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Preterm Birth, Fetal Growth, and Age at Menarche among Women Exposed Prenatally to Diethylstilbestrol (DES)

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Abstract

Diethylstilbestrol (DES), a synthetic estrogen used in pregnancy during the 1950s and 1960s, provides a model for potential health effects of endocrine disrupting compounds in the environment. We evaluated prenatal exposure to DES, based on medical record review, in relation to gestational length, fetal growth, and age at menarche in 4429 exposed and 1427 unexposed daughters. DES exposure was associated with an increase in preterm birth (odds ratio (OR) = 2.97; 95%CI=2.27, 3.87), and a higher risk of small for gestational age (SGA) (OR=1.61; 95% CI=1.31,1.98). The association between DES exposure and early menarche was borderline, with stronger effects when early menarche was defined as <= 10 years (OR = 1.41 95%CI=0.97, 2.03) than defined as <= 11 years (OR=1.16; 95%CI=0.97, 1.39). This study provides evidence that prenatal DES exposure was associated with fetal growth and gestational length, which may mediate associations between DES and health outcomes in later life.

Keywords

diethylstilbestrol; early life factors; birth weight; small for gestational age; gestational length; menarche; endocrine disruptors

1. Introduction

Diethylstilbestrol (DES) is a non-steroidal estrogen widely used beginning in the 1940s as a treatment for threatened abortion and preterm birth. Although clinical trials in the 1950s found that it was ineffective,(1,2) DES was used until 1971 when it was linked to vaginal adenocarcinoma in young women exposed in utero.(3) Approximately two million pregnancies are estimated to have been exposed to DES in the U.S.,(4) and the drug was also used in Europe, most frequently in the Netherlands, Great Britain, and France.(5) The teratogenic and carcinogenic effects of DES exposure in prenatally exposed women are well known.(3,6–10) DES is also associated with adverse pregnancy outcomes,(11) preeclampsia,(9) infertility,(8) and an earlier age at menopause.(12) Associations of DES with cervical dysplasia(13) and breast cancer are also suspected.(14)

Early clinical trials of the effectiveness of DES in preventing miscarriage and preterm birth were suggestive of adverse effects on pregnancy outcomes.(1,2) Brackbill and Berendes evaluated the data from the Dieckmann clinical trial and noted that “the published data clearly show that DES significantly increased abortions, neonatal deaths, and premature births” (15). However, a detailed analysis of the effects of prenatal exposure to DES on preterm birth or fetal growth retardation has not been published. The effect of prenatal exposure to DES on timing of puberty has also not been evaluated. DES serves as a model for potential effects of endocrine disrupting chemicals in the environment, some of which have been related to low birth weight(16–18) and early age at menarche.(19–22)

Studies of prenatally DES exposed female mice, which show outcomes such as infertility and reproductive tumors (23), are remarkably consistent with outcomes seen in exposed women. I*n utero* growth retardation(24) and earlier vaginal opening(25,26) are also seen in mouse studies, but the corresponding outcomes, including birth size and age at menarche, have not been assessed in women exposed to DES prenatally. There is convincing evidence that many adult diseases, including cancer and cardiovascular disease, are related to early life factors such as birth weight and timing of puberty.(27) Alterations in these early life factors could affect future health outcomes in DES-exposed daughters, and may provide insights into the potential mechanisms by which DES causes cancer and other adverse outcomes. In addition, results found for DES may help elucidate effects of current exposure to other endocrine disrupting chemicals with estrogen like mechanisms that occur at much lower levels but from multiple environmental sources of exposure.

2. Methods

2.1. Study population

This analysis combines data from two cohort studies, the National Cooperative Diethylstilbestrol Adenosis (DESAD) Project (28) and follow-up of a University of Chicago clinical trial (Dieckmann Study),(1) to evaluate the effects of prenatal DES exposure on birth weight, length of gestation, and timing of menarche. The methods of the DESAD project have been previously described.(28) Briefly, 4015 prenatally exposed and 1034 unexposed daughters were enrolled in the project at five medical centers throughout the U.S. from 1974–1978. All women included in DESAD had their DES exposure status documented by prenatal records. At entry, the daughters completed a questionnaire on

baseline medical history, including age at menarche. Their mothers (directly exposed to DES during the pregnancy or without evidence of prenatal DES exposure) completed a questionnaire on pregnancy-related events, including smoking history. Data on birth weight were available from the obstetrical charts for 80% of the prenatally exposed and unexposed daughters. For the remaining 20%, these data were ascertained from the mother when their daughter first enrolled in the study (average age of the daughter at enrollment =24 years). Gestational length was estimated by subtracting the date of the last menstrual period (LMP) from the date of delivery from obstetric charts.

The Dieckmann clinical trial enrolled pregnant women at the University of Chicago prenatal clinic between 1950 and 1952 to receive either DES (n=414) or placebo (n=393), following the recommended high dose regimen.(29) Data on gestational length and birth weight were abstracted from the original trial records. Age at menarche of the prenatally DES-exposed and placebo-exposed groups was self-reported at first enrollment into the follow-up of the Dieckmann cohort from 1974–1977.

2.2. Dose and timing of DES exposure

Detailed data on total dose of DES was known for 39% of the combined cohorts and gestational week of first exposure was known for 81%. Because complete dose data were missing for the a majority of participants, we conducted secondary analyses in which we classified women into high and low dose groups, based on knowledge about regional prescribing practices in the U.S. and on actual dose data from the Dieckmann cohort and DESAD participants identified at different medical centers.(14) Women originally from Chicago (Dieckmann cohort), and two DESAD sites (Los Angeles and Boston) were classified as high dose, and the remaining three DESAD sites (Texas, Minnesota and Wisconsin) were classified as low dose. The available dose data from the original cohorts supported our classification: the median total cumulative doses were 12,442, 8,675, and 7,550 mg for the Chicago, Boston, and California sites (high dose) and were 2,572, 1,520, and 3,175 mg for the Texas, Minnesota, and Wisconsin sites (low dose) respectively(14).

2.3. Statistical analysis

2.3.1 Length of gestation, birth weight and small for gestational age—We used multivariable linear regression to estimate the effect of DES exposure on mean gestational length and birth weight, controlling for maternal age (<25, 25–29, 30+) and smoking (yes versus no) during the index pregnancy, year of birth (<1950, 1950–54, 1955–59, 1960+), and study cohort. To determine cutpoints for gestational age (SGA) we compared the distribution of birth weights at specific gestational ages in our data with those from a large study conducted in the 1960s among white women and their babies (30). We classified infants as SGA if their birth weight was less than the 10^{th} percentile at each gestational age (data available from week 27 through week 44). Logistic regression analysis was used to estimate the effect of DES on the risk of SGA and preterm birth (<37 weeks), as well as preterm birth before 34 weeks. We examined associations according to high and low dose group, and by cumulative dose and timing of first exposure to DES during gestation in the subset of participants with information on those variables. We also examined whether the association between DES and birth weight was modified by maternal smoking during the index pregnancy. Analyses were conducted in the combined cohorts and separately in the DESAD and Dieckmann cohorts.

2.3.2. Age at menarche—We used logistic regression to evaluate whether DES was associated with an earlier age at menarche. We defined early menarche as ≤11 years, but also examined very early menarche (≤10 years) to determine whether changing the definition of early menarche affected the results. We adjusted for maternal age, maternal

smoking, year of birth and study cohort, as described above. Data were also stratified by the original cohort, and within the DESAD cohort, by age of the woman when menarche was first ascertained (defined by the median age at first report, <23 vs. 23+), birth weight (<3000 g vs. 3000+ g), and maternal age (<25 vs 25+) and smoking during the index pregnancy. We also examined age at menarche in years using a discrete-time analogue of the Cox proportional hazards model to calculate hazard ratios (HRs) and their respective 95% CIs in the combined cohorts and separately in the two cohorts.(31)

3. Results

The majority of women in the current analysis were from the DESAD cohort (Table 1). Exposed women were somewhat younger than unexposed women, and correspondingly, were younger at first enrollment into the study, when age at menarche was ascertained. Highest level of education was similar among the exposed and unexposed daughters, although slightly more exposed had college degrees or higher. DES-exposed mothers were slightly older at the index pregnancy than unexposed mothers. There was little difference in maternal smoking during pregnancy in the DESAD cohort (Table 1). Information on maternal smoking was missing for 62% of the Dieckmann cohort.

DES exposure was associated with a small reduction in the mean length of gestation. Overall, the mean difference in gestational length, comparing DES-exposed with unexposed women, was −0.63 weeks (95% confidence interval (CI) = (−0.78, −0.49), after adjustment for cohort (Table 2). There was a greater reduction in the DESAD (−0.66 weeks) than the Dieckmann (−0.51 weeks) cohort. Further adjustment for maternal age, maternal smoking, and year of birth in the DESAD participants with complete information on those variables (94%) attenuated the estimate somewhat (−0.61 vs. −0.66 weeks). The average gestational length was shorter in the high dose cohort compared to those in the low dose cohort (mean difference between exposed and unexposed: −0.77 vs. −0.43 weeks, respectively, Table 2). In analyses stratified by cumulative total dose in the subset of DESAD women with complete information on dose, higher dose appeared to be associated with shorter gestational length, although the trend was not monotonic by dose category. Earlier exposure to DES during gestation also was associated with shorter gestational length, compared to the unexposed (Table 2). Among exposed women only, exposure before eight weeks was also related to shorter length of gestation compared to those exposed at a gestational age of 15 weeks or later (mean difference=−0.49 weeks, 95%CI=−0.72, −0.27).

Risk of having been born early (<37 weeks) was elevated in the DES exposed, compared to the unexposed (OR=2.97; 95% CI=2.27, 3.87) (Table 2). This association was stronger in the DESAD (OR=3.22; 95% CI=2.39, 4.33) than in the Dieckmann cohort (OR=1.98; 95% CI=1.05, 3.74). The estimates for very preterm birth (<34 weeks) were also elevated in both cohorts (OR=3.92 (95% CI=2.05, 7.50) and 3.07 (95% CI=1.00, 9.48), for DESAD and Dieckmann, respectively). The effect estimates were slightly higher in those originating from the high dose cohorts (OR=3.29) compared to the low dose cohorts (OR=2.54) (Table 2). Control for maternal age, smoking, and year of birth did not have an appreciable effect on the estimates (data not shown). In the DESAD cohort, the effect estimates for preterm birth were higher among those with greater cumulative dose (ORs=2.56, 4.02, and 4.60 for <2500, 2500–9999, and 10,000+ mgs respectively, compared to unexposed). There was also a higher risk of preterm birth among those who had been exposed to DES earlier in gestation (ORs=4.25, 2.76, 2.85, and 2.84 for <=7, 8–10, 11–14, and 15+ weeks at first exposure respectively). Among exposed women only, DES exposure early in gestation (<=7 weeks) was associated with a higher risk of preterm birth compared to late exposure (15+ weeks) (OR=1.50; 95% CI=1.15, 1.95).

DES was also associated with reduced birth weight. The mean difference in birth weight, adjusting for cohort and gestational age, was −105 grams (95% CI=−134, −76) (Table 3). Mean differences in birth weight were greater in the DESAD than in the Dieckmann cohort (−118 grams (95%CI=−151, −86) versus −52 grams (95%CI=−113, −10), respectively; p=0.08 for difference in estimates). The effect of DES on birth weight was slightly stronger among smokers (mean g=−153; 95%CI=−206, −101) than non-smokers (mean g=−103; 95%CI=−145, −61). Except for the large reduction in the effect estimates for birth weight with adjustment for gestational length (−188 grams unadjusted versus −105 grams adjusted), there was little evidence for confounding by other covariates in either cohort. In the subset of 4011 DESAD participants (79% of total cohort) with complete information on birth weight, gestational age, maternal age and smoking, and year of birth, DES exposure was associated with a 122 gram reduction in birth weight after controlling for gestational length (comparable to the estimate of −118 grams in the entire cohort before exclusion of those with missing data on potential confounders); this estimate was virtually the same (−126 grams) after additionally adjusting for maternal age, smoking, and year of birth of the index pregnancy. Among the 769 (95% of the total cohort) Dieckmann participants with complete information on birth weight, gestational length, and maternal age, the mean reduction in birth weight was 52 grams controlling for gestational length only and 53 grams with further control for maternal age at the index birth. (Year of birth was not included as a covariate in these models because of the small range in the Dieckmann cohort and maternal smoking was not included because of the large number of missing values.) In the subset of the DESAD cohort with dose information, birth weight did not appear to be related to cumulative total dose of DES, and there was little difference in birth weight according to membership in a high dose versus low dose cohort. Earlier exposure to DES during gestation also did not appear to be associated with greater reductions in birth weight.

DES exposure appeared to be related to the risk of being born SGA (Table 3), although the results were stronger in the DESAD (OR=1.75; 95% CI=1.37, 2.24) than the Dieckmann cohort (OR=1.26; 95% CI=0.83–1.90). The risk of SGA was not related to gestational age at first exposure to DES or to total dose, assessed either by estimation of low versus high dose cohort or by use of actual cumulative dose values in a subset. We assessed confounding by year of birth and maternal age and smoking in pregnancy within the DESAD cohort and found that the estimates changed very little (data not shown). The effect of DES on SGA among women whose mothers smoked in pregnancy was about the same (OR= 1.76; 95%CI=1.22, 2.52) as among those whose mothers did not smoke in pregnancy (OR= 1.90; 95%CI=1.30, 2.75).

The OR for prenatal DES exposure in relation to menarche ≤10 years was 1.41 (95% CI=0.97, 2.03), and for menarche ≤11 years, the OR was 1.16 (95% CI=0.97, 1.39) (Table 4). When early menarche was defined as ≤11 years, the results differed by cohort, with essentially null results in the Dieckmann cohort but a 25% increase in risk of early menarche in the DESAD cohort. Results were similar in the two cohorts using menarche ≤10 years as the outcome variable (Table 4). We tested for potential confounding in the DESAD cohort by adding maternal age at birth, smoking in pregnancy, and year of birth and found little change in the effect estimates for either menarche ≤10 years or ≤11 years (data not shown). For menarche ≤10 years, the effects were slightly stronger among those categorized as belonging to a high dose cohort (OR=1.50; 95%CI=1.02, 2.21) than those belonging to a low dose cohort (OR=1.24; 95%CI 0.81, 1.91), but there was essentially no difference in associations between the low and high dose cohorts when the outcome variable was menarche ≤11 years (Table 4).

The association between age at menarche and cumulative dose of DES appeared to be U-shaped, with some increase in early menarche in both the low (<2500 mg) and high

(10,000+ mg) groups, and a slight reduction in risk of early menarche in those with moderate doses (total cumulative dose of 2500–9999 mg). Timing of first exposure to DES during gestation did not appear to affect the risk of early menarche, although first exposure later in gestation (15+ weeks) had slightly stronger effects than the other categories. With the exception of birth weight, there was little evidence that the effect of DES on age at menarche was modified by other variables. When the results were stratified by birth weight, there was a slightly reduced association of early menarche (≤11) among exposed women with birth weight <3000 grams (OR=0.91; 95% CI=0.63, 1.30), whereas the association was stronger among women with birth weights of 3000 grams or greater (OR=1.39; 95% CI=1.09, 1.79) (data not shown).

Similar results were found when we modeled age at menarche by individual year using Cox proportional hazards models (Table 5). DES-exposed women were slightly more likely to reach menarche at a younger age, but this association was evident only in DESAD (HR=1.09, 95% CI=1.02, 1.16), and not the Dieckmann (HR=0.97; 95%CI=0.82–1.14), cohort. There were no consistent patterns for dose and timing of exposure to DES in relation to menarcheal age in years.

4. Discussion

In this study, prenatal DES exposure was associated with a small reduction in mean birth weight and length of gestation, and an elevated risk of preterm delivery and SGA birth. The possible mechanisms of action for these associations are speculative. In humans, the timing of parturition is influenced by a change in the ratio of two estrogens estradiol (E2) and estriol (E3) as labor approaches, resulting in a more than 10-fold excess of estriol (32). It is plausible that exposure to exogenous estrogens may interfere with this delicate balance of pregnancy hormones and potentially lead to alterations in the timing of parturition. Another potential mechanism may be through a stress hormone pathway. Synthetic sources of estrogen may induce maternal and fetal stress, stimulating the secretion of corticotropin-releasing hormone (CRH)(33,34). High concentrations of CRH concentrations have been associated with preterm labor and premature rupture of the membranes(35,36).

There were stronger associations with length of gestation when DES was administered early rather than later in pregnancy and for higher versus lower doses, but timing and dose of DES did not appear to be related to birth weight or to the risk of an SGA birth. It is possible that women with first trimester bleeding or threatened miscarriage were prescribed DES very early in pregnancy in comparison to women with other indications for DES use. Pregnancies with first trimester bleeding may be more likely to result in preterm birth,(37) and thus the effects that were seen with timing of exposure during gestation may be due to characteristics of the pregnancy itself, rather than to early DES exposure. The Dieckmann cohort originated from a clinical trial and thus provides a means to evaluate the possibility of confounding by indication in our results. Since the majority of Dieckmann participants received DES relatively early in pregnancy, timing of gestation could not be evaluated separately in this cohort. However, the overall effect for any DES exposure on preterm birth was elevated in the Dieckmann cohort, albeit of smaller magnitude than in the DESAD cohort (OR for preterm birth =1.98 vs. 3.22), suggesting that the association in the DESAD cohort may be partially due to unmeasured confounding, perhaps by confounding by indication. Similarly, the risk of an SGA birth was also smaller in the Dieckmann (OR=1.26) compared to the DESAD cohort (OR=1.75).

DES was also associated with a small elevation in risk of early menarche, although the results were not consistent and varied depending on the definition of early menarche, with stronger and more consistent effects for menarche ≤10 years. It is possible that prenatal DES

exposure may be related to very early menarche, but that it is not associated with timing of puberty in the normative range. DES exposure has been associated with earlier vaginal opening in rodents,(25,26) however this endpoint is more closely associated with thelarche in humans,(38) which we were unable to assess, than with age at menarche. A large amount of data on age at menarche was missing from the Dieckmann cohort (25% of the exposed and 32% of the unexposed), because many of the offspring from the original clinical trial in the early 1950s could not be located during the first follow-up in the mid-1970s. However, it seems unlikely that loss to follow-up would have occurred based on age at menarche and DES exposure status, suggesting that bias due to selective loss to follow-up in the Dieckmann cohort is not an explanation for the findings in that cohort.

Accuracy of recall of age at menarche has been shown to depend on recency of the event.(39) We used the earliest recorded data on age at menarche, and whereas residual misclassification of this variable is a possibility, it seems unlikely that it would be dependent upon DES exposure status. Results did not differ when we stratified according to the age of the women when they reported their age at menarche (<23 vs 23+).

The majority of data on birth weight came from the prenatal medical record (80%) and is likely to have been accurately recorded. Mother's recall of infant birth weight also tends to be well reported.(40,41) Gestational length was missing for 14.8% of the DESAD cohort and may have been less accurate during the study time period because of lack of ultrasound measurements. However, it seems unlikely that the misclassification would be differential by DES exposure status, and thus is more apt to have resulted in bias to the null.

In order to classify our participants as SGA, we used data from a large series of 40,000 singletons births among white women during the 1960s (30). Because birth weight percentiles were not available by gender, we may have slightly underestimated the proportion of infants who were SGA in our population of female infants. However, it is unlikely that the classification would have been differential according to DES exposure.

Because concerns about the health effects of DES did not emerge until 1971 and most of the women were enrolled in the study during their late teens and early twenties, data were not systematically collected during childhood. Therefore, the only measurable influences on age at menarche in our study were from the prenatal period. If DES was related to childhood and early adolescent behaviors, such as physical activity, or conditions, such as obesity, that could affect the timing of menarche,(42) these might mediate the effect on age at menarche that we observed. We also lacked information on several potential confounding variables, including maternal body mass index, pregnancy diet, and socio-economic status. We found similar, although smaller, effects on birth weight and gestational age in the Dieckmann cohort. This cohort originated from a clinical trial which provides some reassurance that confounding is not a major problem. The larger effects in the DESAD cohort are likely to be due to confounding, in particular confounding by indication, or other biases.

A major strength of our study is that DES exposure status was documented by prenatal records, in contrast to other cohorts which have relied on patient recall(43,44) or assessment of DES exposure by physical changes typically found in the reproductive tract of participants.(44) Cumulative total dose of DES was available for only 39% of the exposed in our cohort, but we were able to assess the effect of DES dose by classifying participants into high dose or low dose groups, according to the prescribing patterns typical of the medical centers where they were born.(14) We were also able to assess timing of first exposure during gestation, which was available for 85% of the combined cohorts.

In summary, we found that prenatal DES exposure was associated with a small reduction in mean birth weight, a small increase in the risk of an SGA birth, shorter gestational length,

and an approximately two to three fold greater risk of preterm birth. The larger effects seen in the DESAD compared to the Dieckmann cohort suggest that part of the effect in the former group may be explained by confounding. Results for age at menarche were less clear, but suggest that DES may have been related to a small increase in risk of very early menarche. Because birth weight has been associated with conditions such as cardiovascular disease and cancer in adulthood, it may be important to consider whether birth weight and other early life factors play a role in the causal pathway between DES and outcomes occurring in adulthood. In addition, the results may have implications for the assessing potential effects of estrogenic environmental chemicals that women today are exposed to at lower doses but through many sources.

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Table 1

Characteristics of women prenatally exposed and unexposed to diethylstilbestrol

	DES-exposed	Unexposed
	N (%)	N (%)
Original Cohort		
DESAD	4015 (90.7)	1034 (72.5)
Dieckmann	414 (9.3)	393 (27.5)
Year of Birth		
<1950	671 (15.2)	276 (19.3)
1950–54	2059 (46.5)	785 (55.0)
1955–59	1128 (25.5)	332 (23.3)
1960 +	571 (12.9)	34 (2.4)
Daughter's highest level of education		
High school or less	463 (10.5)	193 (13.5)
Some college	819 (18.5)	260 (18.2)
4 year college	1242 (28.0)	337 (23.6)
Graduate school	986 (22.3)	298 (20.9)
Missing	919 (20.8)	339 (23.8)
Maternal smoking during pregnancy (DESAD)		
Yes	1430 (35.6)	345 (33.4)
No	2353 (58.6)	606 (58.6)
Missing	232 (5.8)	83 (8.0)
Maternal age at index pregnancy		
<25	998 (22.5)	351 (24.6)
25–29	1559 (35.2)	503 (35.3)
30+	1732 (39.1)	506 (35.5)
Missing	140 (3.2)	67 (4.7)
Age at first enrolment		
<20	1265 (28.6)	140 (9.8)
20–22	923 (20.8)	271 (19.0)
23–25	1259 (28.4)	495 (34.7)
26+	870 (19.6)	392 (27.5)
Missing	112 (2.5)	129 (9.0)
Gestational length (weeks)		
<34	137 (3.1)	14 (1.0)
35–36	374 (8.40	52 (3.6)
37–38	852 (19.2)	270 (18.9)
39–42	2248 (50.8)	1001 (70.2)
43–45	81 (1.8)	44 (3.1)
Missing	737 (16.6)	46 (3.2)
Birth weight (gms)		
<2500	562 (12.7)	60 (4.2)

	DES-exposed	Unexposed
	N (%)	N (%)
2500–2999	1098 (24.8)	283 (19.8)
3000–3499	1657 (37.4)	594 (41.6)
3500–3999	822 (18.6)	387 (27.1)
4000+	199 (4.5)	90 (6.3)
Missing	91 (2.1)	13 (0.9)
Age at menarche		
<11	162 (3.7)	37 (2.6)
11	512 (11.6)	151 (10.6)
12–13	2630 (59.4)	776 (54.4)
14–15	894 (20.2)	286 (20.0)
16+	117 (2.6)	47 (3.3)
Missing	114 (2.6)	130 (9.1)

Table 2

Difference in mean gestational length and odds ratios (95% CI) for preterm birth by DES exposure status, cohort, and dose and gestational age of first exposure to DES

	Gestational Length[1] (week)		Preterm birth (<37 weeks)	
	N	β (95% CI)	N (%)	OR (95% CI)
Combined cohorts				
Unexposed	1386	Ref	66 (4.8)	1.00
Exposed	3713	−0.63 (−0.78, −0.49)[2]	511 (13.8)	2.97 (2.27, 3.87)[2]
Dieckmann cohort				
Unexposed	369	Ref	15 (4.1)	1.00
Exposed	400	−0.51 (−0.84, −0.17)	31 (7.8)	1.98 (1.05,3.74)
DESAD cohort				
Unexposed	1017	Ref	51 (5.0)	1.00
Exposed	3313	−0.66 (−0.82, −0.51)	480 (14.6)	3.22 (2.39,4.33)
Low dose cohort	1607	−0.43 (−0.60, −0.26)	206 (12.8)	2.54(1.90,3.40)
High dose cohort	2085	−0.77 (−0.92, −0.61)	305 (14.6)	3.29 (2.45,4.33)
Cumulative dose of DES (mg)[3,4]				
Unexposed	1017	Ref	51 (5.0)	1.00
<2500	384	−0.47 (−0.72, −0.22)	46 (12.0)	2.56 (1.69,3.89)
2500–9999	415	−0.99 (−1.23, −0.74)	73 (17.6)	4.02 (2.75,5.87)
10,000+	367	−0.90 (−1.16, −0.64)	72 (19.6)	4.60 (3.14,6.74)
Gestational age at first exposure to DES (weeks)[4]				
Unexposed	1017	Ref	51 (5.0)	1.00
≤ 7	901	−0.98 (−1.19, −0.78)	166 (18.4)	4.25 (3.06,5.91)
8–10	806	−0.60 (−0.81, −0.40)	103 (12.8)	2.76 (1.95,3.91)
11–14	617	−0.50 (−0.72, −0.28)	81 (13.1)	2.85 (1.97,4.10)
15+	808	−0.49 (−0.70, −0.28)	106 (13.1)	2.84 (2.01,4.03)

[1] 757 observations with missing gestational age and 26 outliers (gestational age > 45 weeks) excluded

[2] Adjusted for cohort

[3] 2094 observations with unknown cumulative dose excluded

[4] DESAD cohort only

Table 3

Difference in mean birth weight and odds ratios (95%CI) for small for gestational age by DES exposure status, cohort, and dose and gestational age of first exposure to DES

	Birth Weight[1,2] (gram)		Small for gestational age	
	N	β (95% CI)	N (%)	OR (95% CI)
Combined cohorts				
Unexposed	1373	Ref	130 (9.5)	1.00 (ref)
Exposed	3640	−105 (−134, −76)[3]	506 (14.0)	1.61 (1.31, 1.98)[3]
Dieckmann cohort				
Unexposed	369	Ref	46 (12.5)	1.00 (ref)
Exposed	400	−52 (−113, −10)	60 (15.2)	1.26 (0.83, 1.90)
DESAD cohort				
Unexposed	1004	Ref	84 (8.4)	1.00 (ref)
Exposed	3240	−118 (−151, −86)	446 (13.8)	1.75 (1.37, 2.24)
Low dose cohort	1592	−101 (−135, −67)	228 (14.4)	1.60 (1.27, 2.01)
High dose cohort	2048	−107 (−137, −76)	278 (13.7)	1.51 (1.21, 1.88)
Cumulative dose of DES (mg)[4,5]				
<2500	381	−120 (−171, −68)	52 (13.8)	1.74 (1.20, 2.51)
2500–9999	404	−151 (−202, −99)	56 (13.9)	1.76 (1.23, 2.52)
10,000+	361	−129 (−182, −75)	46 (12.7)	1.59 (1.09, 2.33)
Gestational age at first exposure to DES (weeks)[5]				
≤ 7	902	−116 (−157, −74)	128 (14.5)	1.85 (1.39, 2.58)
8–10	807	−120 (−162, −78)	108 (13.7)	1.73 (1.28, 2.34)
11–14	623	−150 (−196, −104)	91 (15.0)	1.93 (1.41, 2.65)
15+	819	−89 (−131, −47)	101 (12.7)	1.58 (1.17, 2.15)

[1] 104 observations with missing birth weight excluded

[2] Adjusted for gestational age at birth (missing values and gestational age>45 weeks excluded)

[3] Adjusted for cohort

[4] 2094 observations with unknown cumulative dose excluded

[5] DESAD cohort only

Table 4

Odds ratios (95% CI) for early menarche (defined as ≤10 and≤11 years old) by DES exposure status, cohort, and dose and gestational age of first exposure to DES (DESAD only)

	Menarche <=10 years		Menarche <=11 years	
	N (%)	**OR (95% CI)**	**N (%)**	**OR (95% CI)**
No DES exposure	37 (2.9)	1.00	188 (14.5)	1.00
Any DES exposure[1]	162 (3.8)	1.41 (0.97,2.03)	674 (15.6)	1.16 (0.97,1.39)
Dieckmann[2]				
Unexposed	10 (3.8)	1.00	57 (21.4)	1.00
Exposed	16 (5.2)	1.40 (0.62,3.14)	59 (19.0)	0.87 (0.58,1.30)
DESAD[3]				
Unexposed	27 (2.6)	1.00	131 (12.7)	1.00
Exposed	146 (3.6)	1.41 (0.93,2.13)	615 (15.4)	1.25 (1.02,1.53)
Low dose cohort	61 (3.3)	1.24 (0.81,1.91)	287 (15.4)	1.18 (0.96,1.46)
High dose cohort	101 (4.1)	1.50 (1.02,2.21)	387 (15.8)	1.15 (0.94,1.39)
Cumulative dose of DES (mg)[4]				
<2500	22 (4.9)	1.93 (1.08,3.42)	77 (17.2)	1.43 (1.05,1.94)
2500–9999	11 (2.4)	0.92 (0.45,1.86)	52 (11.3)	0.88 (0.63,1.24)
10,000	21 (5.3)	2.10 (1.17,3.76)	73 (18.5)	1.54 (1.13,2.11)
Gestational age at 1st exposure to DES (weeks)[4]				
<=7	27 (3.0)	1.15 (0.67,1.97)	128 (14.2)	1.14 (0.88,1.48)
8–10	25 (3.1)	1.19 (0.69,2.07)	115 (14.3)	1.15 (0.88,1.50)
11–14	17 (2.7)	1.05 (0.57,1.94)	90 (14.5)	1.17 (0.87,1.56)
15+	40 (4.9)	1.91 (1.16,3.14)	153 (18.7)	1.58 (1.23,2.04)

[1] Adjusted for original cohort (DESAD vs. Dieckmann)

[2] 232 observations with missing values for age at menarche were excluded

[3] 12 observations with missing values for age at menarche were excluded

[4] DESAD cohort only

Table 5

Hazard ratios and 95% confidence intervals for the occurrence of menarche in DES-exposed compared to unexposed daughters, overall, by original cohort, and by dose and timing of first exposure (DESAD only)

	N (%)	HR (95% CI)
Combined cohorts		
No DES exposure	1297 (23.1)	1.00
Any DES exposure[1]	4315 (76.9)	1.05 (0.99,1.12)
Dieckmann[2]		
Unexposed	266 (46.3)	1.00
Exposed	309 (53.7)	0.97 (0.82,1.14)
DESAD[3]		
Unexposed	1031 (20.5)	1.00
Exposed	4006 (79.5)	1.09 (1.02,1.16)
Cumulative dose of DES (mg)[4]		
<2500	447 (34.4)	1.10 (0.99,1.23)
2500–9999	458 (35.3)	1.02 (0.91,1.13)
10,000+	393 (30.2)	1.10 (0.98,1.24)
Gestational age at 1st exposure to DES (weeks)[4]		
<=7	901 (28.7)	1.08 (0.99,1.19)
8–10	805 (25.6)	1.04 (0.95,1.14)
11–14	620 (19.7)	1.10 (0.99,1.21)
15+	818 (26.0)	1.09 (1.00,1.20)

[1] Adjusted for original cohort (DESAD vs. Dieckmann)

[2] 232 observations with missing values for age at menarche were excluded

[3] 12 observations with missing values for age at menarche were excluded

[4] DESAD only