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Prenatal dioxin exposure and glucose metabolism in the Seveso Second Generation Study

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Abstract

Background: Exposure to endocrine disrupting compounds such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) during susceptible developmental windows may alter risk of metabolic disease later in life. Animal studies of *in utero* and lactational TCDD exposure report associations with alterations in insulin sensitivity and energy homeostasis, but epidemiologic evidence is limited. We examined the relationship of prenatal TCDD exposure with markers of glucose homeostasis in the Seveso Second Generation study, a unique cohort of children born to TCDD-exposed women resulting from a 1976 explosion in Seveso, Italy.

Methods: We included 426 children who were 18 years or older with complete follow-up data including a fasting blood draw. Insulin and glucose were measured and the updated homoeostatic model assessment was used to estimate insulin resistance (HOMA2-IR) and beta-cell function (HOMA2-B). Prenatal TCDD exposure was defined in two ways, as initial maternal serum TCDD concentration and TCDD estimated at pregnancy.

Results: The children (222 female, 204 male) averaged 28.6 (±6.0) years. We found a 10-fold increase in TCDD estimated at pregnancy was inversely associated with insulin (adj-β = −1.24 μIU/mL, 95% confidence interval (CI): −2.38, −0.09) and HOMA2-B (adj-β = −10.2% decrease, 95% CI: −17.8, −1.9) among daughters, but not sons (insulin: adj-β = 0.57 μIU/mL, 95% CI: −0.84, 1.98, *P* for interaction = 0.04; and HOMA2-B: adj-β = 0.8% increase, 95% CI −10.7, 13.9, *P* for interaction = 0.11). Similar effect modification was observed for TCDD estimated at pregnancy and HOMA2-IR (*P* for interaction = 0.13). The models for initial maternal serum TCDD showed similar effect modification by child sex. The observed associations in daughters showed evidence of mediation by body mass index, which we have previously found to be associated with prenatal TCDD exposure in female offspring.

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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.

Conclusion: These results suggest prenatal exposure to TCDD is associated with lower insulin resistance and beta compensation in female offspring, and show evidence of mediation by body mass index.

Keywords

dioxin; glucose metabolism markers; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDD; Seveso; insulin

INTRODUCTION

The increasing prevalence of diabetes worldwide is a major public health concern, associated with significant morbidity and mortality.[1] Diabetes is a complex metabolic disease characterized by hyperglycemia arising from inadequate insulin secretion, impaired insulin action, or a combination of both.[2] In addition to well-recognized risk factors such as excess caloric consumption and sedentary lifestyle, there is increasing evidence that environmental exposure to endocrine disrupting compounds plays a role in promoting metabolic disruption.[3–5] In particular, exposure during susceptible developmental windows such as *in utero* or the early postnatal period may program metabolic disease later in life.[4, 5]

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent endocrine disrupting compound and ubiquitous environmental contaminant.[3, 6, 7] It is highly lipophilic, resists metabolism, and bioaccumulates. While environmental levels are declining, due to its long half-life TCDD continues to be detected in lipid stores of humans, and fetal exposure occurs through transplacental transfer.[8–10]

In *in vitro* and animal studies, TCDD exposure has been linked to alterations in insulin secretion and glucose homeostasis.[5] However, depending on the experimental model, TCDD has been shown to cause different effects. TCDD has been shown to impair glucose-stimulated insulin secretion,[11–13] disrupt insulin signaling,[14, 15] and reduce glucose uptake.[16, 17] In models of diabetes in rats, TCDD was shown to cause hypoglycemia.[18] Studies of *in utero* and lactational TCDD exposure in mice also report impairments in insulin sensitivity and energy homeostasis.[19, 20]

Epidemiologic studies of TCDD exposure and glucose homeostasis are inconsistent. Significant positive associations with diabetes have sometimes been reported in Vietnam veterans exposed to TCDD-contaminated Agent Orange,[21–23] but not always.[24, 25] In the Seveso Women's Health Study (SWHS), serum TCDD levels soon after exposure were not associated with diabetes diagnosis about thirty years later.[26] Cross-sectional epidemiologic studies of exposure to TCDD and dioxin-like polychlorinated biphenyls (PCBs) and markers of insulin sensitivity have also reported variable associations.[27–31] Higher serum dioxin levels were associated with lower insulin sensitivity in Operation Ranch Hand veterans,[30] higher insulin levels in residents near a TCDD-contaminated Superfund site,[29] and increased insulin resistance in non-diabetic persons living near a deserted pentachlorophenol factory in Taiwan.[27, 28] Among adult Inuit living in Greenland, dioxin-like PCBs were significantly inversely associated with pancreatic beta cell function.[31]

Only one previous study has examined associations with perinatal TCDD exposure, a sensitive window for programming of all aspects of metabolism.[4] A Dutch longitudinal birth cohort study with follow-up into adolescence found associations between perinatal dioxin exposure and altered glucose metabolism.[32] Specifically, dioxin toxic equivalents (TEQ) in breast milk were inversely associated with fasting insulin, but not glucose, in 33 adolescents (average age 15 years). This study suggests that prenatal dioxin exposure may influence the metabolic parameter insulin secretion later in life. Nonetheless, interpretation is limited by the small sample size and follow-up during adolescence, a period of variable insulin sensitivity.[33]

Using data from the Seveso Second Generation Study, we aim to examine the metabolic consequences of prenatal TCDD exposure that manifest in adulthood. The Seveso Second Generation Study is a cohort of children born to members of the Seveso Women's Health Study (SWHS), a cohort of female residents of Seveso, Italy who were exposed to high levels of TCDD as a result of a nearby chemical factory explosion on 10 July 1976.[34–38] Here, we examine the relationship of prenatal TCDD exposure with biomarkers of glucose metabolism in adult children and determine whether the relationship is modified by child sex.

MATERIALS AND METHODS

Study population

Details of SWHS have been previously presented.[38, 39] Briefly, women eligible for the study were newborn to 40 years of age in 1976 (the time of the chemical explosion), resided in the most highly contaminated areas at the time of the explosion, and had adequate stored sera collected soon afterwards in which to measure individual-level TCDD exposure. Initial enrollment took place from March 1996 to July 1998, and 981 women (80% of those eligible) participated. Between May 2014 and May 2016, children born to SWHS women were eligible for the Seveso Second Generation study if they were born after the explosion in 1976 and at least 2 years of age. A total of 611 children (66.4% of 920 alive and eligible) born to 402 SWHS mothers participated in the study visit. Given the wide age range (2 to 39 years) of children and because puberty is associated with decreased insulin sensitivity,[33, 40] we restricted the analysis to the 431 children who were 18 years or older. We excluded two who refused the blood draw and three who reported current treatment for diabetes, leaving a final analysis sample of 426 adult children born to 303 mothers.

The study was approved by all participating institutions' Institutional Review Boards. Written informed consent was obtained from all children age 18 years or older. Data collection included fasting blood draw, anthropometric (height, weight, waist circumference) and blood pressure measurements, personal interview, and food frequency questionnaire.[41]

Prenatal TCDD exposure

Prenatal TCDD exposure was examined as 1) maternal initial (1976) serum TCDD level and 2) maternal TCDD estimated at pregnancy. For all SWHS participants, TCDD was measured by high-resolution gas chromatography/high-resolution mass spectrometry methods in

archived sera collected soon after the explosion.[42] Details of serum sample selection and 1976 TCDD concentrations have been previously presented.[38, 43] TCDD values are reported on a lipid weight basis as parts-per-trillion (ppt).[44] Values below the limit of detection were assigned values of one-half the detection limit.[45] As previously described, maternal serum TCDD concentrations at pregnancy were estimated by extrapolation from the TCDD concentration closest to but preceding the pregnancy (1976, 1996) via first-order kinetic model and a half-life that varieswith initial dose, age, and other covariates.[39, 46]

Outcome assessment

Fasting blood samples were analyzed for glucose and insulin on the automatic analyzer COBAS 8000 (Roche Diagnostics, Mannheim, Germany) at the University Hospital of Desio Laboratory. Glucose was measured in plasma by the reference enzymatic method (hexokinase). Insulin was measured in serum by electrochemical luminescence immunoassay (ECLIA). We used the computer-based homeostatic model to assess insulin resistance (updated homoeostatic model assessment of insulin resistance, HOMA2-IR) and pancreatic beta cell function (updated homoeostatic model assessment of beta-cell function, HOMA2-B).[47, 48]

Statistical analysis

Measures of prenatal TCDD exposure (maternal 1976 serum TCDD, maternal TCDD estimated at pregnancy) were $\log_{10}$-transformed in order to reduce the influence of outliers and analyzed as continuous variables. The relationship of prenatal TCDD exposure with continuous outcomes (insulin, glucose, HOMA2-IR, HOMA2-B) was examined using multivariable linear regression. Distributions of HOMA2-IR and HOMA2-B variables were highly skewed, so they were log-transformed to achieve a more normal distribution; thus, model coefficients denote a multiplicative factor for HOMA2-IR and HOMA2-B, here presented as a percent change, associated with a 10-fold increase in prenatal TCDD exposure. For all models, we evaluated the shape of the exposure-response curves using generalized additive models (GAMs) with a 3-degree-of-freedom cubic spline; the process was done in the full sample, as well as in males and females separately. As we did not find evidence of non-linearity, all models presented use linear regression with continuous exposures.

Based on a review of the literature, we considered the following variables as potential confounders: maternal age at explosion, age and smoking during pregnancy; household socioeconomic status including education, income, occupation, and marital status; family history of diabetes; and child sex, age, birth weight, tobacco or alcohol use, diet, and physical activity. Covariate data was collected during the interview. The final set of covariates used in the model was determined using a directed acyclic graph (DAG) (Supplementary Figure 1). Final models were adjusted for age at interview, sex, primary wage earner education, and maternal age at pregnancy. In all analyses, we considered effect modification by sex by including a cross-product term between exposure and sex, with interaction p-values < 0.2 considered significant.

Since insulin resistance is correlated with higher body fat, we hypothesized that associations between prenatal TCDD and glucose metabolism biomarkers might be mediated through body mass index (BMI, kg/m^2). We used mediation models (SEM[49] and counterfactual[50]) to assess the total, direct, and indirect (via BMI as a mediator) effects of prenatal TCDD exposure on glucose metabolism biomarkers.

In sensitivity analyses, we reanalyzed the final models after excluding three outliers with standardized residuals greater than 3 or less than −3. We also ran the analysis including some of the younger Seveso Second Generation participants, children ages 14 to 18 only (n=74), a sample similar to Leijs et al.[32] 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.[51]

Results

Select characteristics of the 426 adult child participants are presented in Table 1. Maternal age at pregnancy averaged 27.8 (±4.8) years and about 13% reported smoking during the pregnancy. At interview, children were an average of 28.6 (±6.0) years and about half were female. About one-third of children were current smokers, more than half currently consumed alcohol, and one-third reported a family history of diabetes in a first-degree relative. The average BMI of children was 23.6 (±3.7) kg/m^2, with 32% classified as overweight or obese. SWHS women who gave birth to female children were older at the time of explosion and at pregnancy than women who gave birth to male children (see Supplementary Table 1). Male children had higher average BMI, and consumed more total calories and a lower percentage of calories from fat but a higher percentage from alcohol than female children. When adjusted for body weight, total calories consumed per kilogram body weight did not differ significantly between male and female children (30.0±11.7 kcal/kg vs. 29.1±11.8 kcal/kg, p=0.40). Male children were also more likely to be more physically active than female children.

At the time of the explosion, the average age of SWHS mothers (n=303) was 18.3 (±6.5) years and ranged from 6 to 36. Median maternal TCDD concentrations in 1976 serum and estimated at pregnancy were 53.1 ppt and 20.6 ppt, respectively. Maternal 1976 TCDD levels were higher among female children but did not differ with respect to other child factors. As reported previously, mothers who were youngest at the time of the explosion had higher 1976 TCDD levels.[43] In Table 2, concentrations of glucose metabolism biomarkers are summarized overall and stratified by sex. Fasting glucose levels were lower and HOMA2-B was higher in females than males, but all biomarkers were within normal reference ranges.

In adjusted models, in the full sample prenatal TCDD exposure was not associated with glucose metabolism biomarkers (Table 3, Figure 1). However, we observed evidence of effect modification by sex. A 10-fold increase in TCDD estimated at pregnancy was inversely associated with insulin (adj-β = −1.24 μIU/mL, 95% confidence interval (CI): −2.38, −0.09) and HOMA2-B (adj-β = −10.17 percent, 95% CI: −17.76, −1.88), in females, but not males (insulin: adj-β = 0.57 μIU/mL, 95% CI: −0.84, 1.98, P for interaction = 0.04;

and HOMA2-B (adj-β = 0.83 percent, 95% CI −10.72, 13.88, *P* for interaction = 0.11). Similar effect modification was observed for TCDD estimated at pregnancy and HOMA2-IR (*P* for interaction = 0.13). The models for maternal 1976 TCDD showed similar indications of effect modification by sex, but was significant only for insulin (*P* for interaction = 0.11).

The observed associations between prenatal TCDD exposure and glucose metabolism biomarkers in females show evidence of mediation through BMI when considered using SEM (Table 4) or counterfactual models (Supplementary Table 2). Results of mediation models suggest that a significant part of the observed associations between both measures of prenatal TCDD exposure and glucose metabolism biomarkers is mediated through BMI. For example, for TCDD estimated at pregnancy, SEMs estimate that about 48% of the total inverse association with HOMA2-IR is mediated thru BMI (Table 4). The extent of mediation seems comparable between the two measures of prenatal TCDD exposure (maternal 1976 TCDD and TCDD estimated at pregnancy), although not all total effects are significant (Table 4 and Supplementary Table 2). Estimates of total effect sizes are different for the counterfactual model, likely due to differences between the two methods; counterfactual models aim to approximate a causal effect of an exposure, while structural equations are more comparable to standard linear regressions. However, while the statistical significance varies between the two types of model, the estimates trend in a similar direction.

In sensitivity analyses, excluding outliers (n=3) did not change the results meaningfully (results not shown). In the sensitivity analysis examining the subset of children of age 14 to 18 years similar to Leijs et al.[32], we found positive associations between prenatal TCDD exposure and some glucose metabolism biomarkers in males, but not females (Supplementary Table 3). While these results diverge from our primary models, it is likely that the estimates were unstable due to small numbers.

Discussion

This is the first epidemiologic study to examine the metabolic disruptive effects of prenatal TCDD exposure in children followed into adulthood. In the Seveso Second Generation study, we observed sex-specific effects on glucose homeostasis, with inverse associations with markers of insulin resistance and beta-cell compensation in daughters but not sons. Specifically, among adult children (18 years or older), prenatal TCDD exposure, as measured by maternal TCDD estimated at pregnancy, was associated with lower insulin levels, HOMA2-IR, and HOMA2-B in daughters only. No associations with prenatal TCDD exposure measures were found for fasting glucose in either daughters or sons. Results were similar for prenatal TCDD exposure as measured by maternal 1976 TCDD. The observed associations with prenatal TCDD exposure, however, were likely mediated by body mass index, which we have previously found to be associated with *in utero* TCDD exposure in female offspring only.[52] In fact, results of mediation models suggest a significant part of the observed associations between prenatal TCDD exposure and glucose metabolism biomarkers in daughters is indirect through BMI.

The majority of studies of dioxin exposure and glucose metabolism in adult populations are cross-sectional.[27–31] Only one previous Dutch study has examined dioxin exposure during

the prenatal period. Our results in daughters only are consistent with Leijs et al.[32] who reported prenatal TEQ exposure was significantly inversely correlated with fasting insulin but not glucose in 33 adolescents (18 girls, 15 boys). However, we were unable to replicate their findings in a sensitivity analysis with children of similar age, 14 to 18 years. Both the Dutch study and the sensitivity analysis in our population suffered from a small number of participants, and thus may have lacked sufficient power to identify sex-specific associations. Further, both studies were not able to consider puberty status, which may affect insulin resistance or its relationship with TCDD.

The observed sex-specific effects of prenatal TCDD exposure on glucose homeostasis and potential mediation via body mass index are biologically plausible. Experimental data demonstrate that TCDD can affect multiple levels of glucose regulation, from β-cell insulin secretion to insulin signaling in metabolically active tissues.[11–17] Animal studies suggest there may be sex-specific programming effects of perinatal TCDD exposure on energy homeostasis.[20, 53] In mouse models, sex-specific effects of perinatal TCDD exposure (designed to mimic human dietary exposure) were reported in offspring followed into adulthood for a wide range of endpoints involved in energy homeostasis.[20] For example, perinatal TCDD exposure led to a leaner phenotype in males and an overweight phenotype in females. In a second mouse study, perinatal exposure to dioxin-like PCB-126 altered body composition in a sex-specific manner.[53] While we observe associations in females only, it is possible there are additional effects on energy homeostasis in males that we were unable to observe; developmental exposure to TCDD may alter set points and long-term trajectories for changes that do not manifest until later in life, when the system is challenged by additional stressors.[4]

Sex-specific effects are frequently observed with exposure to endocrine disrupting compounds such as TCDD, as the endocrine milieu of males and females is different.[4] We have also reported sex-specific effects of *in utero* TCDD exposure with cardiometabolic endpoints.[52] The toxic effects of TCDD are mediated via binding the aryl hydrocarbon receptor (AhR),[54] and AhR has been shown to be involved in metabolism and central regulation of energy balance.[55, 56] AhR activation may thus explain the observed programming effects of TCDD on energy homeostasis in adult offspring. Although mechanisms by which AhR regulates energy homeostasis are not well understood, both direct and indirect mechanisms, including cross-talk with the estrogen receptor (ER), are likely to be involved and may contribute to sex-dependent differences. Estrogens are also known to play a role in regulating energy homeostasis including sex-specific mechanisms in lipid and glucose metabolism.[4] The observed sex-specific effects could also be related to the suppression during development of gonadotropins,[20] which regulate growth in a sex-specific manner via regulation of sex hormones.[60]

This study has several strengths, which include a large sample size, prospective assessment of glucose homeostasis measures, and follow-up into adulthood. Eligible post-explosion SWHS children who participated in the Seveso Second Generation Study did not differ from non-participants with respect to maternal characteristics at explosion or initial TCDD exposure, limiting potential participation bias.[39] We assessed glucose/insulin homeostasis using models that have been shown to correlate well with the euglycemic-clamp method,

which is considered the gold standard.[61] 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. The study population is also relatively homogeneous with regard to potential confounders such as socioeconomic status, breastfeeding, and diet.

The present study has some 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 (data not shown). We had to rely on an extrapolation model to estimate TCDD at pregnancy, but we would expect any exposure misclassification to be non-differential and drive any associations towards the null. The study was limited to maternally-mediated effects of prenatal TCDD exposure; as we lack information on paternal exposure and were unable to estimate its effects. We were also unable to consider sources of postnatal exposure. The wide age range of the Second Generation cohort likely increased variability in outcome measures. However, by limiting the study to participants who had reached adulthood, we were able to minimize variability due to puberty.[62] Finally, HOMA2-IR and HOMA2-B primarily describe hepatic insulin resistance and steady state insulin secretion.[63] It is not known whether similar associations exist between TCDD exposure and measures of insulin resistance and secretion in the post-prandial state.

In summary, this is the first epidemiologic study to examine the metabolic disruptive effects of *in utero* TCDD exposure in a cohort of children followed into adulthood. Prenatal TCDD exposure was associated with lower insulin resistance and beta cell challenge among daughters but not sons, suggesting a sex-specific influence of dioxin exposure in the perinatal period on pancreatic development. The observed associations with prenatal TCDD exposure, however, were likely mediated by body mass index, which we have also previously found to be associated with *in utero* TCDD exposure in female offspring only.[52] Continued follow-up of the unique Seveso Second Generation cohort may inform the long-term impact of prenatal TCDD exposure on metabolic diseases including diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

BMI	body mass index
DAG	directed acyclic graph
HOMA2-B	homoeostatic model assessment to estimate beta-cell function
HOMA2-IR	homoeostatic model assessment to estimate insulin resistance
PCB	polychlorinated biphenyl
SEM	structural equation modeling
SWHS	Seveso Women's Health Study
TCDD	2,3,7,8-tetracholorodibenzo-*p*-dioxin
TEQ	toxic equivalent

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Highlights

- The Seveso Second Generation study is a cohort of children born to TCDD-exposed women
- We examined associations of in utero TCDD exposure with glucose homeostasis
- In utero TCDD exposure was associated with lower insulin resistance in females only
- Observed associations in daughters showed evidence of mediation by body mass index

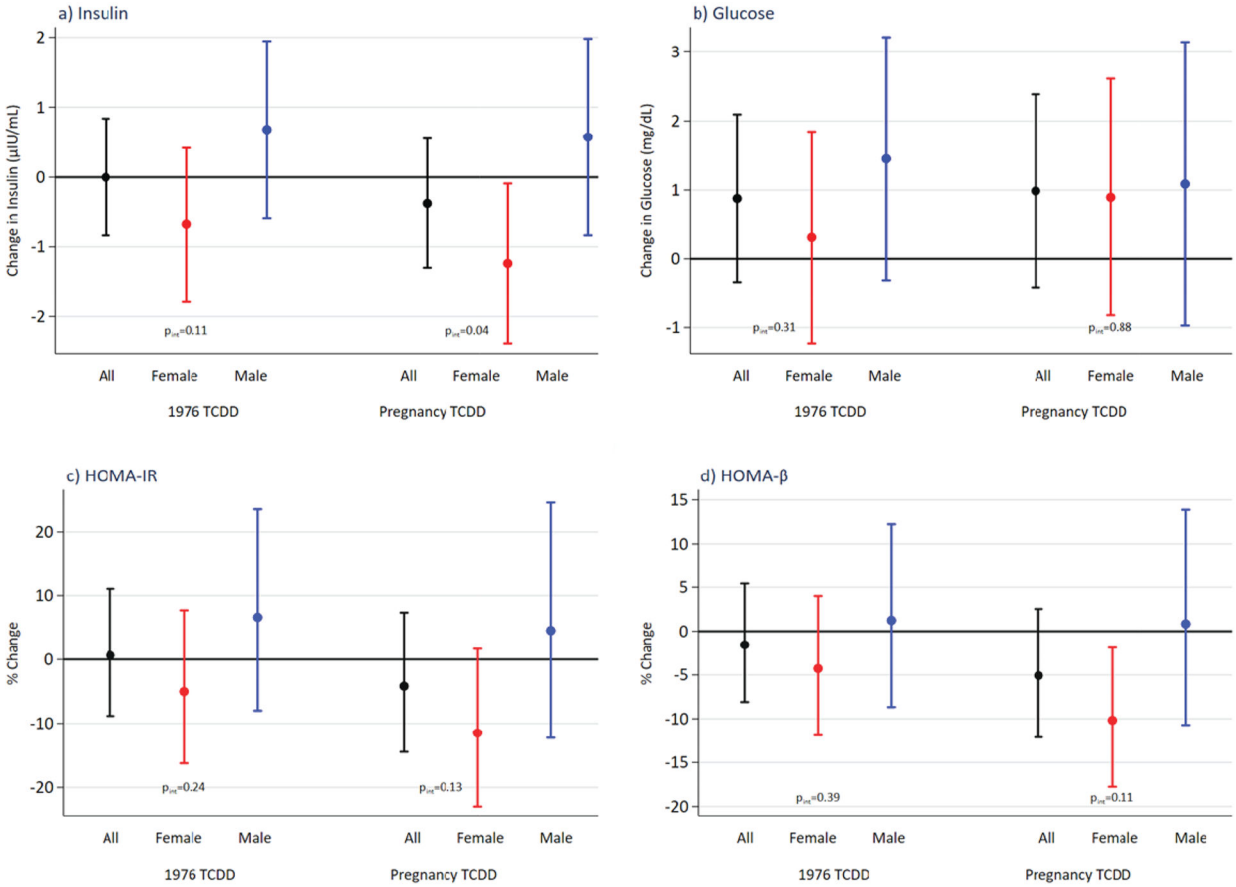

Figure 1.

Associations of prenatal TCDD exposure measures with a) insulin, b) glucose, c) HOMA-IR, and d) HOMA2-β stratified by sex, Seveso Second Generation Study, Seveso, Italy, 2014–2016.

Table 1.

Summary of participant characteristics, Seveso Second Generation Study, Seveso, Italy, 1976–2016.

Characteristic	Total n (%)
N	426 (100.0)
Maternal age (years) at pregnancy	
<25	104 (24.4)
25–29	188 (44.1)
30–34	94 (22.1)
35+	40 (9.4)
Maternal smoking during pregnancy	
No	372 (87.3)
Yes	54 (12.7)
Child age (years) at interview	
18–23	114 (26.8)
24–29	113 (26.5)
30–34	101 (23.7)
35–39	98 (23.0)
Child sex	
Female	222 (52.1)
Male	204 (47.9)
Primary wage earner education	
≤ Required	133 (31.2)
Secondary	215 (50.5)
> Secondary	78 (18.3)
Smoking status	
Never	222 (52.1)
Former	67 (15.7)
Current	137 (32.2)
Alcohol status	
Never	142 (33.3)
Former	51 (12.0)
Current	233 (54.7)
Physical activity	
Less active than others	154 (36.2)
About the same	172 (40.4)
More active than others	100 (23.5)
Body mass index	
Underweight	25 (5.9)
Normal	265 (62.2)
Overweight	106 (24.9)
Obese	30 (7.0)
Dietary intake	

Characteristic	Total n (%)
Total calories[a]	1,953 (±772)
% carbohydrates[a]	45.7 (±7.2)
% protein[a]	16.5 (±2.9)
% fat[a]	35.2 (±5.5)
% alcohol[a]	2.5 (±3.2)
Family history of diabetes	
No	259 (60.8)
Yes	152 (35.7)
DK	15 (3.5)
Maternal 1976 serum TCDD (ppt)[b]	53.1 (25.1, 112.0)
TCDD estimated at pregnancy (ppt)[b]	20.6 (9.4, 47.1)

Abbreviation: DK, don't know; TCDD, 2,3,7,8-tetrachloro-*p*-dibenzodioxin; ppt, part-per-trillion

[a]mean ± standard deviation

[b]median (interquartile range)

Table 2.

Summary of glucose metabolism biomarkers, overall and by sex, Seveso Second Generation Study, Italy, 2014–2016.

	Total	Female	Male
N	426	222	204
Insulin (μIU/mL)	8.4 ±4.9	8.6 ±4.6	8.1 ±5.1
Glucose (mg/dL)	89.7 ±7.5	87.6 ±6.5	92.0 ±8.0
HOMA2-IR	0.93 ±1.8	0.97 ±1.7	0.90 ±1.8
HOMA2-B	92.5 ±1.5	99.6 ±1.4	85.4 ±1.5

Abbreviations: HOMA2-IR, homeostatic model assessment of insulin resistance; HOMA2-B, homeostatic model of beta-cell function

Values are mean ± SD for continuous, geometric mean ± GSD for HOMA2-IR and HOMA2-B.

Table 3.

Adjusted[a] linear regression models of the associations of prenatal TCDD exposure with glucose metabolism biomarkers, overall and stratified by sex, Seveso Second Generation Study, Seveso, Italy, 2014–2016.

		Total (n=426)		Female (n=222)		Male (n=204)		
Exposure[b]	**Outcome**	**Adjusted β (95% CI)**		**Adjusted β (95% CI)**		**Adjusted β (95% CI)**		***P*-int**
Maternal 1976 serum TCDD								
	Insulin	0.00	(−0.84, 0.84)	−0.68	(−1.78, 0.42)	0.68	(−0.58, 1.95)	0.11
	Glucose	0.88	(−0.34, 2.09)	0.31	(−1.23, 1.85)	1.45	(−0.31, 3.21)	0.31
	HOMA2-IR	0.62%	(−8.84, 11.05)	−5.01%	(−16.24, 7.71)	6.61%	(−8.00, 23.54)	0.24
	HOMA2-B	−1.56%	(−8.09, 5.43)	−4.27%	(−11.89, 4.00)	1.24%	(−8.68, 12.24)	0.39
TCDD estimated at pregnancy								
	Insulin	−0.37	(−1.30, 0.55)	−1.24	(−2.38, −0.09) *	0.57	(−0.84, 1.98)	0.04
	Glucose	0.99	(−0.41, 2.39)	0.90	(−0.82, 2.61)	1.09	(−0.97, 3.15)	0.88
	HOMA2-IR	−4.18%	(−14.46, 7.34)	−11.51%	(−22.97, 1.66)	4.55%	(−12.26, 24.58)	0.13
	HOMA2-B	−5.08%	(−12.12, 2.53)	−10.17%	(−17.76, −1.88) *	0.83%	(−10.72, 13.88)	0.11

Abbreviation: HOMA2-IR, homeostatic model assessment of insulin resistance; HOMA2-B, homeostatic model of beta-cell function

* $P<0.05$

[a] Adjusted for age at interview, sex, primary wage earner education, and maternal age at pregnancy.

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

Table 4.

Results of structural equation models[a,b] to assess mediation by body mass index of relationship between prenatal TCDD exposure and glucose metabolism biomarkers among female participants, Seveso Second Generation Study, Seveso, Italy, 1976–2016.

		Direct		Indirect		Total	
Exposure[c]	**Outcome**	**Adjusted β (95% CI)**		**Adjusted β (95% CI)**		**Adjusted β (95% CI)**	
Maternal 1976 serum TCDD							
	Insulin	−0.14	(−1.15, 0.87)	−0.54	(−1.08, 0.00)*	−0.68	(−1.72, 0.36)
	Glucose	0.69	(−0.79, 2.16)	−0.38	(−0.79, 0.03)	0.31	(−1.20, 1.82)
	HOMA2-IR	−0.11%	(−10.52, 11.52)	−4.91%	(−9.65, 0.07)	−5.01%	(−15.49, 6.76)
	HOMA2-B	−1.77%	(−8.63, 5.60)	−2.55%	(−5.13, 0.11)	−4.27%	(−11.17, 3.16)
TCDD estimated at pregnancy							
	Insulin	−0.63	(−1.69, 0.42)	−0.60	(−1.15, −0.06)*	−1.24	(−2.32, −0.15)*
	Glucose	1.33	(−0.30, 2.95)	−0.43	(−0.86, −0.01)*	0.90	(−0.78, 2.57)
	HOMA2-IR	−6.39%	(−17.34, 6.00)	−5.46%	(−10.26, −0.41)*	−11.51%	(−22.23, 0.69)
	HOMA2-B	−7.57%	(−14.69, 0.14)	−2.81%	(−5.45, −0.09)*	−10.17%	(−17.16, −2.59)**

Abbreviation: HOMA2-IR, homeostatic model assessment of insulin resistance; HOMA2-B, homeostatic model of beta-cell function

*$P<0.05$

**$P<0.01$

[a]Analysis based on Baron and Kenny (1986).

[b]Models adjusted for age at interview, primary wage earner education, maternal age at pregnancy.

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