Prenatal Glucocorticoids and Maternal Smoking During Pregnancy Independently Program Adult Nicotine Dependence in Daughters: A 40-Year Prospective Study

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Background: Maternal smoking during pregnancy (MSDP) is an independent risk factor for offspring nicotine dependence (ND), but mechanisms remain unknown. We investigated prenatal glucocorticoid (cortisol) and androgen (testosterone) associations with offspring ND over 40 years and the possibility that prenatal glucocorticoids and androgens would mediate links between MSDP and offspring ND.

Methods: Participants were 1086 mother-adult offspring pairs (59% female) from the New England Family Study, a 40-year longitudinal follow-up of the Collaborative Perinatal Project. MSDP was assessed prospectively at each prenatal visit. Maternal cortisol, testosterone, and cotinine (nicotine metabolite) were assayed from third trimester maternal sera. Offspring lifetime ND was assessed via structured interview.

Results: Significant bivariate associations emerged for: 1) MSDP/cotinine and lifetime ND; and 2) maternal cortisol and lifetime ND, for daughters only. In multivariate models, maternal cortisol and MSDP/cotinine remained significantly and independently associated with increased odds of lifetime ND of daughters. However, cortisol did not mediate the MSDP-lifetime ND relation. No associations emerged between maternal testosterone and offspring ND.

Conclusions: Results provide the first evidence in support of prenatal glucocorticoid programming of adult ND over 40 years in daughters only. Our study highlights two independent prenatal pathways leading to increased risk for ND in daughters: elevated prenatal glucocorticoids and MSDP/nicotine exposure. Daughter-specific effects of glucocorticoid and MSDP programming over 40 years highlight the breadth and persistence of sexually dimorphic programming effects in humans. Results do not support androgen programming of offspring ND.

Key Words: Androgen, cortisol, cotinine, glucocorticoid, maternal smoking during pregnancy, nicotine dependence, programming, testosterone

Maternal smoking during pregnancy (MSDP) remains a major public health problem. Despite pervasive medical and societal sanctions, 13%–30% of pregnant mothers continue to smoke in the United States, with highest rates in poor, less educated, underserved mothers (1–3). Maternal smoking during pregnancy has been linked to numerous adverse medical and behavioral outcomes in offspring, including low birth weight and sudden infant death syndrome, and attention deficits/attention-deficit/hyperactivity disorder and disruptive behaviors/behavioral/psychiatric problems in later life (4–8). Maternal smoking during pregnancy has also been linked to a significantly increased risk for offspring smoking uptake and regular smoking (9–20). Most pronounced links have emerged between MSDP and progression to regular/heavy smoking and nicotine dependence (ND) (16,17,21,22), phenotypes associated with resistance to quit attempts, nicotine craving, and alterations in neural processes and circuitry (23). In some studies, effects of MSDP were more pronounced for female offspring (12–14,16,17,19,24). Although findings supporting the MSDP–offspring smoking/ND links have been replicated across samples and measures, mechanisms remain largely unknown.

Over the last decade, a large body of human and preclinical research has highlighted the profound importance of the fetal environment in “programming” physiologic systems and structures leading to adult health and disease (25–27). Programming has been defined as permanent alterations in fetal tissues and physiological systems as a function of the prenatal environment (28). Programmed physiological changes are believed to lead to adjustments in developmental trajectories that might predispose offspring to either positive outcomes or impairments/diseases, depending on congruence with postnatal environmental demands (26,29,30). Steroid hormones including glucocorticoids and androgens have been proposed as prominent candidate mediators of prenatal programming (31,32). Although necessary for fetal development, overexposure to glucocorticoids during the sensitive prenatal period is proposed to alter or “program” numerous fetal physiological and neural systems leading to psychiatric, cardiovascular, and metabolic diseases in offspring (33–37). However, although several human studies have highlighted the impact of prenatal glucocorticoid overexposure on physiological conduct disorder in older children and adults (4–8). Maternal smoking during pregnancy has been linked to a significantly increased risk for offspring smoking uptake and regular smoking (9–20).
and behavioral outcomes in infancy and childhood (38–41), we know of no human studies investigating glucocorticoid programming of adult behavioral disorders including ND.

Several lines of research highlight the plausibility of glucocorticoid programming of ND. First, exposure to MSDP/nicotine has been associated with increased maternal glucocorticoids and alterations in offspring hypothalamic-pituitary-adrenal stress response in animal and human studies (42–52). Second, preclinical studies have shown links between increased maternal glucocorticoids and alterations in reward pathways, including persistent effects on drug sensitivity, altered propensity for drug self-administration, and altered brain dopamine activity in offspring (53–56). Given relationships between brain dopamine activity and reinforcement from nicotine (57) and between prenatal glucocorticoid exposure and alterations in offspring dopamine activity (58), smoking-induced increases in maternal glucocorticoids might alter fetal dopamine activity leading to increased propensity for ND. Thus, we propose that maternal cortisol levels might mediate the relation between MSDP and offspring ND. Given evidence for more pronounced MSDP-offspring smoking links in daughters (12–14, 16, 17, 19, 24) and for sex differences in prenatal glucocorticoid levels (59) and offspring outcomes after prenatal glucocorticoid exposure (60), we also hypothesized that links between prenatal glucocorticoids and offspring ND would be stronger for daughters.

Prenatal androgens have been proposed as an additional candidate mediator of prenatal programming. Several studies support the plausibility of androgenic programming of offspring ND. Lombardo et al. (32) showed associations between fetal testosterone exposure and alterations in responsiveness of neural reward regions and behavioral approach tendencies, both of which have been associated with ND (61). Furthermore, Kandel and Udry (13) published the only human study, to our knowledge, investigating prenatal androgen (testosterone) programming of offspring smoking. They hypothesized that testosterone might program the developing brain, leading to increased offspring testosterone, corresponding to greater sensation-seeking behaviors and increased likelihood of smoking. In their study of mother-daughter pairs, they found significant positive associations between MSDP and maternal prenatal testosterone and between prenatal testosterone adolescent and adult offspring smoking. Their results highlight the plausibility of prenatal androgen programming of offspring smoking, especially in daughters.

In sum, multiple converging lines of evidence support the plausibility of glucocorticoid and androgen programming of offspring smoking/ND. Yet, to our knowledge, only one study (13) has investigated one of these plausible mechanisms (testosterone) and in an all-female sample (n = 240) focusing on offspring smoking but not ND. In the present study, we conduct the first large-scale (n = 1086) investigation of the plausibility of both prenatal glucocorticoid and androgen programming of offspring ND in both daughters and sons in relation to prospectively assessed, biochemically validated MSDP. Specifically, we investigated maternal late third-trimester prenatal cortisol and testosterone as possible mediators between MSDP and offspring ND, capitalizing on mother-offspring pairs taking part in the New England Family Study (NEFS), a 40-year longitudinal follow-up of the Collaborative Perinatal Project (CPP).

Methods and Materials

Participants and Sample Selection

The CPP was a multisite, prospective investigation of the prenatal and familial antecedents of pediatric, neurological, and behavioral disorders of childhood. The CPP enrolled more than 50,000 pregnancies between 1959 and 1966 from 12 university-affiliated medical centers and followed offspring through 7 years of age (62–64). The NEFS was established to locate and interview adult offspring of mothers enrolled in the Boston and Providence cohorts of the CPP. Selection and sampling for 1674 NEFS participants has been previously described (65–67). All NEFS participants provided written informed consent and followed procedures reviewed and approved by Human Subjects Committees at The Miriam Hospital, Brown University, and the Harvard School of Public Health. Participants in the present study were mother-offspring pairs enrolled in the NEFS from live, singleton births who had available maternal prenatal serum sampled between 31 and 36 weeks gestation (1086 of 1674; 65%).

Procedures

After enrollment, CPP mothers completed numerous measures, including socio-demographic and pregnancy characteristics, and cigarette smoking during pregnancy. Nonfasting maternal blood was collected at each prenatal visit. Serum was extracted, and samples were frozen and shipped to a CPP repository in Bethesda, Maryland. Offspring birth characteristics were recorded by study examiners. Adult offspring enrolled in the NEFS follow-up study (1999–2004) completed a number of interview and self-report measures including ND (67).

We obtained late third trimester maternal serum samples from the CPP central storage repository to assay for cotinine (nicotine metabolite), testosterone, and cortisol. Sex hormone binding globulin (SHBG) and cortisol binding globulin (CBG) were also assayed to determine concentrations of free cortisol (free cortisol index [FCI]) and testosterone (free androgen index [FAI]) for a more accurate estimate of fetal exposure (68, 69). We selected NEFS mother-offspring pairs with a serum sample drawn between 31 and 36 weeks after the last menstrual period and at least 14 days before the birth date of the infant, given known effects of labor/delivery on steroid hormone levels (70–72). Weeks 31–36 were selected, because: 1) they provided a relatively tight window within the third trimester to examine hormone levels; 2) they included the greatest number of participants with available serum samples; and 3) prior literature showed links between third trimester stress and hormone levels and offspring neurobehavioral outcomes (73–76). Mean gestational age at sampling was 31 weeks (SD = 1.5) after last menstrual period. Time of day of sampling was not recorded in the CPP. Validity of cotinine, testosterone, and cortisol values from the CPP has been demonstrated previously (77, 78).

Measures

MSDP. Pregnant mothers were queried with regard to cigarette smoking by study physicians at each prenatal visit. Mothers were asked whether they were currently smoking and, if so, the number of cigarettes smoked/day. Validity of CPP maternal smoking reports through comparison with serum cotinine levels has been shown to be excellent (κ = 83%–87%) (78). Following Kandel and Udry (13), bivariate analyses used an ordered categorical MSDP variable: none (did not smoke), low (smoked <15 cigarettes/day), and high (smoked ≥15 cigarettes/day). For multivariate analyses, this three-level ordinal MSDP measure was recoded with 2 dummy variables contrasting low and high MSDP with no MSDP.

Adult Lifetime ND. Adult history of ND was based on DSM-IV criteria (79) and assessed with a modified (80) version of the Composite International Diagnostic Interview (81). Lifetime ND was summarized as a binary variable (nondependent, nicotine
dependent) covering all ages through the adult follow-up interview (mean = 39 years, SD = 2).

Biological Variables. Cotinine was assayed with liquid chromatography–tandem mass spectrometry (82,83), laboratory of Neal Benowitz, M.D., University of California, San Francisco. Limit of quantitation was 1 ng/mL. Cortisol, testosterone, and SHBG were assayed with enzyme-linked immunosorbent assay kits; CBG was measured with radioimmunoassay (laboratory of C. Kirschbaum, University of Dusseldorf; assays described at www.ibl-hamburg.com). Inter/intra-assay coefficients of variability ranged from 3% to 12%. The FAI and FCI were calculated from testosterone and SHBG and cortisol and CBG, respectively (84,85). For further details of sample collection, storage, and analysis, see Stroud et al. (77).

Potential Confounding Variables. Maternal age, race/ethnicity, education, occupation, income, gravida, and parity (number of prior live births) were assessed during the first prenatal visit. A composite index of socioeconomic status (SES) (range: 1 = lowest through 100 = highest) was derived from education (years), occupation (manual, nonmanual, unemployed) of the head of household, and household income (on the basis of US poverty threshold at the time) with methods developed by the US Census Bureau (86). Maternal psychiatric conditions and excessive alcohol and drug use during pregnancy were recorded by study personnel as part of an obstetric diagnostic summary. Maternal history of treatment for mental illness before pregnancy was assessed by maternal report. Gestational age was calculated on the basis of maternal report of last menstrual period. Birth weight was recorded by a nurse observer at delivery.

Statistical Analysis

Bivariate associations between MSDP, cotinine, cortisol (FCI), testosterone (FAI), and offspring ND were estimated with poly-choric, polychoric, polyserial (φ), and Pearson (r) correlations for the full sample (n = 1086), stratified by sex (649 daughters, 437 sons). Following LeWinn et al. (87), given potential for confounding by low birthweight and prematurity (4,88), the sample for mediation modeling was restricted to 986 mother-offspring pairs (584 daughters), with gestational age ≥37 weeks and birthweight ≥2500 g. A causal steps approach was used to test cortisol and testosterone as mediators of the MSDP/offspring ND link (89). This requires significant associations between: 1) predictor and outcome; 2) predictor and mediator; and 3) mediator and outcome adjusted for predictor. Furthermore, it requires attenuated of the predictor-outcome association when the putative mediator is included in the model. Causal steps were tested with multivariate logistic regression analyses in S-plus 8.2 (90), with ordinal MSDP followed by maternal cotinine as predictors. Potential confounders were then tested for inclusion in regression models on the basis of significant associations with both MSDP and offspring ND. These included: gravida/parity, maternal age at delivery (centered at 35 years, scaled by 5 years), maternal race/ethnicity (Caucasian, other), SES, maternal other drug use (use, no use), history of maternal treatment for mental illness (yes, no). Continuous predictors/confounders were centered at the median and scaled by the distance from the median to the third quartile.

Results

Sample Characteristics

Pregnant mothers. Mean maternal age at delivery was 25 years (SD = 6). Racial/ethnic characteristics of mothers included 87.3% Non-Hispanic white, 12.0% black, 2.2% Hispanic, and 5% other. Average gravida was 2 (SD = 2). Mean composite maternal SES was 56 (SD = 19), on a 100-point scale. Fifty-eight percent of mothers endorsed smoking during pregnancy; 43% reported smoking during third trimester. Among smokers, average maximum cigarettes/day during pregnancy was 18 (SD = 11), highly similar to third trimester levels (mean = 18, SD = 10); mean cotinine levels were 95 ng/mL (SD = 78, range 1–526).

Adult Offspring. Fifty-nine percent were women. Mean age at adult follow-up was 39 years (SD = 2; range 34–44). Average gestational age at birth was 40 weeks (SD = 2); 5% of infants were born premature (<37 weeks). Mean birthweight was 3310 g (SD = 506); 6% of infants were born low birthweight (<2500 g). Thirty-nine percent of offspring (42% of daughters) met criteria for lifetime ND.

Bivariate Associations

Bivariate associations between MSDP, maternal cotinine, cortisol (FCI), testosterone (FAI), and offspring ND for daughters (n = 649) and sons (n = 437) are shown in Table 1. As in prior CPP samples (78), MSDP showed strong associations with maternal cotinine for daughters and sons (ψs > .825, ps < .0001). Significant associations between maternal cortisol and testosterone also emerged for both daughters and sons (rs > .222, ps < .0001). For daughters only, increasing MSDP exposure (did not smoke, < 15 cigarettes/day, 15+ cigarettes/day; ns = 254, 132, 258) and maternal cotinine were associated with increased likelihood of offspring lifetime ND (ps < .01). Increased MSDP was associated with increased maternal cortisol (p < .05); increased cortisol was also associated with increased likelihood of lifetime ND (p < .05) in daughters only. No associations emerged between maternal cotinine and cortisol or between testosterone and either MSDP/cotinine or offspring ND for daughters. For sons, no significant associations emerged between MSDP (did not smoke, <15 cigarettes/day, 15+ cigarettes/day, ns = 195, 78, 163) and maternal cotinine with offspring ND, or between cortisol and testosterone with MSDP, cotinine, or offspring ND.

Thus, cortisol was the only putative mediator associated with both MSDP and lifetime ND for daughters only. Accounting for missingness, final mediation sample for daughters was n = 544. Because cortisol was not associated with maternal cotinine in bivariate analyses (r = .062, p = .09), mediation models were not pursued with cotinine. Instead, interest centered on whether bivariate associations were robust to control for potential confounders. Maternal testosterone did not qualify as a possible mediator, because it was not significantly related to MSDP/maternal cotinine in either daughters or sons.

Multivariate Model: Maternal Cotinine as a Predictor of Lifetime ND in Adult Daughters (n = 544). Maternal cotinine remained associated with an increased likelihood of lifetime ND in daughters (β = .114, SE = .055, p = .039) in the restricted sample after controlling for gravida, advanced maternal age at delivery, race, and SES. Specifically, increases from the median to the third quartile of cotinine raised the odds of lifetime ND by 12% (odds ratio [OR]: 1.12, 95% confidence interval [CI]: 1.01–1.25).

Multivariate Model: Maternal Cortisol as a Mediator of Associations Between MSDP and Lifetime ND in Adult Daughters (n = 544). MSDP, dummy coded as low versus none (ns = 110 vs. 218) and high versus none (ns = 216 vs. 218), was entered into the model along with significant covariates (Step 1), followed by cortisol (FCI) as the mediator (Step 2). Logistic regression coefficients for both steps are presented in Table 2. In Step 1, high MSDP (≥ 15 cigarettes/day) was associated with an increased likelihood of lifetime ND.
(β = .419, SE = .207, p = .043). In Step 2, maternal cortisol was significantly associated with lifetime ND in daughters (β = .121, SE = .055, p = .029), with increases from the median to the third quartile, raising the odds of lifetime ND by 13% (OR: 1.13, 95% CI: 1.01–1.26). The association between high MSDP and lifetime ND showed little change when cortisol was added to the model (β = .409, SE = .207, p = .048). High MSDP increased the odds of lifetime ND by approximately 50% both before (OR: 1.52, 95% CI: 1.01–2.28) and after (OR: 1.51, 95% CI: 1.00–2.26) adjustment for maternal cortisol. Low MSDP was not significantly related to offspring ND in daughters before or after adjustment for cortisol (p > .30).

Linear regression coefficients for associations between MSDP and maternal prenatal cortisol are shown in Table 3. Although MSDP was positively correlated with cortisol in bivariate analyses (Table 1, ϕ = .11), it was no longer significant at either low (β = .191, SE = .119, p = .337) or high MSDP (β = .138, SE = .165, p = .403) after adjusting for potential confounders.

Table 2. Parameter Estimates from Regression Analyses to Test Mediation Models Linking Maternal Smoking during Pregnancy, Maternal Prenatal Cortisol, and Offspring Nicotine Dependence in Daughters (n = 544)

<table>
<thead>
<tr>
<th>Maternal Smoking to Lifetime Nicotine Dependence of Daughters*</th>
<th>β</th>
<th>SE</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>−1.554</td>
<td>.382</td>
<td>−4.068</td>
<td>&lt;.001</td>
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<tr>
<td>Gravida*</td>
<td>.199</td>
<td>.049</td>
<td>4.044</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Maternal age at delivery*</td>
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<td>.032</td>
<td>2.490</td>
<td>.013</td>
</tr>
<tr>
<td>Maternal race (white vs. other)</td>
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<td>.343</td>
<td>2.328</td>
<td>.020</td>
</tr>
<tr>
<td>Maternal socioeconomic status</td>
<td>−.135</td>
<td>.067</td>
<td>−2.022</td>
<td>.043</td>
</tr>
<tr>
<td>Maternal smoking (low vs. none)*</td>
<td>.239</td>
<td>.250</td>
<td>.954</td>
<td>.340</td>
</tr>
<tr>
<td>Maternal smoking (high vs. none)*</td>
<td>.419</td>
<td>.207</td>
<td>2.028</td>
<td>.043</td>
</tr>
<tr>
<td>Step 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
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<td>.385</td>
<td>−3.926</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Gravida*</td>
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<td>.050</td>
<td>4.048</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Maternal age at delivery*</td>
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<td>.033</td>
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<td>.020</td>
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<tr>
<td>Maternal race (white vs. other)</td>
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<td>2.307</td>
<td>.021</td>
</tr>
<tr>
<td>Maternal socioeconomic status</td>
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<td>.068</td>
<td>−1.627</td>
<td>.104</td>
</tr>
<tr>
<td>Maternal smoking (low vs. none)*</td>
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<td>.252</td>
<td>.863</td>
<td>.388</td>
</tr>
<tr>
<td>Maternal smoking (high vs. none)*</td>
<td>.409</td>
<td>.207</td>
<td>1.974</td>
<td>.048</td>
</tr>
<tr>
<td>Maternal cortisol (FCI)*</td>
<td>.121</td>
<td>.055</td>
<td>2.177</td>
<td>.029</td>
</tr>
</tbody>
</table>

In Step 1, significant covariates followed by maternal cigarettes smoked/day (low vs. none, and high vs. none) were entered. In Step 2, significant covariates followed by both level of maternal cigarettes/day and maternal cortisol were entered into the model. Sample is restricted to gestational age ≥37 weeks and birthweight ≥2500 g.

*Adapted lifetime nicotine dependence: 1 = nicotine dependent, 0 = non-nicotine dependent.

1Sample sizes for the dummy low vs. non cigarettes smoked/day during pregnancy variable (dummy coded): low, n = 110; none, n = 218.

2Sample sizes for high vs. no cigarettes smoked/day during pregnancy variable (dummy coded): high, n = 216; none n = 218.
and perinatal characteristics, unique distribution of MSDP (58% size (ND (13). Strengths of the present study include the large sample to con
corticoid exposure; and elevated MSDP exposure. Our prenatal pathways to ND of daughters: elevated prenatal gluco-

discussion

The present study provides the first evidence of prenatal glucocorticoid programming of an adult psychiatric disorder, namely, ND, among daughters over a 40-year prospective study. Uniquely, this study also highlights two independent and additive prenatal pathways to ND of daughters: elevated prenatal glucocorticoid exposure; and elevated MSDP exposure. Our findings fail to confirm prior evidence for androgen programming of offspring ND (13). Strengths of the present study include the large sample size (n = 1086), prospective assessment of MSDP and maternal and perinatal characteristics, unique distribution of MSDP (58% smokers), 40-year longitudinal follow-up, interview measure of DSM-IV ND, and availability of third trimester serum samples for neuroendocrine assays. Available serum samples allowed measurement of free cortisol and testosterone, indicating bioavailable cortisol and testosterone, as well as cotinine, a biomarker of MSDP/nicotine exposure (92). Furthermore, roughly equal numbers of female and male offspring allowed us to conduct all analyses stratified by sex.

Increased exposure to maternal prenatal glucocorticoids was associated with a 13% increased odds of lifetime ND of daughters over 40-year follow-up. To our knowledge, this is the first study to reveal effects of prenatal glucocorticoid exposure on risk for nicotine addiction and the first to reveal effects of endogenous glucocorticoid exposure enduring to adulthood in daughters. Evidence for prenatal glucocorticoid programming in the present study complements a number of animal studies highlighting the causal role of overexposure to maternal glucocorticoids in programming CNS dysfunction and disease in adult offspring (33,93). Results also complement an emerging human literature suggesting that overexposure to prenatal glucocorticoids might program early behavioral, physiologic, and neurocognitive outcomes (38–41,87). The present study extends evidence in humans for associations between endogenous prenatal glucocorticoids and offspring outcomes to include adult ND.

Smoking 15 cigarettes/day or more was associated with a 52% increased odds of ND in daughters. Our finding of an independent pathway between MSDP and elevated risk for offspring ND is consistent with numerous prior studies (16,17,21), including a prior study from the CPP (21). Previous studies have shown links between MSDP and all stages of offspring smoking progression (smoking uptake, regular smoking, ND) (10,18,19,21) but with most pronounced effects for progression to regular/heavy smoking and ND (17,21,22). Nicotine dependence specifies a maladaptive pattern of tobacco use involving withdrawal, tolerance, and/or inability to quit smoking that is more closely linked to alterations in neural, affective, and hedonic processes as well as smoking-related diseases and healthcare burden relative to regular smoking (94–97). Results highlight the influence of MSDP on the phenotype of nicotine addiction in female offspring.

Maternal cotinine also predicted ND in adult daughters, highlighting the importance of nicotine in the long-term behavioral consequences of MSDP. Our results contrast with Kandel and Udry (13), who found no effects of maternal cotinine on the smoking of adolescent daughters. Because smoking in adolescence might be a
better representation of smoking initiation versus persistent smoking or ND, it is possible that exposure to maternal nicotine is associated with alterations in fetal neurosterogenes, increasing propensity to ND in adulthood (97,98), whereas other aspects of MSDP might impact smoking initiation in adolescence (13).

Associations between both prenatal glucocorticoids and MSDP/nicotine and offspring ND emerged only for daughters. Results are unlikely to be due to sex differences in ND in the population, because men have shown slightly increased ND prevalence (99), or sex differences in fetal metabolism, because the fetus shows little ability to independently metabolize drugs or glucocorticoids (100). Results are consistent with numerous preclinical and recent human studies revealing sex differences in effects of prenatal insults and in neuroendocrine programming pathways (60,101). Daughter-specific effects of MSDP in the present study complement prior studies revealing more pronounced effects of MSDP on offspring smoking in daughters (9,10,12–16,18–20; see also 21,24). Daughter-specific effects of prenatal glucocorticoid exposure are consistent with a recent study revealing increased late-gestational cortisol levels in mothers carrying daughters versus sons (59) and daughter-specific effects of maternal prenatal cortisol on child amygdalar volume, a neural marker of affective disorders (102). Likely mechanisms are sex differences in placental glucocorticoid regulation and adaptations to environmental insults, as well as differential effects of cortisol and nicotine on a sexually differentiating fetal brain (103). That daughter-specific effects emerged for both MSDP and prenatal glucocorticoids in the present and prior studies highlights consistent sexual dimorphism in programming outcomes across a broad range of prenatal exposures. Future research is needed to further elucidate sexually dimorphic prenatal programming pathways in relation to a broad range of prenatal insults.

Initially, we hypothesized that prenatal glucocorticoid programming would mediate links between MSDP and offspring ND. Instead, our findings suggest that exposure to maternal elevated glucocorticoids and high MSDP were associated with independent and additive increased risk for female offspring ND, together increasing odds of ND by 72%. Results complement emerging epidemiologic theories highlighting the interplay of allostatic load and environmental toxicants on maternal and child health disparities (104). Additive effects of elevated prenatal glucocorticoids and nicotine also complement an intriguing series of preclinical studies of offspring exposed to prenatal nicotine and dexamethasone, a synthetic glucocorticoid (105–107). Dually exposed offspring showed synergistic effects on brain cholinergic, serotonergic, and dopaminergic circuitry, with effects persisting to adulthood and evidence for sex differences in developmental trajectories.

In contrast to Kandel and Udry (13), our results fail to confirm prenatal testosterone as a mechanism linking MSDP and offspring ND, despite a large sample size and prospective assessment of MSDP. We believe it is unlikely that our failure to replicate the findings of Kandel and Udry (13) is due to lack of power. Our sample size was large (n = 1086), even when stratifying by sex with 80% power to detect small effects: r = .11 and .13 for daughters and sons, respectively; however, effect sizes for cortisol were statistically significant and approximately two times greater than those for testosterone. Although prenatal androgens did not show associations with the ND phenotype, they might be more closely linked to other points in offspring smoking trajectories, such as smoking initiation (13).

We acknowledge several key limitations of our study. First, time of day of serum collection and additional factors related to variability in cortisol and testosterone levels (food intake, caffeine) were not recorded in the CPP (108–110). However, if these factors are assumed to be random across prenatal visits (87), variation in hormone levels due to time of day/nutritional status would be included as error variance and would serve to attenuate rather than strengthen links between maternal glucocorticoids and offspring ND. That findings emerged despite likely high error variance suggests that effects would be stronger with more precise measures of maternal glucocorticoids. Second, measures of postnatal environment are lacking, particularly exposure to secondhand smoke, which was not assessed in CPP. Although CPP mothers who smoked during pregnancy likely continued to smoke postpartum, several prior studies of MSDP and offspring smoking/ND have shown associations to be robust to control for postnatal secondhand smoke exposure (14–17) and parental lifetime smoking status (111). Additional postnatal environmental factors (e.g., maternal care, parental sensitivity) have also been shown to mitigate effects of prenatal adversity (112–114). Future studies are needed to investigate postnatal environmental moderators of links between gestational glucocorticoids and MSDP and offspring ND (e.g., parental sensitivity, early life stressors, behavioral modeling).

Third, our study design did not allow assessment of familial confounding factors. Several recent studies revealed evidence for familial confounding of links between MSDP and offspring behavioral outcomes, although offspring ND was not measured, and most did not include biochemical verification of MSDP (115–117). Nonetheless, future studies with genetically informative designs (e.g., sibling pairs differing in MSDP exposure levels), which also include intermediate phenotypes and biological mediators, are needed (118,119). Finally, maternal glucocorticoids in the present study are presumed to indicate fetal glucocorticoid exposure. Future studies of both maternal prenatal and offspring cortisol regulation in relation to risk for adult ND would be a major contribution.

Conclusions

This 40-year longitudinal study reveals the first evidence, to our knowledge, that prenatal exposure to glucocorticoids predicts ND in adult daughters. Specifically, two independent and additive pathways to ND of daughter were identified: exposure to elevated prenatal glucocorticoids; and exposure to high MSDP. Results highlight the enduring influence of gestational glucocorticoid exposure and also support differential vulnerability of daughters to long-term adverse outcomes after gestational exposure to glucocorticoids and MSDP.

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