Pharmacologic Sex Hormones in Pregnancy in Relation to Offspring Obesity

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Objective: To assess the association between in utero exposure to either diethylstilbestrol (DES) or an oral contraceptive in pregnancy and offspring obesity.

Methods: Using data from the Collaborative Perinatal Project (1959-1974), a multicenter prospective study of pregnant women and their offspring, we examined overweight or obesity among 34,419 children with height and weight data at age 7 years. Generalized linear models to estimate the adjusted odds ratio (aOR) for overweight or obesity (≥85th percentile) or obesity (≥95th percentile) in the offspring according to exposure during different months of pregnancy were used.

Results: Oral contraceptive use during pregnancy was positively associated with offspring overweight or obesity and obesity. The magnitude of association was strongest in the first 2 months of pregnancy for obesity (aOR 2.0, 95% CI: 1.1, 3.7). DES use was also associated with offspring overweight or obesity and obesity, with the association being strongest for exposure beginning between months 3 and 5 (e.g., for exposure beginning in months 3-4, the aOR for obesity was 2.8, 95% CI: 1.3, 6.3).

Conclusions: Pharmacologic sex hormone use in pregnancy may be associated with childhood obesity. Whether contemporary, lower dose oral contraceptive formulations are similarly associated with increased risk of childhood obesity is unclear.

Introduction

The possibility that agents with weak estrogenicity are obesogenic has received considerable attention in the epidemiologic literature (1,2). Experiments on animals have shown a positive association between perinatal exposure to diethylstilbestrol (DES) and exogenous 17-β estradiol and offspring development of overweight or obesity (3). One human study identified a positive association between DES exposure in utero and subsequent development of obesity among adult women. Women exposed in utero, at ≥15 weeks of gestation, were at highest risk (7). There have been no published studies evaluating this association in children.

Oral contraceptives were first introduced in 1960 and generally consist of a synthetic estrogen (primarily ethinyl estradiol in contemporary formulations, mestranol at the time oral contraceptives were first introduced) and a progestin component. Since the introduction of oral contraceptives, there have been many different, less potent formulations developed to help minimize the risk for adverse effects (8). DES is no longer in use, but was commonly prescribed in the 1950s and 1960s to pregnant women considered at risk of pregnancy complications—complications such as threatened miscarriage, as evidenced by vaginal bleeding, or having a history of fetal loss. Once a woman initiated use of DES, she typically used it throughout the remainder of pregnancy (9). DES exposure in in vitro and animal models has resulted in a dose-responsive increase in stem cell differentiation into preadipocytes and adipocytes (4). In humans, mesenchymal stem cell differentiation into preadipocytes and, subsequently, adipocytes occurs after the first trimester of pregnancy, between gestational weeks 14 and 22 (13). Studies using cohorts exposed in utero to DES and first generation formulations of oral contraceptives offer the opportunity to study the association given a higher dose exposure and to evaluate possible developmentally sensitive periods of exposure.

In this study, we assessed whether exposure to pharmacologic sources of estrogenic agents, including oral contraceptives in early pregnancy (ethinyl estradiol or mestranol) and diethylstilbestrol (DES) during pregnancy, was associated with early childhood overweight.
reported whether they were using contraception at the time of conception and the type of contraception being used. The first month of pregnancy began with the first day of the last menstrual period. Of the 34,419 pregnancies studied, 196 (0.6%) were exposed to an oral contraceptive and 131 (0.4%) were exposed to DES. For oral contraceptive use, most of the women who were exposed were exposed within the first 8 weeks of pregnancy (159 of the 196).

Once DES use was initiated, women generally continued use through the remainder of their pregnancy. Most women using DES began use within the first 4 months of pregnancy (n = 103). An additional 28 women began using DES in months 5-9. In our primary analysis, we characterized DES exposure by the trimester in which use began. Next, given the literature suggesting increased adipogenesis in weeks 14-22 (13) and an increased susceptibility for obesity in adult women exposed to DES in utero ≥ 15 weeks of gestation (7), we evaluated exposure according to the month of first use. Data for DES use, however, were generally too sparse to characterize exposure for each month. We therefore also present the results for DES exposure based on 2-month, overlapping increments. The overlapping periods of exposure is an approach used to create a smoothed representation of change in association over time and provides a more stable estimation of association when the number of exposed cases is small, the exposure is time varying and there is interest in how the association may vary over time, yet the periods of exposure are correlated (15-17). We also characterized the month of initiation of use as a continuous measure to increase power in evaluating the association with timing. We used the same approach for OC use, characterizing use by trimester, by month, and by overlapping, 2-month increments, through the fourth month of pregnancy.

Overweight or obese or obese only status was ascertained from height and weight data collected by study personnel. Height was measured to the nearest 0.5 cm using a standardized backboard, and weight was recorded in pounds to the nearest 0.25 lbs or g to the nearest 100 g using scales calibrated semianually (18). The mean age at collection was 84 months (SD: 2.5, range 72-96). From height and weight measures we calculated body mass index (BMI) (kg/m²). Each child’s BMI was then expressed as a percentile, based on the Centers for Disease Control reference standards for age and sex (19). Overweight or obese status was characterized as having a BMI of ≥85th percentile. Obesity was characterized as having a BMI of ≥95th percentile. These percentiles correspond to a BMI of ≥17.4 kg/m² (overweight or obese) and ≥19.1 kg/m² (obese only) for boys and ≥17.6 kg/m² (overweight or obese) and ≥19.6 kg/m² (obese only) for girls at age 7 years (19). In this study, 4,954 (14.4%) children were overweight or obese and 1,743 (5.1%) were obese. The mean BMI for boys was 16.0 kg/m² (sd: 1.8) and for girls was 15.9 kg/m² (sd: 2.1).

Covariate selection was informed by a directed acyclic graph (16), constructed based on a review of the literature. Given the potential for differences in prescribing practices by study center and the observed differences in study population demographics by center, all multivariate models included adjustment by study center (12 centers). Additionally, multivariate models included adjustment for maternal age (<20, 20-34, ≥35) (20,21), race (black, white, other) (22,23), education (<high school, high school diploma or equivalent, >high school diploma or equivalent) (22,23), prepregnancy BMI (continuous) (24,25), smoking during pregnancy (22,26) (any vs. none) (21,25), and parity (0, 1, 2-3, ≥4) (22,27).

We used generalized linear models to estimate odds of overweight or obese or obese only for exposed versus unexposed pregnancies...
(logit link) and generalized estimating equations with an exchangeable working correlation matrix to estimate robust errors and account for correlation in the data resulting from sibling clusters (28). For overlapping periods of exposure, each exposure period was modeled separately.

We explored the potential for confounding by indication for DES users, first by comparing the distribution of pregnancy-related events and complications among DES users and non-users and then by conducting a sensitivity analysis to assess the robustness of study results when adjusting on these additional factors associated with DES use.

In a supplementary analysis, we evaluated change in estimates with inclusion of additional model terms for birthweight, year of birth, and gestational diabetes. We also evaluated for possible effect modification by race, including an interaction term for race and OC or DES use. *A priori*, a *P*-value of <0.15 was considered to be evidence in support of possible interaction by race. All statistical analyses were conducted using SAS v9.3 (SAS Institute, Cary, NC). This study was approved by the Institutional Review Board (IRB) of the National Institutes of Environmental Health Sciences.

### Results

Examination of the distribution of the study covariates for the study population by exposure to either an oral contraceptive or DES indicated differences in proportion exposed by race and education (Table 1). Specifically, pregnancies to mothers of white race and

| Characteristic                  | Study population (*n* = 34,419) | Exposed to an OC<sup>b</sup> months 1-4 (*n* = 196) | Exposed to DES<sup>d</sup> months 1-9 (*n* = 131) | Overweight or obese (≥85<sup>th</sup> percentile) at age 7 (*n* = 4,934) |
|--------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Overall                        | -                               | 0.4                             | 0.4                             | 14                              |
| Maternal age (years)           |                                 |                                 |                                 |                                 |
| <20                            | 8,192                           | 0.3                             | 0.1                             | 13                              |
| 20-34                          | 23,516                          | 0.7                             | 0.4                             | 15                              |
| ≥35                            | 2,711                           | 0.1                             | 0.8                             | 19                              |
| Maternal race                  |                                 |                                 |                                 |                                 |
| Black                          | 15,402                          | 0.2                             | 0.2                             | 12                              |
| White                          | 17,663                          | 0.7                             | 0.7                             | 17                              |
| Other                          | 1,354                           | 0.1                             | 0.1                             | 20                              |
| Maternal education (years)     |                                 |                                 |                                 |                                 |
| <12                            | 3,833                           | 0.2                             | 0.2                             | 14                              |
| 12                             | 10,913                          | 0.6                             | 0.6                             | 16                              |
| ≥13                            | 19,673                          | 0.7                             | 0.7                             | 15                              |
| Maternal pre-pregnancy BMI (kg/m<sup>2</sup>) |                                 |                                 |                                 |                                 |
| <18.5                          | 3,103                           | 0.6                             | 0.6                             | 6                               |
| 18.5-24.9                      | 23,383                          | 0.4                             | 0.4                             | 13                              |
| 25.0-29.9                      | 1,792                           | 0.4                             | 0.4                             | 21                              |
| ≥30.0                          | 6,141                           | 0.4                             | 0.4                             | 27                              |
| Maternal smoking               |                                 |                                 |                                 |                                 |
| No                             | 18,550                          | 0.4                             | 0.4                             | 14                              |
| Yes                            | 15,869                          | 0.4                             | 0.4                             | 15                              |
| Parity                         |                                 |                                 |                                 |                                 |
| 0                              | 10,103                          | 0.3                             | 0.3                             | 16                              |
| 1                              | 7,672                           | 0.3                             | 0.3                             | 15                              |
| 2-3                            | 9,607                           | 0.5                             | 0.5                             | 14                              |
| ≥4                             | 7,037                           | 0.3                             | 0.3                             | 12                              |

<sup>a</sup>Complete case analysis sample.<br>
<sup>b</sup>Oral contraceptive.<br>
<sup>c</sup>Denotes percent of study population within a given category who had the given exposure or outcome.<br>
<sup>d</sup>Diethylstilbestrol.
TABLE 2 Odds of overweight or obese among offspring of women using a pharmacologic sex hormone - by trimester of first use

| Exposure | Overweight or obese (≥85th percentile) | Obese (≥95th percentile) |
|----------|---------------------------------------|--------------------------|
|          | Exposed n | Exposed cases n | Crude OR (95% CI) | Adjusteda OR (95% CI) | Exposed cases n | Crude OR (95% CI) | Adjusteda OR (95% CI) |
| Oral contraceptiveb | None | 34,223 | 4,920 | Referent | Referent | 1,731 | Referent | Referent |
|                    | Trimester 1 | 182 | 31 | 1.3 (0.9, 1.8) | 1.4 (0.9, 2.0) | 12 | 1.3 (0.8, 2.4) | 1.7 (0.9, 3.0) |
|                    | Trimester 2 | 14 | 3 | 1.7 (0.5, 6.0) | 1.8 (0.5, 7.0) | 0 | -c | -c |
| DESc | None | 34,288 | 4,929 | Referent | Referent | 1,730 | Referent | Referent |
|                    | Trimester 1 | 86 | 15 | 1.2 (0.7, 2.1) | 0.8 (0.5, 1.4) | 8 | 2.0 (0.9, 4.0) | 1.3 (0.6, 2.8) |
|                    | Trimester 2 | 39 | 9 | 1.6 (0.7, 3.4) | 1.5 (0.7, 3.2) | 4 | 2.0 (0.7, 6.0) | 2.0 (0.6, 6.5) |
|                    | Trimester 3 | 6 | 1 | 1.2 (0.1, 10.1) | 1.2 (0.1, 12.3) | 1 | 3.7 (0.4, 31.9) | 4.4 (0.3, 55.9) |

aAdjusted for maternal smoking, education, parity, race, prepregnancy BMI, maternal age, and study center.
bTrimester of first documented use in pregnancy—trimester 1 corresponds to months 1-3, trimester 2 corresponds to months 4-6, and trimester 3 corresponds to months 7-9.
cNot estimable.

with a high school education or more were more likely to have been exposed (Table 1). Additionally, pregnancies to women with higher parity were, in general, more likely to have been exposed to an oral contraceptive. The distribution of child overweight or obesity status at age 7 by covariate status indicated a higher proportion of overweight or obese children were born to women of white or other race or who had a prepregnancy BMI ≥ 25.0 (kg/m²) (Table 1). The distribution of covariates among those pregnancies not included in the study, either because of missing covariates or loss to follow-up (n = 21,321), compared to the study population (n = 34,419) was similar (Supporting Information Table 1S) as was the distribution of exposure status (Supporting Information Table 2S).

Oral contraceptives

Use of an oral contraceptive in the first trimester was positively associated with both overweight or obese (adjusted OR: 1.4, 95% CI: 1.4, 2.0) and obese (adjusted OR: 1.7, 95% CI: 0.9, 3.0). Estimates obtained for exposure in the second trimester were highly imprecise for overweight or obese and not estimable for obese, a reflection of the sparseness of data in this exposure period (Table 2). Evaluation of exposure by month of initiation further illustrated the sparseness of the data for exposure in months 3 and 4, but provided the benefit of increasing precision for estimates in gestational month 1 (Supporting Information Table 3S). Finally, in characterizing exposure in 2 months, overlapping exposure periods, we observed that contraceptive use in early pregnancy was weakly, positively associated with overweight or obese at exposure periods 1-2, 2-3, and 3-4, and, more strongly, positively associated with offspring obese for exposure during gestational months 1-2 (adjusted OR: 2.0, 95% CI: 1.1, 3.7) (Table 3).

DES

Use of DES was positively associated with both overweight or obese and obese only for all trimesters of initiation of use. However, estimates were highly imprecise (Table 2). Evaluation of DES use by month was suggestive of a higher period of susceptibility in months 3 or 4, although again, estimates were imprecise and cell counts were sparse (Supporting Information Table 3S). For several months of exposure, data were too sparse for estimation. Applying the 2-month, overlapping periods of exposure approach, described above, we observed more stable and more precise estimates (Table 3). These results indicated that DES was positively associated with overweight or obesity for exposure beginning at 3-4 months and 4-5 months. Similarly, for DES and obesity, there was a positive association between initiation of use at 2-3, 3-4, and 4-5 months, with the strongest association observed at 3-4 months (adjusted OR: 2.8, 95% CI: 1.3, 6.3) (Table 3). With exposure modeled as a continuous measure, a positive trend was observed in the association between timing of use and offspring obesity, with a 10% increase in odds of obesity for each increase in month of initiation of use (Table 3). Inclusion of a quadratic term for month of initiation did not improve model fit.

A higher proportion of women prescribed DES had vaginal bleeding during pregnancy, a history of prior pregnancy loss, a longer period of time to achieve pregnancy, or a history of infertility investigation. Women using DES in early pregnancy were also more likely to report that the pregnancy was planned (Supporting Information Table 4S). We also observed differences in the indications for prescribing DES in early versus later pregnancy (trimester 1 vs. trimester 2). Women initiating DES use in the first trimester were more likely to have a history of infertility (54% for first trimester users vs. 15% for second trimester users) and were somewhat less likely to have experienced vaginal bleeding in the index pregnancy (58% for first trimester users vs. 72% for second trimester users). To explore whether the association between DES use and offspring obesity could be confounded by these differences, we conducted a sensitivity analysis whereby we adjusted for these possible confounding factors. An additional 414 participants had to be excluded from the sensitivity analyses for missing one or more of these study covariates. The estimates obtained from these analyses (Supporting Information Table 5S) were similar to estimates observed in our primary analyses.
Sex Hormones and Offspring Obesity

In our study, exposure to either an oral contraceptive or to DES during specific periods of pregnancy was associated with offspring overweight or obese only. This result is compatible with effects observed in animal studies. Although estrogenic agents are believed to sometimes act in non-receptor driven pathways, their primary action is through binding with nuclear receptors, for example, estrogen receptors (ER) and peroxisome proliferator-activated receptor gamma (PPAR-γ), and activating or deactivating steroid receptor-mediated transcription (29). In vitro models, DES has activated expression of both ER and PPAR-γ receptors required for adipogenesis (11). Similarly, 17β-estradiol has resulted in increased preadipocyte proliferation, likely through upregulation of PPAR-γ (30). The process of preadipocyte formation can be initiated at any stage of life, but perturbation has been demonstrated to occur as early as in the blastocyst stage (29). In humans, adipocyte formation has been identified to be most active in gestational weeks 14 through 22 (13). Recently, there has been interest in possible epigenetic effects of obesogens, whereby alterations in gene expression are driven by DNA methylation or histone modifications. These epigenetic effects may perturb priming of multipotent stem cells to preadipocyte formation (31).

In this study, the associations were strongest for DES, particularly DES exposure in months 3-4 and 4-5. We were only able to evaluate oral contraceptive use for exposure in early pregnancy, but for exposure in 1-2 months or duration of use 2-3 months, the magnitude of association observed was weaker than that observed for DES. The difference in association for these two agents may be attributable to differences in their capacity to alter receptor signaling, in epigenetic effects, in potency, or in pharmacokinetic changes as pregnancy advances. DES is considered a more potent estrogenic agent than ethinyl estradiol or mestranol, the two forms of synthetic estrogen in oral contraceptives in this study. The relative difference in potency may explain differences observed in the teratogenicity of DES and oral contraceptives. Whereas DES has been consistently reported to have numerous teratogenic effects on offspring (32,33), there is little to no evidence of a teratogenic effect for offspring of oral contraceptive users (34).

Another important distinction is that the oral contraceptive formulation includes a progestin component in addition to the estrogen component. Unopposed exposure to an estrogen may elicit a different

**Supplementary analyses**

In our supplementary analyses, evaluating change in estimate with inclusion of model terms for birthweight, year of birth, and gestational diabetes, an additional 156 participants were excluded from the analysis due to missing data on one or more of these covariates. Estimates obtained, for both DES or OCs, were substantively unchanged from estimates obtained in analyses including model terms for birthweight, year of birth, and gestational diabetes, an additional 156 participants were excluded from the analysis due to missing data on one or more of these covariates.
response than exposure to opposed estrogen. Finally, the results observed may reflect differences in duration in exposure, as opposed to differences in timing of exposure. Once initiated, DES use occurred throughout pregnancy, while oral contraceptive use generally ended early in pregnancy. Duration of DES use during pregnancy and timing of DES initiation was strongly correlated (Spearman \( r = 0.99 \)) and thus the independent effects of each could not be distinguished in these analyses. Given the observation that DES use in months 1-2 and 2-3 were not associated with offspring obesity, timing of use may be more important than duration. However, given the sparse data and the strong correlation between timing and duration, the possibility that both components are necessary cannot be ruled out.

There is very little information in the published literature on the association between oral contraceptive use and offspring over-weight or obesity. There has been one study, published as an abstract, suggesting a positive association between DES exposure \textit{in utero} and offspring obesity in adult women (7). A pooled analysis of women exposed \textit{in utero} to DES indicated no difference (unadjusted) in mean body mass index in adulthood compared to unexposed women (35). The difference in this study findings, and the findings from this pooled analysis, may be attributable to differences in study population, or indicative of a washing out of effect over time. It may be too, that in examining exposure at any point in time, any differences would have been unobservable, as opposed to this study for which there is indication of differences specific to timing of exposure. In Hatch et al., the association between DES and obesity was strongest for use at \( \geq 15 \) weeks gestation (7). In this study, we assessed for and found no evidence of an interaction between oral contraceptive use or DES use and sex. However, given the relatively small number of women using either of these agents, the study was underpowered to conclude an absence of interaction with sex. Attenuation of effects overtime has been demonstrated in animal models of obesogens, such as that described by Ryan et al. in evaluating the association between BPA and offspring obesity in mice followed to adulthood (36). This same study showed that \textit{in utero} exposure to DES decreased body fat in adult mice relative to controls (36).

The results observed, or differences in results observed between DES and oral contraceptives, could be attributable to unmeasured or residual confounding. Underlying maternal metabolic factors could contribute to poorer pregnancy outcomes and DES use. Underlying maternal factors could also contribute to conceiving while taking an oral contraceptive. These factors, if associated with offspring adiposity, could bias estimates. As described previously, adjustment for pregnancy-related factors associated with DES use did not substantively change estimates obtained in the primary analyses (Supporting Information Table 5S). Finally, the estimates observed could reflect chance.

The results might have been influenced by misclassification. Either DES or oral contraceptives could have been under- or over-reported due to poor recall of use. Although the exposure was self-reported, exposure ascertainment occurred relatively close to the time at which exposure occurred. Furthermore, validation studies of self-reported oral contraceptive use indicate women are generally accurate in their recall of oral contraceptive use (37,38). For example, in a validation study of self-reported oral contraceptive use occurring within 12 months of interview, the weighted kappa for self-reported – versus medical record-indicated use was 0.8 (38). Notwithstanding, potential for misclassification of exposure exists, with the greatest potential for misclassification of use in early pregnancy, the most distal point of recall for enrolled women. There is also the potential that initiation of oral contraceptive in months 3 or 4 reflects misclassification of exposure. The 37 women reporting initiation of an oral contraceptive in months 3-4 were generally younger (16% less than 20 years of age vs. 9% for those reporting use in months 1 or 2) and somewhat less educated (35% with less than 12 years of education vs. 24% for those reporting use in months 1 or 2). Women initiating use in months 3 or 4 were also less likely to be Black (24% vs. 36% for women reporting use in months 1 or 2). Any misclassification would likely result in a bias of estimates toward the null. Attenuation of effect estimates in months 3 and 4, for oral contraceptive use, could be an artifact of greater misclassification of exposure in this period.

All height and weight measures were obtained from trained study personnel. Furthermore, the association between DES use and obesity was stronger than the association with overweight or obesity. Obesity is a more sensitive measure of clinically relevant adiposity status as some children who are identified as overweight are children with increased muscle mass, as opposed to fat mass and thus obesity is likely to represent a more valid measure of childhood adiposity (39).

Although this study was large enough to provide an opportunity to study exposure of relatively low occurrence (failed contraception), sample size limitations precluded exploring, in greater detail, the influence of timing, duration, or drug formulation of exposure on observed associations. These details could yield additional etiologic clues in the role of estrogenic agents in development of overweight or obesity. Power calculations after a study has already been conducted may have limited utility (40); however, we conducted a power calculation to assess adequacy of study sample size and whether there was a potential that lack of association observed at some time periods could be attributable to a Type II error. With a prevalence of obesity of 0.06 at age 7, a proportion exposed to an oral contraceptive of 0.005 for exposure in months 1-2, power of 0.80, and a Type 1 error of 0.05, we would have needed approximately 27,000 subjects in our study population to detect an odds ratio of 2.0. For DES use, with a proportion exposed of 0.002 in months 3-4, we would have needed approximately 29,000 subjects to detect an odds ratio of 2.8. For exposure periods with fewer exposed or for smaller effect sizes, our study would have been underpowered to reject the null hypothesis of no effect. Therefore, although the study results are suggestive of timing-specific effects, the small sample sizes at some exposure periods may limit the interpretability of results.

Future studies, with a larger number of births followed (>55,000), would benefit from additional exposure details to estimate the association with greater precision and explore possible dose-response relationships. Furthermore, future studies would benefit from assessing the association with contemporary, lower dose oral contraceptive formulations. The findings from this study generally support those obtained in animal models. However, lower dose formulations may more closely approximate the exposures incurred through environmental sources of estrogenic compounds than the doses documented in this cohort.
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