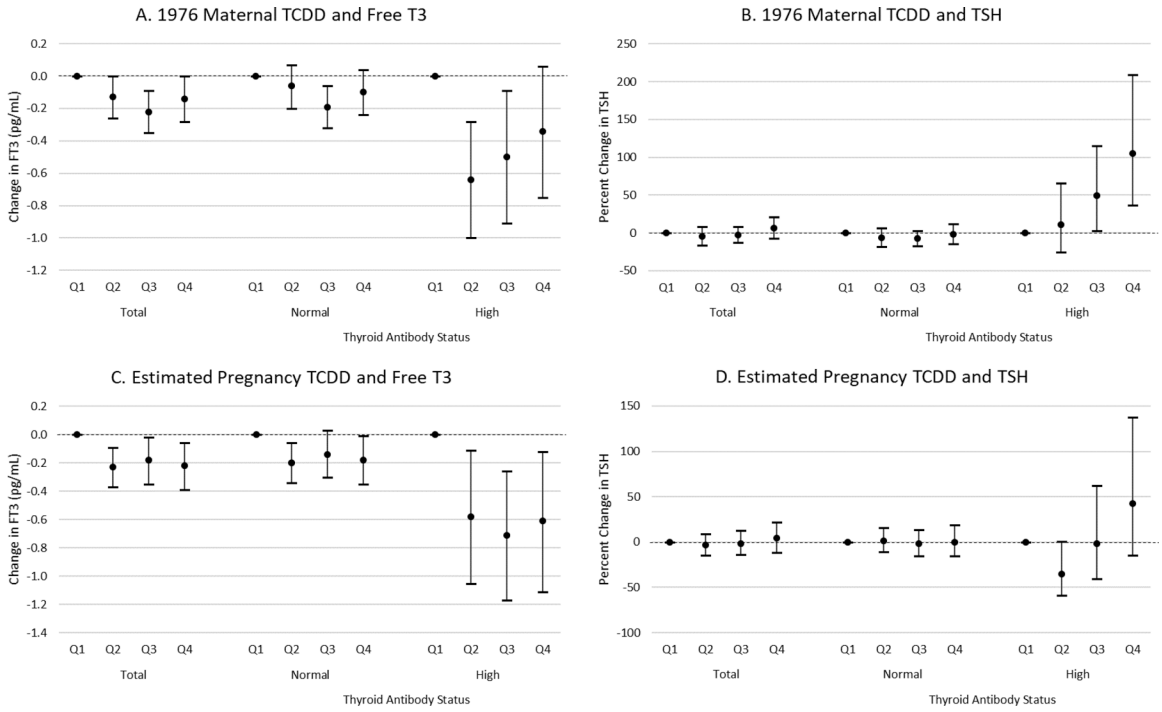

Figure 1.

Associations between prenatal TCDD exposure quartiles and free T_3 and TSH hormone levels, overall and stratified by thyroid antibody status, Seveso Second Generation Study, Italy, 1976–2016. Models adjusted for child's age at interview and household primary wage earner education.

Table 1.

Select maternal and child characteristics, Seveso Second Generation Study, Italy, 1976–2016.

Characteristic	N (%)
Total Mothers	383 (100.0)
Maternal age at explosion (years)	
0–10	90 (23.5)
11–20	185 (48.3)
21–30	99 (25.8)
31+	9 (2.4)
Maternal menarche status at explosion	
Premenarche	127 (33.2)
Postmenarche	256 (66.8)
Maternal age at pregnancy (years) *	
<25	103 (18.1)
25–29	212 (37.2)
30–34	159 (27.9)
35+	96 (16.8)
Maternal smoking during pregnancy	
No	509 (89.3)
Yes	61 (10.7)
Total Children	570 (100.0)
Child sex	
Male	282 (49.5)
Female	288 (50.5)
Child birth order	
1	342 (60.1)
2	195 (34.3)
3+	32 (5.6)
Child birthweight	
<2500 g	40 (7.0)
≥2500 g	530 (93.0)
Child age (years) at interview *	
2–17	152 (26.7)
18–29	224 (39.3)
30+	194 (34.0)
Body mass index category at interview[a]	
Normal	412 (72.3)
Overweight	123 (21.5)
Obese	35 (6.1)
Household primary wage earner education	
≤ Required	169 (29.7)
Secondary	310 (54.4)

Characteristic	N (%)
> Secondary	91 (16.0)
Child smoking status (18+ years only)	
Never	217 (52.0)
Former	65 (15.6)
Current	135 (32.4)
Regular alcohol consumption (18+ years only)	
Never	136 (32.6)
Former	48 (11.5)
Current	233 (55.9)
TSH (μIU/ml)[b]	2.2 ±1.7
Free T_3 (ρg/ml)[c]	3.6 ±0.6
Free T_4 (ρg/ml)[c]	12.1 ±1.5
Total T_4 (μg/dl)[c]	6.6 ±1.8
Thyroid antibody status	
TgAb >115 IU/mL	42 (7.4)
TPOAb >34 IU/mL	38 (6.7)
TRAb >1.8 IU/mL	5 (0.9)
High antibody status	64 (11.2)
Maternal initial (1976) serum TCDD (ppt)[d]	60.2 (28.4, 156.0)
TCDD estimated at pregnancy (ppt)[d]	14.4 (6.4, 33.3)

Note: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; ppt, part-per-trillion; TSH, thyroid-stimulating hormone; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TRAb, TSH receptor antibody

[a] Obese (BMI≥30.0 kg/m^2 or BMI *Z*-score ≥95^{th} percentile); Overweight (BMI 25.0–29.9 kg/m^2 or BMI Z-score ≥85^{th} -< 95^{th} percentile); Normal (BMI< 25.0 kg/m^2 or BMI *Z*-score <85^{th} percentile)

[b] geometric mean ± GSD

[c] mean ± standard deviation

[d] median (interquartile range)

* p<0.05

Table 2.

Adjusted linear regression models of the associations of prenatal TCDD exposure with thyroid hormones levels, Seveso Second Generation Study, Italy, 1976–2016.

Prenatal TCDD Exposure	TSH[a] (n=570) Adjusted[c] β[d] (95% CI)	Total T_4 (n=568)[b] Adjusted[c] β[d] (95% CI)	Free T_4 (n=570) Adjusted[c] β[d] (95% CI)	Free T_3 (n=570) Adjusted[c] β[d] (95% CI)
Maternal initial (1976) serum TCDD				
Ql (2.8–27.9 ppt)	Reference	Reference[†]	Reference	Reference[†]
Q2 (28.0–60.9 ppt)	−4.9 (−16.3, 8.1)	−0.20 (−0.61, 0.22)	−0.42 (−0.78, −0.05)[*]	−0.13 (−0.26, 0.00)[*]
Q3 (61.0–149.0 ppt)	−2.7 (−12.6, 8.3)	0.10 (−0.37, 0.58)	−0.08 (−0.46, 0.30)	−0.22 (−0.35, −0.09)[*]
Q4 (150.0–9,140.0 ppt)	6.2 (−6.9, 21.1)	−0.21 (−0.67, 0.24)	−0.52 (−0.92, −0.11)[*]	−0.14 (−0.28, 0.00)[*]
	p-trend=0.34	*p*-trend=0.66	*p*-trend=0.06	*p*-trend=0.02
TCDD estimated at pregnancy				
Ql (0.1–6.4 ppt)	Reference	Reference[†]	Reference	Reference[†]
Q2 (6.5–13.9 ppt)	−3.5 (−14.8, 9.3)	−0.35 (−0.78, 0.09)	−0.05 (−0.44, 0.34)	−0.23 (−0.37, −0.09)[*]
Q3 (14.0–32.9 ppt)	−1.4 (−14.0, 13.1)	0.20 (−0.30, 0.71)	−0.42 (−0.87, 0.02)	−0.18 (−0.35, −0.02)[*]
Q4 (33.0–1,786.1 ppt)	4.1 (−11.2, 22.0)	−0.04 (−0.61, 0.54)	−0.29 (−0.79, 0.22)	−0.22 (−0.39, −0.06)[*]
	p-trend=0.55	*p*-trend=0.61	*p*-trend=0.14	*p*-trend=0.02

[a] Percent change in serum TSH concentration was calculated using the following formula $(10^{\beta} - 1) \times 100$.

[b] Two participants (1 male, 1 female) are missing Total T4 measurements due to insufficient sample volume.

[c] Adjusted for child's age at interview and household primary wage earner education.

[d] Coefficients represent the mean (free T3, free T4, total T4) or percent (TSH) change in thyroid hormone levels for exposure quartiles 2–4 versus quartile 1

[*] $p<0.05$

[†] evidence of non-linearity with $p<0.1$

Table 3.

Adjusted linear regression models of the associations of prenatal TCDD exposure as measured by maternal initial 1976 serum TCDD with thyroid hormones levels, overall and stratified by thyroid antibody status, Seveso Second Generation Study, Italy, 1976–2016.

Thyroid Hormone	TCDD exposure quartile[a]	Total (n=570) Adjusted[b] β[c] (95% CI)	Normal Antibody Status (n=506) Adjusted[b] β[c] (95% CI)	High Antibody Status (n=64) Adjusted[b] β[c] (95% CI)	*p*-int
TSH[d]					
	Ql	Reference	Reference	Reference	<0.01
	Q2	−4.9 (−16.3, 8.1)	−6.5 (−17.9, 6.6)	11.4 (−25.2, 66.1)	
	Q3	−2.7 (−12.6, 8.3)	−7.7 (−17.2, 2.9)	49.0 (3.0, 115.5)*	
	Q4	6.2 (−6.9, 21.1)	−2.0 (−14.3, 11.9)	105.5 (36.6, 209.2)*	
		p-trend=0.34	*p*-trend=0.71	*p*-trend<0.01	
Total T_4[e†]					
	Ql	Reference	Reference	Reference	0.46
	Q2	−0.20 (−0.61, 0.22)	−0.30 (−0.76, 0.16)	0.62 (−0.52, 1.76)	
	Q3	0.10 (−0.37, 0.58)	0.02 (−0.47, 0.51)	0.74 (−1.00, 2.47)	
	Q4	−0.21 (−0.67, 0.24)	−0.25 (−0.74, 0.24)	−0.08 (−1.23, 1.07)	
		p-trend=0.66	*p*-trend=0.58	*p*-trend=0.97	
Free T_4					
	Ql	Reference	Reference	Reference	0.50
	Q2	−0.42 (−0.78, −0.05)*	−0.48 (−0.88, −0.09)*	0.06 (−0.92, 1.04)	
	Q3	−0.08 (−0.46, 0.30)	−0.07 (−0.48, 0.33)	−0.13 (−0.98, 0.71)	
	Q4	−0.52 (−0.92, −0.11)*	−0.49 (−0.92, −0.06)*	−0.77 (−1.89, 0.35)	
		p-trend=0.06	*p*-trend=0.12	*p*-trend=0.16	
Free T_3[†]					
	Ql	Reference	Reference	Reference	0.02
	Q2	−0.13 (−0.26, 0.00)*	−0.06 (−0.20, 0.07)	−0.64 (−1.00, −0.28)*	
	Q3	−0.22 (−0.35, −0.09)*	−0.19 (−0.32, −0.06)*	−0.50 (−0.91, −0.09)*	
	Q4	−0.14 (−0.28, 0.00)	−0.10 (−0.24, 0.04)	−0.34 (−0.75, 0.06)	
		p-trend=0.02	*p*-trend=0.06	*p*-trend=0.17	

Table 4.

Adjusted linear regression models of the associations of prenatal TCDD exposure as measured by TCDD estimated at pregnancy with thyroid hormones levels, overall and stratified by thyroid antibody status, Seveso Second Generation Study, Italy, 1976–2016.

Thyroid Hormone	TCDD exposure quartile[a]	Total (n=570) Adjusted[b] β[c] (95% CI)	Normal Antibody Status (n=506) Adjusted[b] β[c] (95% CI)	High Antibody Status (n=64) Adjusted[b] β[c] (95% CI)	*p*-int
TSH[d]					
	Ql	Reference	Reference	Reference	<0.01
	Q2	−3.5 (−14.8, 9.3)	1.4 (−11.1, 15.6)	−35.4 (−58.5, 0.5)	
	Q3	−1.4 (−14.0, 13.1)	−2.0 (−15.4, 13.4)	−2.0 (−40.8, 62.4)	
	Q4	4.1 (−11.2, 22.0)	0.2 (−15.4, 18.8)	42.2 (−14.9, 137.6)	
		p-trend=0.55	*p*-trend=0.93	*p*-trend=0.06	
Total T_4[e†]					
	Ql	Reference	Reference	Reference	0.38
	Q2	−0.35 (−0.78, 0.09)	−0.35 (−0.82, 0.11)	−0.01 (−1.19, 1.18)	
	Q3	0.20 (−0.30, 0.71)	0.14 (−0.39, 0.66)	1.16 (−0.71, 3.02)	
	Q4	−0.04 (−0.61, 0.54)	−0.20 (−0.80, 0.41)	1.41 (−0.44, 3.26)	
		p-trend=0.61	*p*-trend=0.93	*p*-trend=0.06	
Free T_4					
	Ql	Reference	Reference	Reference	0.90
	Q2	−0.05 (−0.44, 0.34)	−0.03 (−0.45, 0.39)	−0.22 (−1.27, 0.83)	
	Q3	−0.42 (−0.87, 0.02)	−0.45 (−0.94, 0.04)	−0.22 (−1.20, 0.76)	
	Q4	−0.29 (−0.79, 0.22)	−0.28 (−0.82, 0.27)	−0.30 (−1.30, 0.69)	
		p-trend=0.14	*p*-trend=0.17	*p*-trend=0.58	
Free T_3[†]					
	Ql	Reference	Reference	Reference	0.10
	Q2	−0.23 (−0.37, −0.09)*	−0.20 (−0.34, −0.06)*	−0.58 (−1.05, −0.11)*	
	Q3	−0.18 (−0.35, −0.02)*	−0.14 (−0.30, 0.03)	−0.71 (−1.17, −0.26)*	
	Q4	−0.22 (−0.39, −0.06)*	−0.18 (−0.35, −0.01)*	−0.61 (−1.11, −0.12)*	
		p-trend=0.02	*p*-trend=0.09	*p*-trend=0.01	

[a] Exposure quartiles: Q1 (0.1–6.3 ppt); Q2 (6.4–13.9 ppt); Q3 (14.0–32.9 ppt); Q4 (33.0–1,786.1 ppt)

[b] Adjusted for child's age at interview and household primary wage earners education.

[c] Coefficients represent the mean (free T3, free T4, total T4) or percent (TSH) change in thyroid hormone levels for exposure quartiles 2–4 versus quartile 1

[d] Percent change in serum TSH concentration was calculated using the following formula $(10^{\beta} - 1) \times 100$.

[e] Two participants (1 male, 1 female) are missing Total T4 measurements due to insufficient sample volume.

[*] $p<0.05$

[†] evidence of non-linearity with $p<0.1$