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In utero dioxin exposure and cardiometabolic risk in the Seveso Second Generation Study

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Abstract

Background/Objectives: *In utero* exposure to endocrine-disrupting compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) may alter risk of obesity and related metabolic disease later in life. We examined the relationship of prenatal exposure to TCDD with obesity and metabolic syndrome (MetS) in children born to a unique cohort of TCDD-exposed women resulting from a 1976 explosion in Seveso, Italy.

Subjects/Methods: In 2014, nearly 40 years after the explosion, we enrolled 611 post-explosion offspring, 2 to 39 years of age, in the Seveso Second Generation study. *In utero* TCDD exposure was defined primarily as TCDD concentration measured in maternal serum collected soon after the explosion and alternately as TCDD estimated at pregnancy. We measured height, weight, waist circumference, body fat, blood pressure, and fasting blood levels of lipids and glucose, which were combined to assess body mass index (BMI) and MetS.

Results: Children (314 female, 297 male) averaged 23.6 (±6.0) years of age. Among the 431 children ≥18 years, a 10-fold increase in initial maternal TCDD concentration was inversely associated with BMI in daughters (adj-β=−0.99 kg/m^2; 95% CI −1.86, −0.12), but not sons (adj-β=0.41 kg/m^2; 95% CI −0.35, 1.18) (*p*-int=0.02). A similar relationship was found in the younger children (2-17 years); a 10-fold increase in initial maternal TCDD was inversely associated with BMI *z*-score (adj-β = −0.59 kg/m^2; 95% CI −1.12, −0.06) among daughters, but not sons (adj-β = 0.04 kg/m^2; 95% CI −0.34, 0.41) (*p*-int=0.03). In contrast, in sons only, initial maternal TCDD was associated with increased risk for MetS (adj-RR = 2.09, 95% CI 1.09, 4.02). Results for TCDD estimated at pregnancy were comparable.

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CONFLICT OF INTEREST

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

Conclusions: These results suggest prenatal TCDD exposure alters cardiometabolic endpoints in a sex-specific manner. In daughters, *in utero* TCDD is inversely associated with adiposity measures. In sons, *in utero* TCDD is associated with increased risk for MetS.

INTRODUCTION

The increasing prevalence of obesity worldwide is a major public health concern, associated with significant morbidity and mortality.[1-3] Obesity is frequently associated with a cluster of cardiometabolic risk factors, including hyperglycemia, hypertension, and dyslipidemia, which together comprise metabolic syndrome (MetS), a condition that affects an estimated 25% of the global adult population.[4, 5] While excess caloric consumption and physical inactivity are well-recognized risk factors, a role for environmental exposure to endocrine-disrupting compounds in metabolic disruption has been hypothesized.[6, 7] In particular those exposed *in utero*, a critical period of development and epigenetic programming, may be more susceptible to metabolic diseases that manifest later in life.[8]

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a widespread environmental contaminant and potent endocrine disruptor.[7, 9, 10] A highly persistent and lipophilic compound, TCDD has a long half-life in humans (~9 years) and fetal exposure can occur through transplacental transfer.[11, 12] In animal and experimental studies, TCDD causes a wide range of metabolic disruptive effects.[13] Alterations in cholesterol biosynthesis, fatty acid synthesis, glucose metabolism, and adipocyte differentiation have been reported in experimental studies.[14-16] In adult mice, TCDD exposure has been shown to increase serum triglycerides, cholesterol, and blood pressure, and promote atherosclerosis.[17] High-dose TCDD exposure is associated with wasting syndrome in rodents fed a normal diet,[18] and with accelerated weight gain when fed a high-fat diet suggesting an interaction between diet and exposure.[19] Studies of perinatal TCDD exposure also have been linked to a wide range of cardiometabolic health impairments in cardiac physiology,[20] lipid metabolism,[21] glucose homeostasis,[22, 23] and adiposity.[22-24] Sex-specific effects have been noted in some studies of perinatal exposure.[22, 23]

Several longitudinal birth cohort studies have examined associations between prenatal exposure to dioxin-like compounds and child adiposity with inconsistent results.[25-30] With follow-up ranging from 3 to 15 years, reported associations with body mass index (BMI) have been positive,[26] negative,[27] and null.[25, 28-30] Inconsistent sex-specific effects have also been noted, with significant positive associations found in females only[26] and negative associations found in males only.[27] In the two studies that considered additional adiposity measures such as waist circumference, results were similarly inconsistent.[27, 28] No studies have followed children into adulthood. Further, no epidemiologic studies have examined the association of prenatal TCDD exposure on offspring cardiovascular traits or metabolic syndrome.

On July 10, 1976, an explosion at a chemical plant near Seveso, Italy resulted in a toxic plume that exposed nearby residents to high levels of TCDD.[31-33] The Seveso Women's Health Study (SWHS), a cohort of women exposed to a single high dose of TCDD during or before their childbearing years, is unique, with initial, individual-level TCDD exposure

measured in serum collected soon after the explosion. Previously in SWHS, we found a significant positive association between initial serum TCDD levels and MetS thirty years later, but only among women who were youngest at exposure (≤12 years in 1976).[34] Findings were similar for individual components of MetS, with significant interactions between TCDD and age at exposure for increased waist circumference and blood pressure. In contrast, TCDD concentration was non-significantly inversely associated with BMI. Overall, these data support the hypothesis that TCDD exposure during critical developmental windows, in this case prior to puberty, may increase susceptibility to MetS.

In 2014, nearly 40 years after the explosion, we enrolled SWHS post-explosion offspring in the Seveso Second Generation study. Here we examine the relationship of *in utero* TCDD exposure with several measures of adiposity and cardiometabolic risk in the Seveso Second Generation cohort. We also examine whether these relationships are modified by child sex.

SUBJECTS AND METHODS

Study population

Details of the SWHS and the Seveso Second Generation study have been presented elsewhere.[35, 36] Briefly, 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. We enumerated 943 liveborn children (453 females, 490 males) who were born after the explosion to 574 SWHS mothers, ranging in age from newborn to 39 years. Of these, 16 were deceased, 7 were less than 2 years, 76 could not be located, and 611 children (66.4% of 920 alive and eligible) born to 402 SWHS mothers participated in the study visit.

Data collection

The study was approved by the Institutional Review Boards of the participating institutions. Before participation, we obtained written informed consent from all children 18 years or older and all mothers of children less than 18 years, written assent from all children who were 13 to 17 years, and oral assent from all children who were 7 to 12 years of age. Data collection for SWHS women included a fasting blood draw, anthropometric and blood pressure measurements, personal interview, and medical record abstraction. For women with children <18 years, the interview also included questions about the health of her children. Data collection for children 2 to 6 years included a fasting blood draw and anthropometric measurements. Data collection for children 7 to 17 years included a fasting blood draw, anthropometric and blood pressure measurements, and an online self-administered questionnaire (10 to 17 years only). Data collection for children 18 years or older included a fasting blood draw, anthropometric and blood pressure measurements, personal interview and food frequency questionnaire,[37] and medical record abstraction. Information collected during the interview included demographic and lifestyle characteristics as well as medical histories. All interviews were conducted in private by trained nurse-interviewers who were unaware of zone of residence and serum TCDD levels of mothers.

We measured barefoot standing height to the nearest 0.1 cm using a stadiometer and standing weight to the nearest 0.1 kg using a bioimpedence scale (Tanita TBF-300A Body Composition Analyzer) that also measured percent body fat (children 7 years and older) using “foot-to-foot” bioimpedance technology. The scale was set to standard mode and the manufacturer’s algorithm was used for calculation of percent body fat. We measured waist circumference (children 7 years and older) to the nearest 0.1 cm by placing a measuring tape around the abdomen in a horizontal plane midway between the inferior margin of the ribs and the iliac crest. Height and waist circumference were measured in duplicate and averaged for analysis. We measured resting blood pressure (children 7 years and older) at three 1-minute intervals using an automatic digital sphygmomanometer following American Heart Association recommendations;[38] the last two measurements were averaged for analysis.

Triglycerides, high-density lipoprotein cholesterol (HDL-C), total cholesterol, and glucose were measured in fasting plasma (lithium heparin) or serum samples on the automatic analyzer COBAS 8000 (Roche Diagnostics, Mannheim, Germany) at the Hospital of Desio Laboratory. Triglycerides were measured in plasma by glycerophosphate oxidase-phenol aminophenazone method without glycerol correction. Total cholesterol was measured in plasma by enzymatic-colorimetric method (CHOD-PAP). HDL-C was measured in plasma directly using cyclodestrin sulphate and polyethylene glycol-modified enzymes. Glucose was measured in plasma by reference enzymatic method (hexokinase).

Exposure measures

We examined *in utero* TCDD exposure in two ways: 1) maternal initial (1976) serum TCDD level, to test the hypothesis that the primary dose produces a persistent and, if involving the epigenetics of her oocytes, possibly a heritable change to the woman’s reproductive system impacting the health of her offspring; and 2) maternal TCDD estimated at pregnancy, to test the hypothesis that the toxicologically-relevant dose is the maternal body burden at the time of pregnancy. For all SWHS mothers, TCDD was measured in archived sera collected soon after the explosion by high-resolution gas chromatography/high-resolution mass spectrometry methods.[39] Details of serum sample selection are presented elsewhere.[40] For a subset of SWHS mothers 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.[41] Details of TCDD concentrations measured in 1996 or 2008 serum are presented elsewhere.[36, 42] All values are reported on a lipid weight basis as picograms-per-gram lipid or parts-per-trillion (ppt).[43] Non-detectable values were assigned a value of one-half the detection limit.[44] 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.[36, 42] As a result, estimates were extrapolated from TCDD levels measured in 1976 samples for 431 children, 1996 samples for 165 children, and 2008 samples for 15 children.

Outcome measures

For children 18 years and older, we calculated body mass index (BMI, kg/m^2) and classified participants as "overweight" or "obese" if they had a BMI ≥ 25 and <30 kg/m^2, or ≥ 30 kg/m^2, respectively.[45] MetS cases were diagnosed based on the presence of three or more of the following five criteria: (1) increased waist circumference ≥ 80 cm (female) or ≥ 94 cm (male); (2) elevated triglycerides ≥ 150 mg/dL or report of current use of drug treatment for elevated triglycerides; (3) low HDL-C < 50 mg/dL (female) or < 40 mg/dL (male) or report of current use of drug treatment for reduced HDL-C; (4) increased systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥ 85 mmHg, or report of current use of antihypertensive medication; (5) elevated fasting glucose ≥ 100 mg/dL or report of current use of diabetes medication.[4]

For children less than 18 years, we calculated age- and sex-specific weight, height, and BMI *z*-scores and percentiles for each child using SIEDP-2006 Italian growth charts for Northern Italy.[46] Children who were in the 85th percentile of BMI or higher but lower than the 95th percentile were classified as "overweight", and children who were in the 95th percentile or higher were classified as "obese". There were no Italian reference values for waist circumference for this age group, therefore children who were in the 90th percentile or above for age and sex using NHANES III reference data were classified as having increased waist circumference or "abdominal obesity" and considered "at-risk" for metabolic syndrome.[47, 48]

Statistical analyses

Both measures of *in utero* TCDD exposure, maternal initial (1976) serum TCDD and maternal TCDD estimated at pregnancy, were $\log_{10}$-transformed and analyzed as continuous variables. We examined the relationship of *in utero* TCDD exposure with continuous outcomes (weight, height, BMI, waist circumference, body fat percent) using multivariable linear regression and with categorical outcomes (overweight or obese (BMI ≥25kg/m^2 vs. <25 kg/m^2, BMI *z*-score ≥85th percentile vs. <85th percentile), increased waist circumference (≥90th percentile vs. <90th percentile), MetS (case vs non-case) and its five individual criteria (increased waist circumference, elevated triglycerides, low HDL-C, increased blood pressure, elevated glucose) using multivariable Poisson regression. Given the wide age range of participants (2 to 39 years), and the need for sex- and age-specific *z*-scores for participants under age 18, we examined associations for the two age groups, ≥18 years and <18 years, separately.

Based on our review of the obesity and MetS literature, we considered the following variables as potential confounders: maternal age at explosion, maternal age at pregnancy, maternal smoking at pregnancy, maternal BMI, household socioeconomic status including education, occupation, income, and marital status, family history of hypertension or diabetes, child age and sex, child birthweight, child tobacco or alcohol use and environmental tobacco smoke exposure, child diet and physical activity, and menarche and parity status of female children. The final set of covariates was determined using directed acyclic graphs (DAG). Final models for children 18 years and older were adjusted for primary wage earner education, maternal age at explosion, maternal age at pregnancy,

maternal smoking at pregnancy, maternal BMI, family history of hypertension, child age, child sex, and child smoking. Final models for children less than 18 years were adjusted for primary wage earner education, maternal age at explosion, maternal age at pregnancy, maternal smoking at pregnancy, maternal BMI, child age, and child sex. For all outcomes, we considered effect modification by child sex in all analyses by including a cross-product term between exposure and sex. Interaction p-values < 0.2 were considered significant.

In sensitivity analyses, we reanalyzed the final models after excluding outliers with standardized residuals greater than 3 or less than −3. We also reanalyzed the final models for children < 18 years excluding children ages 2 to 6 years (n=19). We calculated alternate *z*-scores using growth standards for all of Italy[46] as well as International Obesity Task Force (IOTF),[49] and reanalyzed final models for children < 18 years.

For all outcomes, we used generalized additive models with a 3-degrees-of-freedom cubic spline to evaluate the shape of the exposure-response curves in the full sample and in males and females separately. Standard errors for all models were estimated using the robust Huber-White sandwich estimator. All statistical analyses were performed using STATA 13.1.[50] The datasets analyzed during the current study are available from the corresponding author on reasonable request.

RESULTS

Select characteristics of the 611 children born post-explosion to 402 SWHS mothers are presented in Table 1(see Supplementary Table 1 for characteristics by child sex). At the time of the explosion, the 402 mothers were an average of 14.9 (±7.4) years of age, 35% were premenarche, and the majority were nulliparous. Mothers were an average of 29.4 (±5.2) years of age at pregnancy and about 10% reported smoking during pregnancy. At last follow-up, mothers averaged 42.5 (±6.3) years, the average BMI of mothers was 26.5 (±5.5) kg/m^2 and 24% were obese. At interview, the 611 children were an average of 23.7 (±9.4) years (range: 2-39) and 51% were female (see Supplementary Table 2). Among children 18 years or older, about one-third were current smokers.

In utero TCDD exposure based on maternal initial (1976) TCDD level was high (median = 63.2 ppt). Maternal initial TCDD levels were higher among women who were youngest or who were still premenarche at the time of explosion, as reported previously.[40] Maternal initial serum TCDD levels were higher in the children who were youngest (2-17 years) since they were more likely to be born to mothers who were younger at explosion. They were also higher among mothers of female children, but did not differ by other child factors (see Supplementary Table 1). With birth years spanning 1976 to 2014, *in utero* TCDD exposure based on maternal estimated TCDD at pregnancy was lower [median (IQR) = 13.4 (6.1, 32.4) ppt], but with a wide range (0.2, 1,786 ppt). Maternal estimated TCDD at pregnancy was significantly higher among mothers who were older and postmenarche at explosion, and among children who were older (30+ years) since they were born sooner after the explosion. The overall correlation between the two indices of *in utero* TCDD exposure was moderate (r=0.51), and higher within each age group (r = 0.79 for children 18 years or older, r = 0.68 for children 2 to 17 years).

The majority of children in the second generation cohort were normal weight (Table 2). Among children 18 years and older, mean BMI was 23.6 (±3.7) kg/m^2, with 24.6% and 7.2% classified as overweight and obese, respectively (Table 2). Mean waist circumference was 79.8 (±10.6) cm and 21.4% had increased waist circumference. The prevalence of MetS in this age group was low (5.6%), however, the prevalence of individual criteria was higher ranging from 8.9% to 21.4%. The most prevalent individual criteria were increased waist circumference (21.4%) and high blood pressure (15.8%). Among children less than 18 years, mean BMI *z*-score was −0.03 (±1.15), with 13.3% and 3.3% classified as overweight and obese, respectively. In this age group, 12.5% of children were above the age-adjusted waist circumference threshold or "at risk" for MetS.

Among children 18 years and older, *in utero* TCDD exposure was not associated with measures of adiposity overall (Table 3a). However, we observed evidence of effect modification by sex. A 10-fold increase in maternal initial serum TCDD was inversely associated with BMI (adj-β = −0.99 kg/m^2, 95% CI −1.86, −0.12) and body fat percent (adj-β = −1.82 percent, 95% CI −3.56, −0.09) among daughters, but not sons (BMI: adj-β = 0.41 kg/m^2, 95% CI −0.35, 1.18, *p*-interaction = 0.02; body fat percent: adj-β = 0.33, 95% CI −1.25, 1.91, *p*-interaction = 0.07). TCDD estimated at pregnancy was also inversely associated with BMI (adj-β = −1.14 kg/m^2, 95% CI −2.06, −0.22) among daughters, but not sons (*p*-interaction < 0.01). Scatterplots of continuous outcomes and *in utero* TCDD exposure are presented in Supplementary Figures 1 through 3. Also among daughters, when BMI was categorized (Table 3b), TCDD estimated at pregnancy was associated with reduced risk of overweight (adj-RR = 0.59, 95% CI 0.39, 0.89) and obese status (adj-RR = 0.39, 95% CI 0.17, 0.93), which was not observed in sons (*p*-interaction = 0.02 and <0.01, respectively). The models for maternal initial serum TCDD showed similar, significant effect modification by sex.

As presented in Table 3b, among children 18 years and older, *in utero* TCDD exposure was not associated with MetS or individual criteria in the full sample. However, we observed some evidence of effect modification by sex. A 10-fold increase in maternal initial serum TCDD was positively associated with MetS risk among sons (adj-RR = 2.09, 95% CI 1.09, 4.02), but not daughters (adj-RR = 0.75, 95% CI 0.11, 5.05). A similar difference by sex was noted for maternal 1976 serum TCDD and some individual criteria including increased blood pressure (sons: adj-RR = 1.42, 95% CI 1.00, 2.02; daughters: adj-RR = 0.68, 95% CI 0.22, 2.11; *p*-interaction = 0.23) and low HDL-C (sons: adj-RR = 1.61, 95% CI 0.96, 2.68; daughters: adj-RR = 0.71, 95% CI 0.30, 1.65; *p*-interaction = 0.13). Similar sex-specific associations were found for TCDD estimated at pregnancy and elevated triglycerides (sons: adj-RR = 1.89, 95% CI 1.08, 3.30; daughters: adj-RR = 0.56, 95% CI 0.18, 1.75; *p*-interaction = 0.05) and low HDL-C (sons: adj-RR = 1.47, 95% CI 0.83, 2.60; daughters: adj-RR = 0.63, 95% CI 0.27, 1.45; *p*-interaction = 0.09).

Among children less than 18 years, results for maternal initial serum TCDD were largely consistent with those reported in the older age group (Table 4). A 10-fold increase in maternal initial serum TCDD was inversely associated with BMI *z*-score (adj-β = −0.59, 95% CI −1.12, −0.06) and body fat percent (adj-β = −6.76, 95% CI −11.43, −2.09) among daughters, but not sons (BMI *z*-score: adj-β = 0.04, 95% CI −0.34, 0.41, *p*-interaction =

0.03; body fat percent: adj-β = 0.10, 95% CI −2.89, 3.09, *p*-interaction < 0.01). In contrast, no associations were found for TCDD estimated at pregnancy, although model estimates were in the same direction. Neither measure of *in utero* TCDD exposure was associated with overweight or obese status, but the number of cases was small, limiting statistical power. However, maternal initial serum TCDD was associated with risk of increased waist circumference in sons (adj-RR = 2.58, 95% CI 1.13, 5.87) but not daughters (*p*-interaction = 0.05).

In sensitivity analyses, we reanalyzed the final models for children ≥18 years excluding outliers (n=7) with standardized residuals greater than 3 or less than −3, and the results did not change meaningfully (data not shown). We calculated alternate *z*-scores for children < 18 years using growth standards for all of Italy[46] as well as IOTF,[49] then reanalyzed the final models; results were similar (data not shown). Finally, we repeated the final models for children < 18 years excluding children ages 2 to 6 years (n=19) and results were similar (data not shown).

DISCUSSION

To our knowledge, this is the first epidemiologic study to examine the metabolic disruptive effects of *in utero* TCDD exposure in children followed into adulthood. In this study, we observed sex-specific effects, with inverse associations for adiposity measures among daughters but not sons. Specifically, among children who had reached adulthood (≥18 years), prenatal TCDD exposure was associated with lower BMI and body fat percent as well as reduced risk of overweight in daughters only. In contrast, prenatal TCDD exposure was associated with increased risk of MetS and individual risk factors (blood pressure, triglycerides) in sons, but not daughters. Associations with adiposity measures among the younger children (2-17 years) were similar; prenatal TCDD exposure was associated with lower BMI *z*-score and body fat percent in daughters only. We could not diagnose MetS in the younger children, but in this group, prenatal TCDD exposure was associated with increased waist circumference or “at risk” for MetS in sons only.

Our consistent findings of inverse associations between prenatal TCDD exposure and adiposity measures in female children have not been reported in previous studies.[25-30] However, assessment of prenatal exposure to dioxin-like compounds varied widely across previous studies and included total dioxin equivalents (TEQ),[29] based on measurement of all dioxin-like compounds (polychlorinated dibenzodioxins (PCDDs), furans (PCDFs) and polychlorinated biphenyls (PCBs) in breast milk, PCDD/F TEQ based on measurement of a subset,[25, 27, 30] and TEQ based on chemical-activated luciferase gene expression bioassay (CALUX-TEQ).[26, 28] We were able to estimate maternal total TEQ at pregnancy for a subset of children less than 18 years in the second generation cohort and the results were largely null (see Supplementary Table 3). Other potential reasons for the inconsistency include the lack of a standardized measure of child growth[25, 26, 30] and limited sample size.[25, 28, 30] Only one longitudinal birth cohort study in Vietnam reported results for prenatal TCDD exposure; with follow-up to 3 years of age, an inverse association, albeit non-significant, was reported between breastmilk TCDD and BMI *z*-score in girls.[27] In this same study, however, a significant inverse association was also reported in boys.[27]

Our finding of increased risk for MetS with prenatal TCDD exposure among sons, but not daughters, has not been previously reported. In a small prospective birth cohort study in the Netherlands, energy metabolism parameters including fasting glucose, insulin, and HbA1c were measured in 33 children in early adolescence (mean=15 years, 14 to 18 years).[25] PCDD/F TEQ in breast milk was negatively associated with insulin, but no associations were found with fasting glucose levels or HbA1c. We also found no association between prenatal TCDD exposure and the individual MetS indicator, elevated fasting glucose (≥100 mg/dL), among adult children.

The observed metabolic disruption effects of *in utero* TCDD exposure are biologically plausible. Most effects of TCDD are mediated via binding the aryl hydrocarbon receptor (AhR),[51] which is involved in the metabolism and central regulation of energy balance.[52, 53] In experimental studies, TCDD alters the expression of genes associated with hepatic circadian rhythm,[54] cholesterol biosynthesis, glucose metabolism and adipose differentiation.[55, 56] Mechanisms by which AhR regulates energy metabolism are not yet well described, but various direct and indirect mechanisms, including cross-talk with the estrogen receptor, may be involved and contribute to sex-dependent differences. In addition, AhR indirectly affects adipogenesis through inhibition of PPAR-γ expression, a key regulator of normal adipocyte development.[57]

This study has several strengths, including the large sample size, prospective design with multiple cardiometabolic measures, and follow-up into adulthood for the majority of offspring. We were able to measure initial TCDD exposure in maternal serum collected near the time of the explosion, and there was a wide range of exposure. Given the significant decline in background TCDD levels since 1976, postnatal exposure is expected to be low.[58] The study population is relatively homogeneous with regard to factors such as diet, breastfeeding, and socioeconomic status, which can minimize confounding. For the younger children, we were able to utilize standardized measures of adiposity based on BMI and waist circumference *z*-scores, facilitating comparison across studies.

This study has some limitations. The participation rate (66.4%) was lower than desired. Nonetheless, participants and non-participants did not differ in terms of maternal characteristics at explosion or maternal initial TCDD exposure. Maternal initial serum TCDD levels were higher among daughters than sons. While this difference could simply be the result of chance, the possibility of selection bias in the study population cannot be ruled out. However, maternal initial TCDD levels were not related to either sex ratio, fetal demise, or birth weight,[36, 59, 60] and there were few other demographic differences between female and male children. The wide age range of the second generation cohort likely increased variability in outcome measures, especially in the younger age group, although we attempted to minimize this by utilizing age-standardized measures. Finally, our reliance on a modeled estimate of maternal TCDD at the time of pregnancy is likely a source of exposure misclassification, but we expect any bias to be non-differential.

In conclusion, this is the first prospective epidemiologic study to examine the relationship of *in utero* TCDD exposure and cardiometabolic risk in offspring born to a highly-exposed maternal population. Our results suggest *in utero* TCDD exposure alters metabolic endpoints

in a sex-specific manner. In daughters, *in utero* TCDD is inversely associated with adiposity measures including lower BMI and percent body fat and reduced risk of overweight. In sons, *in utero* TCDD is associated with increased risk for MetS and some individual components. These results are generally consistent with effects of *in utero* TCDD exposure that have been noted in animal studies and with greater sensitivity to TCDD during development. Continued follow-up of this unique cohort as it ages will be informative.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Select maternal and child characteristics by *in utero* TCDD exposure measures, maternal initial (1976) serum TCDD levels and TCDD estimated at pregnancy, Seveso Second Generation Study, Italy, 1976-2016.

		Maternal 1976 serum TCDD (ppt)	TCDD estimated at pregnancy (ppt)
Characteristic	**N (%)**	**Median (interquartile range)**	**Median (interquartile range)**
Total Mothers	402 (100.0)		
Maternal zone of residence at explosion[a*†]			
A	74 (18.4)	242.5 (82.3, 960.0)	55.5 (16.7, 162.6)
B	328 (81.6)	51.0 (24.4, 108.0)	10.6 (5.5, 25.3)
Maternal age at explosion (years)[a*†a]			
0-10	104 (25.9)	148.5 (54.4, 303.5)	5.2 (2.7, 10.0)
11-20	188 (46.8)	53.1 (23.9, 108.0)	14.0 (7.5, 30.6)
21-30	101 (25.1)	41.9 (23.9, 90.4)	32.4 (18.2, 69.9)
31+	9 (2.2)	40.4 (29.4, 122.0)	46.5 (16.3, 88.9)
Maternal menarche status at explosion[a*†]			
Premenarche	141 (35.1)	120.0 (52.9, 268.0)	6.4 (3.2, 14.2)
Postmenarche	261 (64.9)	44.4 (22.5, 98.9)	20.1 (9.2, 47.1)
Maternal age at pregnancy (years)[*†]			
<25	106 (17.3)	44.1 (20.7, 109.0)	25.5 (11.7, 56.5)
25-29	219 (35.8)	57.6 (27.2, 131.0)	17.4 (8.0, 35.7)
30-34	172 (28.2)	73.9 (31.1, 211.5)	9.2 (4.3, 24.4)
35+	114 (18.7)	71.1 (33.2, 157.0)	6.5 (3.2, 16.7)
Maternal smoking during pregnancy[†]			
No	547 (89.5)	63.5 (29.0, 162.0)	12.8 (5.6, 32.0)
Yes	64 (10.5)	55.1 (20.7, 105.4)	17.5 (8.4, 36.9)
Maternal BMI category at last follow-up[a*]			
Underweight	3 (0.8)	48.8 (33.2, 49.5)	22.0 (17.5, 25.6)
Normal	196 (48.8)	73.2 (37.3, 191.0)	13.2 (5.5, 34.8)
Overweight	107 (26.6)	46.9 (21.3, 118.0)	11.7 (5.6, 25.8)

Characteristic	N (%)	Maternal 1976 serum TCDD (ppt) Median (interquartile range)	TCDD estimated at pregnancy (ppt) Median (interquartile range)
Obese	96 (23.9)	52.9 (26.7, 151.5)	15.9 (8.1, 35.6)
Total Children	611 (100.0)	63.2 (28.6, 157.0)	13.4 (6.1, 32.4)
Child sex[†]			
Male	297 (48.6)	54.2 (25.4, 122.0)	11.1 (5.3, 28.6)
Female	314 (51.4)	70.0 (30.6, 174.0)	15.8 (6.5, 35.5)
Child birth order[†]			
1	369 (60.4)	64.7 (30.3, 164.0)	16.7 (7.4, 38.8)
2	206 (33.7)	57.7 (26.7, 131.0)	9.9 (4.8, 23.4)
3+	36 (5.9)	50.2 (20.8, 95.9)	7.6 (3.2, 16.1)
Child birthweight[†]			
<2500 g	41 (6.7)	61.2 (23.9, 100.0)	8.0 (4.1, 21.3)
≥2500 g	570 (93.3)	63.3 (29.0, 162.0)	13.8 (6.2, 32.6)
Child age (years) at interview[*†]			
2-17	180 (29.5)	98.2 (44.6, 247.0)	4.5 (2.4, 9.1)
18-29	229 (37.5)	64.4 (29.2, 122.0)	14.0 (8.0, 27.7)
30+	202 (33.0)	41.3 (21.3, 90.4)	31.2 (16.9, 68.6)
Primary wage earner education			
≤ Required	181 (29.6)	65.3 (29.9, 157.0)	11.2 (6.3, 26.8)
Secondary	331 (54.2)	63.3 (29.9, 156.0)	12.8 (5.5, 32.1)
> Secondary	99 (16.2)	52.6 (22.5, 157.0)	18.7 (7.9, 56.4)
Child physical activity level			
Less active than others	190 (31.1)	51.2 (25.1, 122.0)	18.0 (8.0, 35.8)
About the same	263 (43.0)	64.0 (29.4, 167.0)	11.6 (5.2, 32.3)
More active than others	158 (25.9)	69.8 (29.9, 157.0)	10.1 (6.1, 27.6)
Family history of hypertension[†b]			
No	295 (48.4)	63.3 (27.0, 135.0)	15.5 (7.1, 35.7)
Yes	315 (51.6)	63.2 (28.6, 163.4)	11.9 (5.2, 29.5)
Child smoking status (18+ years only)			

Characteristic	N (%)	Maternal 1976 serum TCDD (ppt) Median (interquartile range)	TCDD estimated at pregnancy (ppt) Median (interquartile range)
Never	224 (52.0)	59.4 (27.9, 126.0)	20.0 (9.6, 39.9)
Former	69 (16.0)	46.8 (21.1, 102.0)	21.6 (12.8, 56.5)
Current	138 (32.0)	49.9 (21.5, 88.0)	19.2 (8.8, 43.9)

[*] ANOVA $p < 0.05$ for $\log_{10}$(maternal 1976 serum TCDD)

[†] ANOVA $p < 0.05$ for $\log_{10}$(TCDD estimated at pregnancy)

[a] Maternal 1976 serum TCDD are mother-specific (n=402) and TCDD estimated at pregnancy are child-specific (n=611).

[b] Missing n=1

Table 2.

Summary of cardiometabolic outcomes,[a] overall and by child sex, Seveso Second Generation Study, Italy, 2014-2016.

	Total	Female	Male
Total, N	611	314	297
Children 18+ years, n	431	225	206
Weight (kg)	67.7 ±13.8	60.4 ±11.2	75.6 ±11.9
Height (cm)	169.1 ±9.7	162.2 ±6.2	176.5 ±6.9
Body mass index (kg/m^2)	23.6 ±3.7	23.0 ±4.0	24.3 ±3.2
Waist circumference (cm)	79.8 ±10.6	75.5 ±9.5	84.5 ±9.8
Body fat (%)	22.6 ±8.4	26.8 ±8.1	17.9 ±5.8
Overweight (25.0-29.9 kg/m^2)	106 (24.6)	45 (20.0)	61 (29.6)
Obese (≥ 30 kg/m^2)	31 (7.2)	15 (6.7)	16 (7.8)
Metabolic syndrome[b]	24 (5.6)	4 (1.8)	20 (9.7)
Increased waist circumference[b,c]	92 (21.4)	58 (25.9)	34 (16.5)
Elevated triglycerides[b,c]	38 (8.9)	14 (6.3)	24 (11.7)
Low HDL cholesterol[b,c]	50 (11.7)	22 (9.9)	28 (13.6)
Increased blood pressure[b]	68 (15.8)	11 (4.9)	57 (27.7)
Elevated glucose[b,c]	41 (9.6)	12 (5.4)	29 (14.1)
Children 2-17 years, n	180	89	91
Weight *z*-score	0.05 ±1.16	0.24 ±1.37	−0.13 ±0.87
Height *z*-score	0.13 ±1.03	0.26 ±1.15	0.01 ±0.89
Body mass index *z*-score	−0.03 ±1.15	0.13 ±1.31	−0.18 ±0.94
Waist circumference (cm)[c]	67.6 ±10.1	67.4 ±11.2	67.8 ±8.9
Body fat (%)[c]	20.4 ±10.5	25.5 ±10.5	15.6 ±7.9
Overweight (≥ 85th-<95th percentile)	24 (13.3)	14 (15.7)	10 (11.0)
Obese (≥ 95th percentile)	6 (3.3)	6 (6.7)	0 (0.0)
Increased waist circumference[c]	21 (12.5)	16 (19.5)	5 (5.8)

[a] Values are mean ± SD for continuous and n (%) for categorical variables.

[b] Metabolic syndrome, 3 or more of the following individual criteria: waist circumference ≥ 80 cm (female) or ≥ 94 cm (male); triglycerides ≥ 150 mg/dL; HDL-cholesterol < 50 mg/dL (female) or < 40 mg/dL (male); blood pressure ≥ 130/85 mmHg; glucose ≥ 100 mg/dL.

[c] Missing data for children 18+ years: waist circumference (n=1 female), body fat (n=2 females), blood draw (n=2 females). Waist circumference and body fat were not measured for children 2-6 years (n=19; 10 females, 9 males).

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Table 3.

Adjusted[a] linear and Poisson regression models of the associations of *in utero* TCDD exposure with obesity-related outcomes, overall and stratified by child sex, among children 18 years and older, Seveso Second Generation Study, Italy, 1976-2016.

a) Linear regression models

	Total (n=431)[c]	Female (n=225)[c]	Male (n=206)	
	Adjusted β[b] (95% CI)	Adjusted β[b] (95% CI)	Adjusted β[b] (95% CI)	*p*-int
Maternal initial (1976) serum TCDD				
Weight	−0.20 (−2.13, 1.73)	−1.81 (−4.33, 0.72)	1.46 (−1.39, 4.31)	0.09
Height	0.64 (−0.58, 1.86)	0.96 (−0.59, 2.50)	0.31 (−1.46, 2.08)	0.57
BMI	−0.30 (−0.90, 0.30)	−0.99 (−1.86, −0.12) *	0.41 (−0.35, 1.18)	0.02
Waist circumference	−0.73 (−2.45, 1.00)	−1.99 (−4.24, 0.25)	0.59 (−1.78, 2.95)	0.10
Body fat percent	−0.76 (−1.97, 0.45)	−1.82 (−3.56, −0.09) *	0.33 (−1.25, 1.91)	0.07
TCDD estimated at pregnancy				
Weight	−0.48 (−2.71, 1.75)	−2.54 (−5.32, 0.24)	1.90 (−1.20, 5.00)	0.02
Height	0.65 (−0.80, 2.11)	0.77 (−1.00, 2.55)	0.51 (−1.49, 2.51)	0.83
BMI	−0.38 (−1.07, 0.31)	−1.14 (−2.06, −0.22) *	0.49 (−0.33, 1.32)	<0.01
Waist circumference	−0.84 (−2.82, 1.13)	−2.33 (−4.89, 0.22)	0.87 (−1.61, 3.34)	0.05
Body fat percent	−0.77 (−2.16, 0.63)	−1.91 (−3.86, 0.05)	0.55 (−1.18, 2.27)	0.05

b) Poisson regression models

		Total (n=431)[c]	Female (n=225)	Male (n=206)	
	cases	Adjusted RR[b] (95% CI)	Adjusted RR[b] (95% CI)	Adjusted RR[b] (95% CI)	*p*-int
Maternal 1976 serum TCDD					
Overweight	106	0.83 (0.64, 1.09)	0.61 (0.42, 0.88) *	1.08 (0.79, 1.49)	0.02
Obese	31	0.96 (0.51, 1.81)	0.47 (0.20, 1.13)	1.74 0.91, 3.34)	0.03
Metabolic syndrome	24	1.72 (0.92, 3.21)	0.75 (0.11, 5.05)	2.09 (1.09, 4.02) *	0.34
Increased waist circumference	92	0.81 (0.58, 1.14)	0.71 (0.48, 1.06)	1.03 (0.59, 1.79)	0.29
Elevated triglycerides	38	1.31 (0.76, 2.27)	0.96 (0.39, 2.38)	1.54 (0.87, 2.73)	0.35
Low HDL cholesterol	50	1.16 (0.73, 1.85)	0.71 (0.30, 1.65)	1.61 (0.96, 2.68)	0.13

b) Poisson regression models

		Total (n=431)[c]	Female (n=225)	Male (n=206)	
	cases	Adjusted RR[b] (95% CI)	Adjusted RR[b] (95% CI)	Adjusted RR[b] (95% CI)	*p*-int
Increased blood pressure	68	1.28 (0.90, 1.83)	0.68 (0.22, 2.11)	1.42 (1.00, 2.02)	0.23
Elevated glucose	41	1.22 (0.68, 2.19)	1.43 (0.38, 5.35)	1.14 (0.63, 2.08)	0.76
TCDD estimated at pregnancy					
Overweight	106	0.81 (0.60, 1.09)	0.59 (0.39, 0.89)*	1.09 (0.77, 1.54)	0.02
Obese	31	0.82 (0.41, 1.62)	0.39 (0.17, 0.93)*	1.58 (0.89, 2.82)	<0.01
Metabolic syndrome	24	1.54 (0.79, 3.03)	0.83 (0.09, 7.79)	1.82 (0.94, 3.52)	0.53
Increased waist circumference	92	0.83 (0.57, 1.20)	0.77 (0.49, 1.20)	0.96 (0.57, 1.63)	0.49
Elevated triglycerides	38	1.26 (0.71, 2.23)	0.56 (0.18, 1.75)	1.89 (1.08, 3.30)*	0.05
Low HDL cholesterol	50	1.02 (0.60, 1.73)	0.63 (0.27, 1.45)	1.47 (0.83, 2.60)	0.09
Increased blood pressure	68	1.28 (0.85, 1.93)	0.76 (0.24, 2.42)	1.41 (0.95, 2.10)	0.31
Elevated glucose	41	1.14 (0.59, 2.20)	1.84 (0.52, 6.47)	0.89 (0.42, 1.89)	0.32

*p<0.05

[a]Adjusted for primary wage earner education, maternal age at explosion, maternal age at pregnancy, maternal smoking at pregnancy, maternal BMI, family history of hypertension, child age, child sex, and child smoking.

[b]Results are for a 10-fold increase in exposure.

[c]Missing data: waist circumference (n=1 female), body fat (n=2 females), blood draw (n=2 females).

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Table 4.

Adjusted[a] linear and Poisson regression models of the associations of *in utero* TCDD exposure with obesity-related outcomes, overall and stratified by child sex, among children 2 to 17 years, Seveso Second Generation Study, Italy, 1976-2016.

a) Linear regression models

		Total (n=180)	Female (n=89)	Male (n=91)	
Exposure	**Outcome**	**Adjusted β[b] (95% CI)**	**Adjusted β[b] (95% CI)**	**Adjusted β[b] (95% CI)**	***p*-int**
Maternal initial (1976) serum TCDD					
	Weight *z*-score	−0.33 (−0.69, 0.02)	−0.74 (−1.28, −0.20)*	0.05 (−0.26, 0.37)	<0.01
	Height *z*-score	−0.25 (−0.52, 0.03)	−0.54 (−0.96, −0.12)*	0.03 (−0.25, 0.31)	0.01
	BMI *z*-score	−0.27 (−0.64, 0.11)	−0.59 (−1.12, −0.06)*	0.04 (−0.34, 0.41)	0.03
	Body fat percent[c]	−3.05 (−6.09, −0.01)*	−6.76 (−11.43, −2.09)*	0.10 (−2.89, 3.09)	<0.01
TCDD estimated at pregnancy					
	Weight *z*-score	−0.20 (−0.67, 0.28)	−0.48 (−1.42, 0.46)	0.12 (−0.23, 0.47)	0.22
	Height *z*-score	−0.32 (−0.72, 0.08)	−0.58 (−1.34, 0.17)	−0.03 (−0.38, 0.32)	0.16
	BMI *z*-score	−0.04 (−0.46, 0.39)	−0.26 (−1.04, 0.52)	0.21 (−0.22, 0.63)	0.28
	Body fat percent[c]	−1.47 (−5.52, 2.58)	−3.58 (−11.11, 3.96)	0.72 (−3.10, 4.54)	0.28

b) Poisson regression models

		Total (n=180)[c]	Female (n=89)	Male (n=91)	
	cases	**Adjusted RR[b] (95% CI)**	**Adjusted RR[b] (95% CI)**	**Adjusted RR[b] (95% CI)**	***p*-int**
Maternal 1976 serum TCDD					
Overweight or obese	30	0.84 (0.48, 1.49)	0.72 (0.34, 1.52)	1.14 (0.51, 2.58)	0.38
Increased waist circumference	21	1.09 (0.57, 2.10)	0.79 (0.32, 1.94)	2.58 (1.13, 5.87)*	0.05
TCDD estimated at pregnancy					
Overweight or obese	30	0.97 (0.56, 1.68)	1.03 (0.57, 1.84)	0.83 (0.38, 1.82)	0.66
Increased waist circumference	21	1.37 (0.78, 2.42)	1.28 (0.64, 2.57)	1.93 (0.62, 6.01)	0.53

*p<0.05

[a]Adjusted for primary wage earner education, maternal age at explosion, maternal age at pregnancy, maternal smoking at pregnancy, maternal BMI, child age, and child sex.

[b]Results are for a 10-fold increase in exposure.

[c]Missing data: body fat (7-17 years n=1 female). Waist circumference and body fat were not measured for 2-6 years (n=19; 10 females, 9 males).