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Prenatal DDT Exposure and Testicular Cancer: A Nested Case-Control Study

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Abstract

We examined maternal serum levels of DDT-related compounds in relation to son's risk of testicular cancer 30 years later. Fifteen of 9,744 live-born sons were diagnosed with germ cell testicular cancer and had maternal serum samples. Cases were matched to three controls on race and birth year. Maternal serum DDT-related compounds, measured in the early postpartum, were associated with her son's risk of testicular cancer. Despite low statistical power, we observed that mothers of cases had a significantly higher ratio of *p,p'*-DDT to *p,p'*-DDE and lower *o,p'*-DDT. These findings are consistent with earlier exposure to DDT and with slower *p,p'*-DDT elimination among mothers of cases. Whether these associations could be direct, or operate via other pathways is unknown. Further research on inter-individual differences in DDT metabolism could provide clues to testicular cancer etiology.

Introduction

Testicular cancer is more common in Caucasians and among men with a family history of testicular cancer, a personal history of cryptorchidism or testicular cancer and possibly among men of tall stature.¹ Testicular cancer incidence rose dramatically worldwide among birth cohorts born after 1945,² implicating a widespread environmental exposure. Exposures that influence estrogen/androgen balance *in utero* have been suggested as a possible explanation³, and this hypothesis is consistent with the role of androgens in directing testicular descent.⁴⁻⁵ The widespread introduction of commercial DDT in 1945⁶ coincides with the increase in testicular cancer incidence. The persistence of *p,p'*-DDE, a potent anti-androgenic DDT metabolite, is of particular concern.⁷

Commercial grade DDT is one of the most ubiquitous exposures in history. In the United States, nearly all persons had measurable levels of DDT-related compounds in blood or tissue samples obtained in the 1960s-1980's.⁸⁻¹¹ Thus the analytic strategy for studying DDT exposure in relation to human health is not simple; it is not possible to compare the exposed with the unexposed. Instead, simultaneous consideration of several DDT-related compounds can be informative in devising a measure of exposure.

The relevant DDT compounds are *p,p'*-DDT [1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane] the primary component and active ingredient of commercial DDT, *o,p'*-DDT [1,1,1-Trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)ethane] a low level contaminant of commercial DDT, and *p,p'*-DDE [1,1'-Dichloro-2, 2'-bis(*p*-chlorophenyl)ethylene] the

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primary metabolite of *p,p'*-DDT. *o,p'*-DDT is the least persistent of these compounds and is a marker of recent exposure to commercial grade DDT. *p,p'*-DDE is the most persistent of these compounds.⁹

The *p,p'*-DDT/*p,p'*-DDE ratio (DDT/DDE ratio) in maternal serum was chosen *a priori* as the primary risk variable in the present study, based on previous observations that this ratio may be an informative indicator of exposure source and timing.¹²⁻¹⁶ A higher DDT/DDE ratio in maternal serum could be due to slower elimination of *p,p'*-DDT, or alternatively, more recent exposure to commercial DDT. To distinguish between these alternatives, we also examined maternal *o,p'*-DDT as an additional indicator of recent exposure to commercial DDT. We were particularly interested in whether findings would support the hypothesis that cases eliminate *p,p'*-DDT more slowly, resulting in higher cumulative exposure of the fetus to *p,p'*-DDT. This hypothesis would predict that the mothers of cases would have a higher DDT/DDE ratio, but lower levels of *o,p'*-DDT.

Methods

This study is based on existing data available from a 40- year follow-up of more than 20,000 Northern California pregnancies between 1959 and 1967: The Child Health and Development Studies (CHDS). Maternal blood samples were obtained during the 1960's, at the period of peak DDT use in the United States.^{6, 17} Prior studies in CHDS mothers demonstrate that *p,p'*-DDT, the primary ingredient of commercial grade DDT, can be measured in all CHDS mothers, eliminating the need to use the more persistent metabolite, *p,p'*-DDE, as a proxy for past DDT exposure.^{16, 18} *o,p'*-DDT, a low concentration contaminant of commercial DDT that is less persistent, can also be measured as an indicator of recent exposure in the majority of CHDS serum samples.^{16, 18}

Participants were the sons of women who participated in the Child Health and Development Studies.¹⁹ Women who were members of the Kaiser Foundation Health Plan, residing in the Oakland, California area and pregnant between 1959 and 1967, were eligible for the CHDS. 98 % of eligible pregnancies were enrolled. Archived maternal pregnancy and postpartum serum samples are available in addition to prenatal characteristics obtained from maternal interview and medical records. Mother's weight and height were measured at the first prenatal interview. Rate of maternal pregnancy weight gain was calculated as the last third trimester weight measured before delivery minus weight measured at the prenatal interview divided by days elapsed between the visits. Son's birth weight was measured and recorded in the delivery room according to a standardized protocol developed for the CHDS, using a regularly calibrated scale. Date of last menstrual period and number of previous pregnancies were also reported by the mother during the prenatal interview. Gestational age was calculated as days from last menstrual period to delivery. A description of CHDS data files can be found at http://www.chdstudies.org/index.php/overview_of_data_files.html.

All names for each CHDS subject were submitted for cancer linkage to the California Cancer Registry using fixed (i.e. birth date, sex, race and name) and changeable (i.e. address and patient record number) identifiers. A rigorous protocol was used to verify cases, comparing fixed versus changeable identifiers by manual review.^{18, 20} The California Cancer Registry is reported to be >99 percent complete after a lag time of about 2 years.²¹ Record abstraction is performed by highly trained abstractors, and is based primarily on review of pathology reports, most usually required for cancer diagnoses (<http://seer.cancer.gov/tools/codingmanuals/index.html>). False positives are highly unlikely, particularly for testicular cancer where the diagnosis is made by examination of a biological specimen.

All members of the CHDS cohort were additionally linked to the California Department of Motor Vehicles (DMV) files on a regular basis to determine residence history allowing us to assess their control status and to update any name changes. All names registered with the DMV were used in establishing a match. Simultaneous linkage of multiple family members enhanced matching. The regular DMV matching provided a history of location for each subject which is used to determine the population at risk for cancer, corresponding with geographic surveillance by California's cancer registry. Participants who cannot be located were considered lost to follow-up at the date of their last definitive classification as a California resident.

Germ cell testicular cancer cases (equivalent to ICD 10 No. C62) were identified by linking study records to data in the California Cancer Registry through 2000. Nineteen CHDS sons (born 1959-1967) were diagnosed with testicular cancer in adulthood (age range of 17 to 37 years). One additional case was diagnosed in infancy.

For the adult-onset cases, three controls, known to be free of testicular cancer and to reside in the California Cancer Registry surveillance area on the date of diagnosis of the case, were matched to the case on race and year of birth. Controls were selected at random from the pool of matches if more than three were available. A life table analysis of CHDS male birth cohorts using published cohort specific incidence rates for testicular cancer was performed to estimate the number of cases expected from follow-up through 2000. The expected number estimated was 18.4 cases compared to 20 observed. (See Supplementary Materials on the Internet for details of life-table construction). The Institutional Review Board of the Public Health Institute approved the protocols for this study.

CHDS mothers generally donated blood samples during each trimester of pregnancy and in the early postpartum, 1 to 3 days after delivery. However, samples for each trimester are not available for all women. In 2004, we measured DDT-related compounds in serum obtained between 1959 and 1967 from CHDS mothers, preferentially in their postpartum serum sample. Organochlorine levels assayed across all trimesters of pregnancy and the early postpartum correspond,²² therefore we chose postpartum samples whenever available, in order to conserve case serum obtained during pregnancy for future studies where timed samples might be more critical. The mean age of mothers when blood was drawn was 26 years. Samples collected within one to three days of delivery were used for 13 of 15 cases and 40 of 45 controls. One case had only first trimester serum and one case had only third trimester serum samples. Two controls had only first trimester serum and three controls had only third trimester serum samples.

Serum samples had been stored at -20 degrees centigrade, and were first thawed to prepare an aliquot of 1.5 mL for organochlorine assays. The aliquots were shipped frozen to the laboratory. *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT were assayed in the laboratory of Dr. Mary Wolff, using methods developed previously.²³ Sample order was randomly assigned within and across batches. Cases and controls within strata were analyzed in the same batches to minimize differences due to laboratory drift. The laboratory was blind as to case status of the samples. Limits of detection were approximately 0.07 ug/L for individual compounds based on three times the standard deviation of the levels found in the blanks or the lowest quality control pool.²⁴ As described previously, we used all observed positive values of *o,p'*-DDT in analyses, even those reported to be below the limit of detection.²⁵ Seven *o,p'*-DDT measurements with reported negative values were recoded as the lowest measured value (i.e., 0.04 ug/L in this study). All values of *p,p'*-DDT and *p,p'*-DDE were above the limit of detection (0.07 ug/L) as were 46 of the 60 values of *o,p'*-DDT. Inter-batch and intra-batch coefficients of variation in this study, based on a quality control pool created from non-critical CHDS samples were 8.9% and 5.4% respectively for *p,p'*-DDT, 9.7% and 5.9% for *p,p'*-DDE and 29.3% and 19.5% for

o,p'-DDT. The coefficient of variation for *o,p'*-DDT is higher because the concentrations were lower than for the other DDT-related compounds. Total cholesterol and total triglycerides were measured by Quest Diagnostics. Teterboro, New Jersey according to a standardized protocol; autoanalyzer for total cholesterol and a custom in-house reagent incorporating a coupled enzymatic reaction of lipase, glycerol kinase, glycerol phosphate oxidase, and peroxidase, with blanking of endogenous free glycerol for triglycerides.

The Wilcoxon matched pairs, signed ranks test was used to compare the case value of each risk factor to the mean of the control values within each matched set. An exact p-value was obtained by comparing the signed rank statistic to its permutation distribution. The method was implemented using SAS Proc Univariate for SAS 9.1.

Due to the small number of case-control strata, the associations between risk factors and case/control status were analyzed using exact conditional logistic regression. Stratified analysis was performed to control for the correlations within matched sets. All tests were performed using the two sided alternative. To avoid excessive stratification in these data we generally limited analyses to univariate and bivariate models. These methods were implemented using the exact method of LogXact 7 Procs for SAS Users in conjunction with SAS 9.1.²⁶

Results

The median age at diagnosis for 20 testicular cancer cases was 30 years, including one case diagnosed in infancy. Only one case had a history of cryptorchidism. Sixteen cases were born to Caucasian mothers, two were born to Asian mothers, one was born to a Hispanic mother, and one was born to a multi-racial mother. Fourteen cases were seminomas of which one case also had an earlier non-seminoma primary, and 6 were non-seminomas. Cases lacking maternal pregnancy or postpartum serum were excluded (N=5, including the only case diagnosed in infancy).

Table 1 presents the distribution of risk variables investigated in this study. Testicular cancer cases had significantly shorter gestations, and their mothers had lower levels of *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE, but a higher DDT/DDE ratio than their matched controls. A larger DDT/DDE ratio in maternal serum was observed in 11 of 15 case-control strata, $p<0.03$ for the Wilcoxon matched pairs signed ranks test (Table 1) and was significantly associated with testicular cancer risk in exact conditional logistic regression analyses ($p<0.01$, Table 2).

Accounting for recent exposure to commercial DDT, by controlling for *o,p'*-DDT, increased the strength of this association (Model 6 compared to Model 5 in Table 2). There was no indication that this association was explained by other perinatal risk factors examined including mothers' body mass index, pregnancy weight gain, or son's gestational age. (Model 8,9,13, Table 2).

Discussion

The metabolism of DDT has not been fully described²⁷ and this gap limits our ability to fully interpret our findings. However, available evidence does establish several features of DDT metabolism relevant to the present study.

First, metabolic studies,²⁷⁻²⁸ primate feeding experiments,²⁸ human feeding experiments,⁹ and human survey studies¹⁰⁻¹¹ support the concept that *p,p'*-DDT is more rapidly metabolized and excreted than *p,p'*-DDE. The higher DDT/DDE ratio observed in mothers of cases is consistent with recent exposure to commercial DDT or alternatively, slower elimination of *p,p'*-DDT.

Second, although there is a paucity of metabolic studies of *o,p'*-DDT,²⁷ human feeding experiments and human survey studies have also established that *o,p'*-DDT is more rapidly metabolized and excreted than *p,p'*-DDT.⁹⁻¹¹ Since *o,p'*-DDT is acquired as a low level contaminate of commercial DDT, it would be expected that mothers with recent exposure to DDT would have both a higher DDT/DDE ratio and higher levels of *o,p'*-DDT.

In the present study, mothers of sons with testicular cancer had a higher DDT/DDE ratio, but lower *o,p'*-DDT, presenting an informative paradox. Possible explanations for this paradox include: 1) Case mothers had higher exposures earlier in life at a critical period, possibly even prior to pregnancy. 2) Case mothers have slower *p,p'*-DDT elimination so that their internal dose of *p,p'*-DDT is greater even for equivalent or lower total exposure. 3) Case mothers have accelerated elimination of *o,p'*-DDT associated with exposure to a toxic intermediate metabolite.

Human studies of experimental ingestion of DDT showed “marked differences” in excretion among individuals following ingestion of DDT.^{9, 28} We suggest that this observation supports the plausibility of individual differences in DDT metabolism for case mothers compared to control mothers in this study.

This picture is certainly further complicated by the finding that DDT itself induces enzymes that facilitate its own metabolism and excretion,²⁷ further supported by human feeding experiments that showed rates of elimination of *p,p'*-DDT increased as dose increased.⁹ Controls, who showed evidence of recent exposure (higher *o,p'*-DDT), paradoxically also have a lower ratio of *p,p'*-DDT to *p,p'*-DDE. We speculate that high and recent DDT exposure could have led to faster *p,p'*-DDT elimination in controls. Additionally, the metabolism of *p,p'*-DDT may depend on the CYP system²⁷ which can be highly polymorphic. At this time there is not sufficient understanding of DDT metabolism to fully explain study findings, nor can we establish the time course of DDT exposure and elimination.

Our findings suggest that any single DDT-related compound is an inadequate proxy for exposure. High correlations among DDT-related compounds and varying biological activities⁷ suggest that multiple compounds should be measured and considered where possible. For example, the strong positive correlation of *p,p'*-DDE with *p,p'*-DDT confounds associations for each compound in this study (See Table 2 models 1,2 and 4).

To our knowledge there are no other prospective studies of prenatal organochlorine exposure and testicular cancer. However, Hardell and colleagues measured maternal serum levels of organochlorines at the time of the diagnosis of testicular cancer, decades after birth, between 1997 and 2000.²⁹ That study found that case mothers had higher levels of polychlorinated biphenyls, chlordanes and hexachlorobenzene, but not DDT-related compounds. Levels of DDT-related compounds in humans declined even before the ban on the pesticide DDT in 1972.^{11, 18} For this reason, levels of DDT-related compounds found in mothers' blood between 1997 and 2000, decades after commercial DDT was banned, do not necessarily reflect levels during gestation 30 years earlier. Differences in timing of blood sampling could explain the inconsistency between this study and that of Hardell and colleagues.

In contrast, McGlynn et al. used the United States Department of Defense Serum Repository to conduct a large nested case-control study that reported an association between persistent organochlorines and testicular cancer.³⁰ The average time between sample donation and diagnosis was 5 years. This design has the advantage of good power, and the disadvantage of being unable to assess exposure during prenatal life, as noted by the authors.

The present study was undertaken to test the hypothesis that there is a sizable effect of maternal DDT exposure in pregnancy on risk of testicular cancer in sons. Although the CHDS is one of

the largest U.S. pregnancy cohorts with cancer follow-up of parents and children, conclusions drawn from this study are limited to detection of sizable effects due to the rarity of testicular cancer and the small number of cases observed over four decades even among the 9,744 live born sons in the CHDS. To our knowledge there have been no studies that directly measure maternal exposure to DDT-related compounds during pregnancy in relation to subsequent testicular cancer in sons. The current study addresses this gap and has several unique features: 1) Direct measurement of three DDT-related compounds from maternal serum samples which were obtained one to three days after delivery. 2) Maternal serum samples collected at the peak period of active commercial DDT use (1960's). 3) Prospective follow-up for testicular cancer incidence beginning before birth.

The biological effects of commercial DDT have been considered largely with reference to classical steroid hormone activity via binding to estrogen or androgen receptors.⁷ The validity of this approach has been questioned, as *in vitro* studies may not represent actions of exposures in the entire organism.³¹ The balance of exposures, including the DDT/DDE ratio, has been shown to influence effects *in vivo* and may differ between species and across tissues.³¹ Moreover, toxicity of DDT-related compounds may be independent of endocrine disruption effects and could occur via changes in gene expression that control metabolism of many exogenous exposures by cells.³² It has also been suggested that DDT may be a tumor promoter through its strong inhibition of intercellular communication.³³

Our conclusions are tempered by the small sample size available for study, despite a sizeable source population. This limitation is inherent in prospective testicular cancer studies due to the rarity of this cancer. We were also unable to control for multiple confounding factors due to sample size limitations. However, the ratio of *p,p'*-DDT to *p,p'*-DDE was also recently reported to be a risk factor for liver cancer,¹² and for delayed time to pregnancy in CHDS daughters following *in utero* exposure.¹⁶ Higher levels of *p,p'*-DDT at each level of *p,p'*-DDE also predicted greater breast cancer risk in CHDS mothers.¹⁸ The similar findings for time to pregnancy in women and testicular cancer in men of the same generation of the CHDS cohort are unlikely to be serendipitous, and support the alternative idea that *p,p'*-DDT, an intermediate metabolite, or another exposure that shares its metabolic pathway, may be a reproductive toxin and/or a carcinogen.

The associations we observed in this study are of considerable interest given the low statistical power of this study, and the lack of other strong risk factors for testicular cancer identified to date.¹ We suggest that our ability to observe DDT associations is due to timing of the maternal blood collection allowing measurement of exposure relevant to early testicular development, in a time period when DDT was being actively acquired by mothers. A better understanding of individual differences in DDT metabolism and the relationship of DDT to the induction of enzymes that metabolize other endogenous and exogenous exposures will be important for full interpretation of our findings. Such information will be helpful for informing the continuing debate on the cost and benefits of using DDT for malaria control³⁴ and for understanding the human health effects of one of the most universal man-made exposures in history.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CHDS Child Health and Development Studies

DMV	Department of Motor Vehicles
<i>p,p'</i> -DDT	1,1,1-Trichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane
<i>p,p'</i> -DDE	1,1'-Dichloro-2, 2'-bis(<i>p</i> -chlorophenyl)ethylene
<i>o,p'</i> - DDT	1,1,1-Trichloro-2-(<i>p</i> -chlorophenyl)-2-(<i>o</i> -chlorophenyl)ethane
DDT/DDE	<i>p,p'</i> -DDT/ <i>p,p'</i> -DDE

Table 1
Characteristics of testicular cancer cases and their matched controls*

Characteristics [†]	Controls (N=45)			Cases (N=15)			Difference within Matching Group Case - Mean of 3 Controls		
	Median	Interquartile Range	Median	Median	Interquartile Range	Median	Mean Difference	Standard Error	p-value for Matched Wilcoxon Signed Rank Test for Cases vs. Controls
Maternal Characteristics									
<i>p,p'</i> -DDT (ug/L)	11.89	7.12	8.74	15.95			-1.20	1.06	0.25
<i>p,p'</i> -DDE (ug/L)	53.84	26.46	35.81	33.95			-15.29	3.45	0.00
<i>o,p'</i> -DDT (ug/L)	0.36	0.49	0.19	0.46			-0.15	0.15	0.05
Ratio of <i>p,p'</i> -DDT to <i>p,p'</i> -DDE	0.25	0.10	0.27	0.11			0.05	0.02	0.03
Age (years)	26	9	25	9			-2.33	1.49	0.81
Body Mass Index (kg/m ²)	21.6	3.33	21.3	3.32			-0.44	0.98	0.19
Pregnancy Weight Gain (kg/week)	0.23	0.09	0.16	0.10			-0.06	0.03	0.09
Parity (number prior pregnancies)	1	3	2	2			0.36	0.42	0.55
Total Cholesterol (mg/dL)	162	75	143	77			-22.33	13.50	0.55
Total Triglycerides (mg/dL)	150	85	116	81			-34.67	23.60	0.39
Son's Characteristics									
Birth Weight (kg)	3.49	0.77	3.35	0.74			-0.02	0.17	0.46
Length of Gestation (days)	284	13.5	278.5	14			-7.37	2.60	0.02

* Each case is matched to three controls on race and year of birth

[†] DDT-related compounds were collected from mothers' postpartum serum taken within 1-3 days after delivery

Table 2

Associations between the ratio of DDT-related compounds in maternal serum 1-3 days after delivery and son's testicular cancer incidence over 40 years

Model*	Variable	Odds Ratio for Interquartile Range [†]	95% Confidence Interval	P-Value
1	<i>p,p'</i> -DDT (ug/L)	0.70	0.26, 1.64	0.46
2	<i>p,p'</i> -DDE (ug/L)	0.19	0.04, 0.62	0.00
3	<i>o,p'</i> - DDT (ug/L)	0.77	0.37, 1.33	0.59
4	<i>p,p'</i> -DDT (ug/L)	4.81	0.92, 48.62	0.06
	<i>p,p'</i> -DDE (ug/L)	0.08	0.01, 0.41	0.00
5	Ratio of <i>p,p'</i> -DDT to <i>p,p'</i> -DDE	3.56	1.34, 11.88	<0.01
6	Ratio of <i>p,p'</i> -DDT to <i>p,p'</i> -DDE	7.58	1.97, 47.61	0.00
	<i>o,p'</i> - DDT (ug/L)	0.43	0.17, 0.94	0.03
7	Ratio of <i>p,p'</i> -DDT to <i>p,p'</i> -DDE	3.66	1.35, 12.36	<0.01
	Maternal Age (years)	0.64	0.20, 1.82	0.42
8	Ratio of <i>p,p'</i> -DDT to <i>p,p'</i> -DDE	5.02	1.37, 39.32	0.01
	Maternal Body Mass Index (kg/m ²)	0.56	0.19, 1.36	0.23
9	Ratio of <i>p,p'</i> -DDT to <i>p,p'</i> -DDE	5.39	1.51, 28.17	<0.01
	Maternal Pregnancy Weight Gain (kg/week)	0.38	0.13, 0.84	0.01
10	Ratio of <i>p,p'</i> -DDT to <i>p,p'</i> -DDE	3.51	1.30, 11.88	0.02
	Maternal Parity	1.13	0.74, 1.70	0.59
11	Ratio of <i>p,p'</i> -DDT to <i>p,p'</i> -DDE	3.87	1.46, 17.30	<0.01
	Maternal Total Cholesterol (mg/dL)	1.10	0.29, 4.03	0.79
	Maternal Total Tri-glycerides (mg/dL)	0.80	0.27, 2.44	0.72
12	Ratio of <i>p,p'</i> -DDT to <i>p,p'</i> -DDE	4.32	1.46, 17.47	<0.01
	Son's Birth Weight (kg)	0.98	0.95, 1.01	0.16
13	Ratio of <i>p,p'</i> -DDT to <i>p,p'</i> -DDE	5.21	1.41, 28.96	0.01
	Son's Length of Gestation (days)	0.41	0.16, 0.90	0.02

* All models contain 15 case-control strata (one case matched to three controls on race and year of birth) with the exception of the model containing maternal body mass index which has 13 strata due to missing values on maternal weight and maternal pregnancy weight gain which has 14 strata due to a missing value for last third trimester weight.

[†] All models contain only continuous variables. All models estimated odds ratios and 95% confidence intervals from exact conditional logistic regression with contrasts for the interquartile range (75th percentile – 25th percentile) for controls, with the exception of the model containing maternal parity which contrasts one or more prior pregnancies to no prior pregnancies. The interquartile range (IQR) for the ratio of *p,p'*-DDT to *p,p'*-DDE is 0.10. *p,p'*-DDT IQR = 7.12 ug/L, *p,p'*-DDE IQR = 26.46 ug/L, *o,p'*-DDT IQR = 0.49 ug/L, maternal age IQR = 9 years, maternal body mass index IQR = 3.33 kg/m², maternal pregnancy weight gain IQR = 0.09 kg/week, maternal total cholesterol IQR = 75mg/dL, maternal total tri-glycerides IQR = 85mg/dL, son's birth weight IQR = 0.77 kg, son's length of gestation IQR = 13.5 days.