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Prenatal glucocorticoids and maternal smoking during pregnancy independently program adult nicotine dependence in daughters: A 40-year prospective study

Laura R. Stroud, Ph.D.,
Department of Psychiatry and Human Behavior, Alpert Medical School, Brown University

George Papandonatos, Ph.D.,
Center for Statistical Sciences, Brown University

Edmond Shenassa, Sc.D.,
Program in Maternal-Child Health, School of Public Health, University of Maryland

Daniel Rodriguez, Ph.D.,
Department of Psychiatry and Human Behavior, Alpert Medical School, Brown University

Raymond Niaura, Ph.D.,
Schroeder Institute for Tobacco Research and Policy Studies, American Legacy Foundation

Kaja LeWinn, Ph.D.,
Department of Psychiatry, University of California, San Francisco

Lewis P. Lipsitt, Ph.D., and
Department of Psychology, Brown University

Stephen L. Buka, Sc.D.
Department of Epidemiology, School of Public Health, Brown University

Abstract

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 1,086 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.

Corresponding Author: Laura R. Stroud, Ph.D., Centers for Behavioral and Preventive Medicine, Department of Psychiatry and Human Behavior, The Miriam Hospital and Alpert Medical School, Brown University. Coro West, Suite 309, 1 Hoppin Street, Providence, RI 02903, Telephone: (401) 793-8194, Fax: (401) 793-8056), lstroud1@lifespan.org.

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Results—Significant bivariate associations emerged for: a) MSDP/cotinine and lifetime ND, and b) 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 daughters' lifetime ND. 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.

Keywords

Maternal smoking during pregnancy; nicotine dependence; cotinine; cortisol; testosterone; programming; glucocorticoid; androgen

INTRODUCTION

Maternal smoking during pregnancy (MSDP) remains a major public health problem. Despite pervasive medical and societal sanctions, thirteen to thirty percent of pregnant mothers continue to smoke in the United States, with highest rates in poor, less educated, underserved mothers (1–3). MSDP 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, disruptive behaviors/conduct disorder in older children and adults (4–8). MSDP 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 may predispose offspring to either positive outcomes or impairments/disease 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, over-exposure 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 over-exposure on physiological 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 HPA 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 may alter fetal dopamine activity leading to increased propensity for ND. Thus, we propose that maternal cortisol levels may 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 following 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 androgen 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). Further, 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=1,086) 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, a 40-year longitudinal follow-up of the Collaborative Perinatal Project.

METHODS AND MATERIALS

Participants and Sample Selection

The Collaborative Perinatal Project (CPP) was a multi-site, 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 New England Family Study (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 1,674 NEFS participants has previously been 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

Following enrollment, CPP mothers completed numerous measures, including socio-demographic and pregnancy characteristics, and cigarette smoking during pregnancy. Non-fasting maternal blood was collected at each prenatal visit. Serum was extracted, and samples were frozen and shipped to a CPP repository in Bethesda, MD. 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 following the last menstrual period and at least 14 days prior to the infant's birth date given known effects of labor/delivery on steroid hormone levels (70–72). Weeks 31–36 were selected because: a) they provided a relatively tight window within third trimester to examine hormone levels; b) they included greatest number of participants with available serum samples; and c) 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

Maternal Smoking during Pregnancy (MSDP)—Pregnant mothers were queried regarding 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 per day. Validity of CPP maternal smoking reports through comparison with serum cotinine levels has been shown to be excellent (kappas=83–87%) (78). Following Kandel and Udry (13), bivariate analyses utilized an ordered categorical MSDP variable: none (did not smoke), low (smoked <15 cigarettes per day), and high (smoked ≥15 cigarettes per day). For multivariate analyses, this 3-level ordinal MSDP measure was recoded using 2 dummy variables contrasting low and high MSDP with no MSDP.

Adult Lifetime Nicotine Dependence (ND)—Adult history of ND was based on Diagnostic and Statistical Manual IVth Edition (DSM IV) criteria (79) and assessed using a modified (80) version of the Composite International Diagnostic Interview (81). Lifetime ND was summarized as a binary variable (non-dependent, nicotine dependent) covering all ages through the adult follow-up interview (*M*=39 years, *SD*=2).

Biological Variables—Cotinine was assayed using liquid chromatography – tandem mass spectrometry (LC-MS/MS) (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 using enzyme-linked immunosorbent assay (ELISA) kits; CBG was measured using radioimmunoassay (laboratory of C. Kirschbaum, University of Duesseldorf; assays described at www.ibl-hamburg.com).. Inter/intra-assay coefficients of

variability ranged from 3–12%. Free Androgen Index (FAI) and Free Cortisol Index (FCI) were calculated from testosterone and SHBG, and cortisol and CBG, respectively (84, 85). (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 socio-economic status (SES; range: 1= lowest through 100=highest) was derived from education (years), occupation (manual, non-manual, unemployed) of the head of household, and household income (based on US poverty threshold at the time) using 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 prior to pregnancy was assessed by maternal report. Gestational age was calculated based on 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 using polychoric, polyserial (ϕ), and Pearson correlations for the full sample (n=1086), stratified by gender (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 grams. A causal steps approach was utilized to test cortisol and testosterone as mediators of the MSDP/offspring ND link (89). This requires significant associations between: a) predictor and outcome, b) predictor and mediator; and c) mediator and outcome adjusted for predictor. Further, it requires attenuation of the predictor-outcome association when the putative mediator is included in the model. Causal steps were tested using multivariate logistic regression analyses in Splus 8.2 (90), using ordinal MSDP followed by maternal cotinine as predictors. Potential confounders were then tested for inclusion in regression models based on 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 median to 3rd 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, 0.2% Hispanic, and 0.5% other. Average gravida was 2 (*SD*=2). Mean composite maternal SES was 56 (*SD*=19), on a 100-point scale. 58% of mothers endorsed smoking during pregnancy; 43% reported smoking during third trimester. Among smokers, average maximum cigarettes per day during pregnancy was 18 (*SD*=11), highly similar to third trimester levels (*M*=18, *SD*=10); mean cotinine levels were 95 ng/ml (*SD*=78, range=1–526).

Adult Offspring—59% were female. 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 grams (*SD*=506); 6% of infants were born low birthweight (<2500 grams). 39% 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 (ϕ's>0.825, p's<.0001). Significant associations between maternal cortisol and testosterone also emerged for both daughters and sons (r's>.222, p's<.0001). For daughters only, increasing MSDP exposure (did not smoke, <15 cigarettes/day, 15+ cigarettes/day; n's=254, 132, 258) and maternal cotinine were associated with increased likelihood of offspring lifetime ND (p's<.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 either MSDP (did not smoke, <15 cigarettes/day, 15+ cigarettes/day, n's=195, 78, 163) or maternal cotinine with offspring ND, or between either cortisol or 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 daughters' lifetime ND (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 3rd quartile of cotinine raised odds of lifetime ND by 12% (OR=1.12, 95% 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 vs. none (n's=110, 218) and high vs. none; (n's=216, 218), was entered into the model along with significant covariates (Step 1), followed by cortisol (FCI) as the mediator (Step 2). Logistic regression coefficients (log odds β) for both steps are presented in Table 2. In Step 1, high MSDP (≥15 cigarettes per day) was associated with an increased likelihood of lifetime ND (β=0.419, SE=.207, p=.043). In Step 2, maternal cortisol was significantly associated with daughters' lifetime ND (β=0.121, SE=.055, p=.029), with increases from the median to the 3rd quartile raising 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=0.409, SE=.207, p=.048). High MDSP 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 daughters' ND before or after adjustment for cortisol (p's>.30).

Although MSDP was positively correlated with cortisol in bivariate analyses (Table 3, 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.

Results do not support maternal cortisol as a mediator of links between MSDP and offspring ND, but suggest that high MSDP and cortisol are independently associated with daughters' lifetime ND. Figure 1 presents path coefficients (log *OR*s, significance levels) for high MSDP and maternal cortisol, with covariate paths omitted for simplicity. Path coefficients were identical when Structural Equation Modeling (SEM) (91) was utilized to simultaneously estimate all paths.

DISCUSSION

The present study provides the first evidence of prenatal glucocorticoid programming of an adult psychiatric disorder, namely, nicotine dependence (ND), among daughters over a 40 year prospective study. Uniquely, this study also highlights two independent and additive prenatal pathways to daughters' ND: elevated prenatal glucocorticoid exposure and elevated maternal smoking during pregnancy (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). Further, roughly equal numbers of female and male offspring allowed us to conduct all analyses stratified by gender.

Increased exposure to maternal prenatal glucocorticoids was associated with a 13% increased odds of daughters' lifetime ND 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 over-exposure to maternal glucocorticoids in programming CNS dysfunction and disease in adult offspring (33, 93). Results also complement an emerging human literature suggesting that over-exposure to prenatal glucocorticoids may 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 per 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 Collaborative Perinatal Project (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). ND 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 health care 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 adolescent daughters' smoking. Because smoking in adolescence may 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 neuroteratogenesis increasing propensity to ND in adulthood (97, 98), whereas other aspects of MSDP may 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 as men have shown slightly increased ND prevalence (99), or sex differences in fetal metabolism as 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 vs. 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 Kandel and Udry's (13) findings, is due to lack of power. Our sample size was large (n=1086), even when stratifying by gender with 80% power to detect small effects: r's=.11 and .13 for daughters and sons; 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 may 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 the majority 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 daughters' ND 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 following gestational exposure to glucocorticoids and MSDP.

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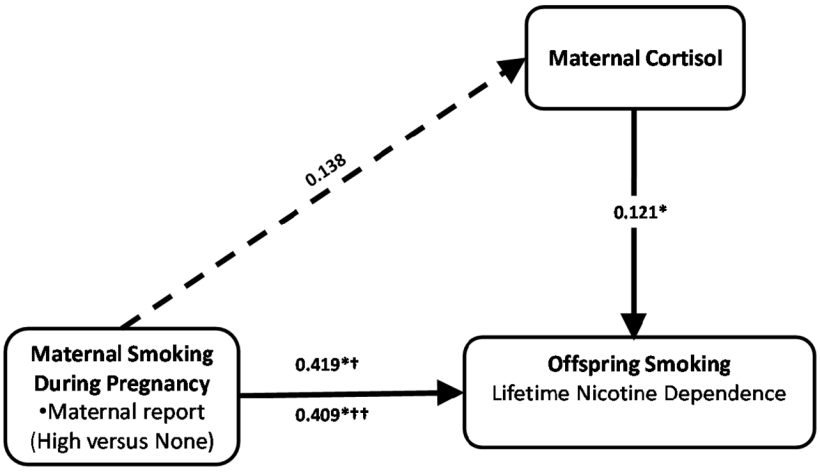

Figure 1.

Prenatal glucocorticoids and maternal smoking independently predict nicotine dependence in adult daughters (*n* =544).

NOTES. Separate regression models were analyzed to derive path coefficients (standard errors) for each endogenous variable (normal linear regression for maternal cortisol, logistic regression for offspring ND). Results were identical using Structural Equation Modeling to simultaneously estimate all path coefficients. Each regression model controlled for maternal race (Caucasian vs. Other), maternal age at delivery (pure quadratic term), gravida (square root transformed), and socioeconomic status (continuous). Maternal smoking included 3 categories: none (did not smoke; *n*=218), low (<15 cigarettes per day; *n*=110), high (15+ cigarettes per day; *n*=216). Dashed line from MSDP to maternal cortisol highlights the non-significance of this path coefficient.

*p<.05

† before including maternal cortisol in the model;

†† after including maternal cortisol in the model.

Table 1

Bivariate associations between maternal smoking, maternal prenatal cotinine, and maternal prenatal testosterone and cortisol levels and with offspring *daughters'* (N= 649) and *sons'* (N= 437) lifetime nicotine dependence.

	Maternal Smoking[a]	Maternal Cotinine[b]	Maternal Cortisol[b]	Maternal Testosterone[b]
Daughters (N=649)				
Maternal Cotinine[b]	**0.856*****	–		
Maternal Cortisol[b]	**0.110***	0.062	–	
Maternal Testosterone[b]	−0.017	−0.032	**0.222*****	–
Adult Offspring Lifetime ND[c]	**0.161****	**0.136****	**0.145***	−0.079
Sons (N=437)				
Maternal Cotinine[b]	**0.825*****	--		
Maternal Cortisol[b]	0.059	0.080	–	
Maternal Testosterone[b]	0.023	−0.030	**0.264*****	--
Adult Offspring Lifetime ND[c]	0.010	0.010	0.053	−0.020

NOTES. Polychoric correlations were estimated between categorical variables, polyserial correlations between categorical and continuous variables, and Pearson correlations between continuous variables.

[a] Maternal smoking included 3 categories: none (did not smoke), low (<15 cigarettes per day), high (15+ cigarettes per day). *N*'s =254, 132, and 258 for daughters; *n*'s=195, 78, and 163 for sons.

[b] Maternal cotinine is the logarithm of maternal cotinine levels. Maternal cortisol is the logarithm of the free cortisol index (FCI). Maternal testosterone is the logarithm of the free androgen index (FAI).

[c] Adult lifetime nicotine dependence included: nicotine dependent, non-nicotine dependent.

*** $p<.0001$;

** $p<.001$;

* $p<.05$.

Table 2

Parameter estimates for regression analyses to test mediation models linking MSDP, maternal prenatal cortisol, and offspring nicotine dependence in daughters (n=544[a]).

Maternal smoking to daughter's lifetime nicotine dependence (n=544) [b]				
Step 1				
	β	SE β	*z*	*p*
Constant	−1.554	0.382	−4.068	<0.001
Gravida[c]	0.199	0.049	4.044	<0.001
Maternal age at delivery[d]	0.080	0.032	2.490	0.013
Maternal race (White vs. Other)	0.798	0.343	2.328	0.020
Maternal SES	−0.135	0.067	−2.022	0.043
Maternal smoking (low vs. none)[e]	0.239	0.250	0.954	0.340
Maternal smoking (high vs. none)[f]	**0.419**	**0.207**	**2.028**	**0.043**
Step 2				
	β	SE β	*z*	*p*
Constant	−1.512	0.385	−3.926	<0.001
Gravida[c]	0.200	0.050	4.048	<0.001
Maternal age at delivery[d]	0.076	0.033	2.334	0.020
Maternal race (White vs. Other)	0.795	0.345	2.307	0.021
Maternal SES	−0.110	0.068	−1.627	0.104
Maternal smoking (low vs. none)[e]	0.217	0.252	0.863	0.388
Maternal smoking (high vs. none)[f]	**0.409**	**0.207**	**1.974**	**0.048**
Maternal Cortisol (FCI)[g]	**0.121**	**0.055**	**2.177**	**0.029**

NOTES. In Step 1, significant covariates followed by maternal cigarettes smoked per day (low vs. none, and high vs. none) were entered. In Step 2, significant covariates followed by both level of maternal cigarettes per day and maternal cortisol were entered into the model.

[a] Sample is restricted to gestational age ≥ 37 weeks and birthweight ≥ 2500 grams.

[b] Adult lifetime nicotine dependence: (1=nicotine dependent, 0=non-nicotine dependent).

[c] Number of prior pregnancies (square root)

[d] Maternal age in years centered at 35 and scaled by 5 (pure quadratic term)

[e] Sample sizes for the dummy low versus no cigarettes smoked per day during pregnancy variable (dummy coded): low, n=110; none, n=218.

[f] Sample sizes for high versus no cigarettes smoked per day during pregnancy variable (dummy coded): high, n=216; none n=218.

[g] Maternal cortisol (logarithm of the free cortisol index (FCI)).

Table 3

Parameter estimates for normal regression analysis predicting maternal prenatal cortisol (n=544[a]).

Maternal Smoking to Maternal Cortisol (FCI; n=544)[b]				
	β	**SE β**	***z***	***p***
Constant	−0.586	0.283	−2.073	0.039
Gravida[c]	0.016	0.037	0.436	0.663
Maternal age at delivery[d]	0.051	0.025	2.034	0.042
Maternal race (White vs. Other)	0.140	0.256	0.546	0.585
Maternal SES	−0.214	0.053	−4.048	<0.001
Maternal smoking (low vs. none)[e]	0.191	0.199	0.962	0.337
Maternal smoking (high vs. none)[f]	0.138	0.165	0.837	0.403

[a] Sample is restricted to gestational age ≥ 37 weeks and birthweight ≥ 2500 grams.

[b] Maternal cortisol (logarithm of the free cortisol index (FCI)).

[c] Number of prior pregnancies (square root)

[d] Maternal age in years centered at 35 and scaled by 5 (pure quadratic term)

[e] Sample sizes for the dummy low versus no cigarettes smoked per day during pregnancy variable (dummy coded): low, *n*=110; none, *n*=218.

[f] Sample sizes for high versus no cigarettes smoked per day during pregnancy variable (dummy coded): high, *n*=216; none *n*=218.