Prenatal Effects of Maternal Smoking on Daughters' Smoking: Nicotine or Testosterone Exposure?

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ABSTRACT

Objectives. The purpose of this study was to specify the effect of prenatal fetal exposure to maternal cotinine and testosterone on daughters' smoking in adolescence and adulthood.

Methods. Longitudinal causal models were estimated among 240 White mother–daughter pairs from the Child Health and Development Study. Mothers and daughters were reinterviewed when daughters were aged 15 to 17 years, and daughters were interviewed at 27 to 30 years of age. Blood samples were obtained from both parents during pregnancy and from adult daughters.

Results. Testosterone and smoking were positively correlated among mothers during their pregnancy and among adult daughters. Maternal prenatal cotinine had no direct effect on daughters' smoking; self-reported smoking in pregnancy did have a direct effect. Smoking among daughters during adolescence was determined by maternal prenatal testosterone and self-reported maternal smoking during pregnancy and postnatally. Smoking among adult daughters reflected chronic smoking since adolescence and the continuing effect of postnatal maternal smoking. Prenatal maternal testosterone affected adult daughters' testosterone.

Conclusions. Estimates of the impact of prenatal maternal smoking depend on the measure of smoking. Prenatal testosterone exposure is a previously unrecognized risk factor for smoking among female offspring. (*Am J Public Health.* 1999;89:1377–1383)

We have found in 2 cohorts, even after controlling for postnatal maternal smoking, that female adolescents are more likely to smoke if their mothers smoked during pregnancy.[1] We have found no such effect for male offspring. In both samples, maternal smoking in pregnancy was ascertained from maternal self-reports subsequent to pregnancy. The average recall periods were 3 years in one cohort and less than 1 year in the other. Effects of self-reported prenatal smoking on smoking by 10-year-old offspring have also been reported.[2] Because of potential biases in self-reports, a more direct test of the prenatal smoking effect requires biological indicators of maternal smoking during pregnancy.

Data are also needed on postnatal maternal smoking to control for children's modeling of their mothers. The opportunity arose to obtain such data in a longitudinal sample. Although not assayed for cotinine, blood sera were collected in pregnancy, and smoking histories were subsequently obtained from mothers and daughters during the daughters' adolescence and from daughters in adulthood. Furthermore, the prenatal sera had been assayed for testosterone, making it possible to investigate the role of prenatal exposure to testosterone in smoking by female offspring.

Kandel et al. hypothesized that one mechanism underlying the effect of prenatal smoking on offspring might be the impact of nicotine or nicotine breakdown products on the developing brain of the fetus.[1] Maternal nicotine crosses the placental barrier, and fetal cotinine levels reach about 90% of maternal levels.[3,4] Nicotine stimulates the action of cholinergic neurons, increases nicotinic receptors in the brain,[5–7] and enhances activity in dopaminergic systems involved in addictive behavior.[8–11] Such findings raise the possibility that during a critical prenatal period of brain development, nicotine might modify the dopaminergic system and change the threshold of this system, or related systems in the brain, to the effects of nicotine later in life. Fetal exposure to passive smoking could also be relevant.

The Kandel et al. hypothesis has to be evaluated in the context of findings from behavior genetic studies that smoking is influenced by genetic factors,[12,13] as well as the possibility that prenatal exposure to compounds other than nicotine also affects offspring smoking. Testosterone might be one such factor. In male and female adolescents, higher testosterone levels have been observed among smokers than among nonsmokers.[14] Among adults, inconsistent associations between testosterone and smoking have been reported for men,[15,16] while small-sample studies show no relationship in (nonpregnant) premenopausal women.[17,18] Smoking appears to have an anti-estrogenic effect in women.[19] Male sex hormones affect neuronal brain structures and functions that contribute to sex differences in behavior.[20] Udry et al.[21] found that mothers' prenatal testosterone influenced daughters' later gendered behavior.

Prenatal exposure may also lead to lifelong higher testosterone levels that may have other consequences, such as higher novelty and sensation seeking[22,23] and stress sensitivity.[24] In female fetuses, in contrast to male fetuses, testosterone levels are determined by maternal levels. Maternal testosterone may affect the central nervous system of females in utero and thus increase daughters' likelihood of smoking. In addition, mothers' testosterone levels may be related to their own

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smoking and may affect daughters' smoking indirectly. Adolescent testosterone has been found to interact with maternal smoking in predicting increasing rates of smoking by female, but not male, adolescents.[25]

Thus, offspring smoking may be determined by prenatal exposure to cotinine, prenatal exposure to testosterone, or a genetic predisposition. The genetic hypothesis could not be tested with our data.

Methods

Data and Procedures

The sample included 471 White women and their daughters (born in 1960–1963) from the Child Health and Development Study.[26] The women received prenatal care at the Kaiser Foundation Health Plan clinic in the California San Francisco Bay Area. During pregnancy, maternal blood samples were taken at each trimester; 1 sample was obtained from fathers. Mothers and children were reinterviewed when the children were aged 9 to 11 and 15 to 17 years (n = 2020). Adolescent daughters' interviews and prenatal sera were available for 471 of 669 White mothers. In 1990–1991, Udry reinterviewed 351 daughters when they were aged 27 to 30 years (74.5% completion rate) and obtained blood samples from 240. Udry et al.[21] assayed the prenatal maternal sera and the adult daughters' sera for testosterone and sex hormone binding globulin. On average, mothers were aged 29 years at delivery and 45.4 years at the initial adolescent interview; the mean age of adolescents was 16.4 years.

Attrition from birth slightly biased the adolescent sample through disproportionate loss of daughters from families of low socioeconomic status.[26] There were no significant differences between adolescent offspring reinterviewed in adulthood who provided sera and those not reinterviewed (data not presented).

In 1990, Udry obtained the prenatal specimens from the National Cancer Institute Frederick Cancer Research Facility in Frederick, Md, and stored them at –20°C at the University of North Carolina until the assay was conducted. Numbers of specimens by trimester were as follows: trimester 1, 453; trimester 2, 377; and trimester 3, 391. In 1997, the sera were assayed for cotinine, the major metabolite of nicotine. Cotinine circulates in the blood, and cotinine concentration is considered the best indicator of smoking.[27–29]

Other variables included concurrent maternal reports of smoking in pregnancy, self-reports of current smoking by mothers and lifetime and current smoking by daughters when the daughters were aged 15 to 17 years, daughters' current smoking at ages 27 to 30 years, and maternal education (less than eighth grade, 8–12 years or trade school but not high school graduate, high school graduate, high school graduate plus additional training, some college, college graduate). Daughters' lifetime smoking was not ascertained in adulthood.

Quality of Cotinine Assays

Udry et al.[21] documented that the means and distributions of the testosterone and sex hormone binding globulin assays conducted in 1990–1991 were well within the previously published range for expected values at each trimester. The cotinine assays were conducted in 1997 by Dr Nancy Haley at the laboratories of Metropolitan Life Insurance; radioimmunoassay was used,[30] modified by the method of Langone et al.[31] Sodium was not controlled. All sera were assayed twice. The detection limit was 2 ng/mL; the upper limit was 480 ng/mL. The coefficient of variation was less than 2 ng/mL. Sera could not be assayed for 5 women.

A major issue concerns the quality of the assays performed on blood sera aged 34 to 37 years. Evidence from other studies, consultation with experts on nicotine metabolism, evaluation of the samples by Dr Haley, and results of the analysis indicate that the assays were more than adequate to characterize the smoking status of the pregnant women in the sample. Among mothers who smoked throughout their pregnancy, cotinine values were slightly higher in trimester 2 (244 ng/mL) than in trimesters 1 and 3 (233 ng/mL and 218 ng/mL, respectively). The mean cotinine value was 331 ng/mL (SD = 154) for fathers with values above the cutoff point recommended to identify active smokers (i.e., 14 ng/mL or higher).[29,32]

Nine percent of mothers reported smoking 1 to 4 cigarettes per day, 25% reported 5 to 9, 16% reported 10 to 14, 5% reported 15 to 19, 39% reported 20 to 29, 3.5% reported 30 to 39, and 3.5% reported 40 to 60. Discrepancies between maternal self-reported smoking and cotinine levels ranged from 4% in trimester 1 to 8% in trimesters 2 and 3 and were almost evenly divided between (1) those who self-reported as smokers and whose cotinine levels were below 14 ng/mL and (2) those who self-reported as nonsmokers and whose cotinine levels were above 14 ng/mL. The mean cotinine levels of the self-acknowledged maternal smokers were higher by about 50% than those reported for sera collected from 1964 to 1967 Child Health and Development Study cohorts.[33]

We attempted a comparison of cotinine levels with levels reported by others for fresh sera of smokers. Precise comparisons were precluded owing to lack of detailed information in most studies; noncomparability of samples with regard to sex, pregnancy status, age, and ethnicity; and lack of control for trimester of pregnancy. The present values were lower by about 10% than those observed among Scottish women,[34] similar to those of a mixed sex and racial/ethnic sample,[35] and higher than those of a sample of pregnant women.[36] While there probably is some degradation of serum cotinine over time, the levels among the Child Health and Development Study smokers are well above the 14-ng/mL cutoff value. Furthermore, as discussed later, serum values are highly related to self-reported number of cigarettes smoked per day. Misclassification of mothers regarding their smoking status appears to be very low.

Statistical Analyses

Nonsaturated causal models were estimated via LISREL[37] to assess the direct and indirect paths linking the variables of interest: the effects of fetal exposure to maternal smoking and testosterone and of adolescent exposure to maternal smoking on daughters' smoking in adolescence and adulthood. Maximum-likelihood techniques were used in estimating parameters of regression equations. Goodness of fit was assessed with a standard nested χ^2 test. The goodness-of-fit index was adjusted for degrees of freedom relative to the number of variables. Values greater than 0.90 are considered adequate. A Wald test was used in testing regression slopes (and paths) for significance.

LISREL has several advantages over other methods: it can include a mixture of types of correlations, it estimates correlated errors, and it represents a full information method in which all parameters and standard errors are estimated simultaneously. Polychoric, polyserial, and Pearson correlations, as appropriate, were entered by PRELIS in the matrices. Two models were estimated, with cotinine levels and maternal self-reported smoking in pregnancy used as measures of fetal nicotine exposure, for the 227 dyads in which the daughters were reinterviewed in adulthood and for whom there were complete data on the relevant variables. Maternal education was included in the models.

There is no experimental literature to establish the period in pregnancy during which the human fetal brain is most sensitive to cotinine and testosterone. It has been concluded, by extrapolation from animal experiments, that sexual differentiation of

TABLE 1—Correlations Between Trimester-Specific Prenatal Maternal Cotinine and Testosterone Levels: Child Health and Development Study (n≥327)

	Trimester 1 Cotinine	Trimester 2 Cotinine	Trimester 3 Cotinine	Trimester 1 Testosterone	Trimester 2 Testosterone	Trimester 3 Testosterone
Trimester 2 cotinine	0.85****					
Trimester 3 cotinine	0.86****	0.88***				
Trimester 1 testosterone	0.11**	0.17****	0.07			
Trimester 2 testosterone	0.25****	0.21****	0.16***	0.54****		
Trimester 3 testosterone	0.08*	0.13**	0.12**	0.44****	0.74****	

*P<.10; **P<.05; ***P<.01; ****P<.001.

the human brain by testosterone starts in the early midtrimester of gestation.[38] Because of changes in fetal brain development in trimester 2, analyses were based on trimester 2 exposure. Trimester 1 cotinine and testosterone values were substituted among the 20% of mothers for whom trimester 2 values were missing. Complete data were available for 461 mother–adolescent dyads and 323 adult daughters, including 240 with testosterone serum assays.

Results

Prevalence of Smoking Among Mothers and Daughters

Of the mothers, 44.7% had cotinine levels above the cutoff point of 14 ng/mL or lower in at least 1 trimester; in trimester 2, 40.2%—the same percentage that reported smoking during their pregnancy—had levels that exceeded the cutoff point. A lower proportion (32.5%) reported smoking when their daughters were adolescents. Among daughters, 29.0% had smoked sometime in their lifetime, and 15.4% were smoking when they were aged 15 to 17 years; 20.9% were smoking 12 years later.

Prenatal Maternal Smoking, Cotinine, and Testosterone

There was a high association between maternal self-reported smoking in pregnancy and serum cotinine levels in each trimester (r = 0.78–0.79). Cotinine values increased as a function of self-reported level of smoking. In trimester 2, these values were 8 ng/mL for nonsmokers, 161.9 ng/mL for mothers smoking fewer than 10 cigarettes per day, 267.9 ng/mL for those smoking 1 pack per day, and 352 ng/mL for those smoking 2 or more packs per day.

Maternal serum cotinine values were little influenced by fathers' smoking. Maternal self-reports of smoking were cross tabulated against fathers' cotinine levels, with the 14-ng/mL cutoff point used to identify smokers. In trimester 2, maternal values were 4.7 ng/mL when neither mothers nor fathers smoked, 9.7 ng/mL when fathers smoked but mothers did not, 209.5 ng/mL when mothers smoked and fathers did not, and 211.4 ng/mL when both smoked. Passive exposure to fathers' smoking was not considered to be a potential determinant of offspring smoking.

There was a significant relationship, especially in trimester 2, between maternal cotinine and testosterone levels (Table 1). Across the pregnancy, there was more stability in cotinine than testosterone levels. There was no relationship between maternal cotinine and sex hormone binding globulin in any trimester (data not shown).

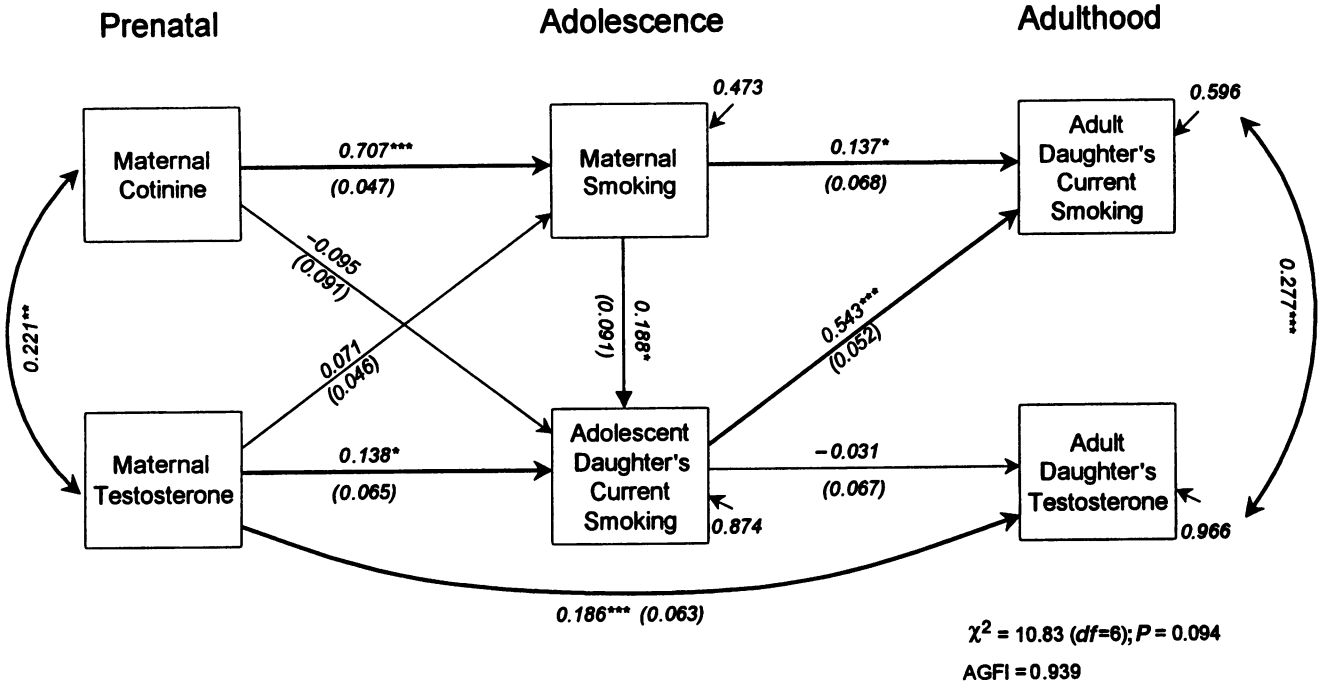
Maternal Prenatal Cotinine and Testosterone Levels and Daughters' Smoking

Three dichotomous measures of daughters' smoking were examined as a function of continuous measures of maternal prenatal

TABLE 2—Correlations of Trimester 2 (T2) Maternal Prenatal Cotinine and Testosterone Levels With Self-Reported Prenatal Smoking and Daughters' Smoking in Adolescence and Adulthood: Child Health and Development Study

	T2 Maternal Cotinine	T2 Maternal Testosterone	Adolescent Daughters' Lifetime Smoking	Adolescent Daughters' Current Smoking	Adult Daughters' Current Smoking
Mother					
T2 testosterone[a] (n = 441)	0.15**				
Self-reported prenatal smoking[b] (n = 441)	0.77**	0.12**	0.09	0.18**	0.33**
Daughter					
Adolescent lifetime smoking[b] (n = 441)	0.04	0.14**			
Adolescent current smoking[b] (n = 441)	0.13**	0.18**	0.68**[c]		
Adult current smoking[b] (n = 325)	0.19**	0.12*	0.57**	0.59**	
Adult testosterone[a] (n = 231)	0.02	0.16*	–0.10	–0.03	0.23**

Note. PRELIS was used to estimate polychoric correlations between categorical variables and polyserial correlations between categorical and continuous variables. Cases missing serum data in trimester 2 were assigned trimester 1 values.
[a]Continuous variable.
[b]Categorical variable: mother, 3 categories (did not smoke, smoked fewer than 15 cigarettes per day, smoked 15 or more cigarettes per day); daughter, 2 categories (did not smoke, smoked).
[c]Pearson r; iteration of polychoric correlation did not converge because of zero value in the never-smoked cell.
*P<.05; **P<.01.



Note. Mother's education is controlled for but not shown. Coefficients are standardized; standard errors are shown in parentheses. Correlated errors between mother's smoking and adult daughter's smoking are estimated but not shown. AGFI = adjusted goodness-of-fit index.
*P<.05; **P<.01; ***P<.001.

FIGURE 1—Predictors of daughters' smoking in adolescence and adulthood (n=227): model including maternal cotinine levels in pregnancy.

cotinine and testosterone levels: lifetime and current smoking at 15 to 17 years of age and smoking at 27 to 30 years of age (Table 2). Statistically significant zero-order associations were observed between each prenatal biological measure and the measures of daughters' smoking with the exception of lifetime smoking in adolescence, which was unrelated to cotinine levels. Adult daughters' testosterone levels were related to maternal prenatal testosterone and daughters' current smoking.

Predictors of Smoking by Daughters in Adolescence and Adulthood

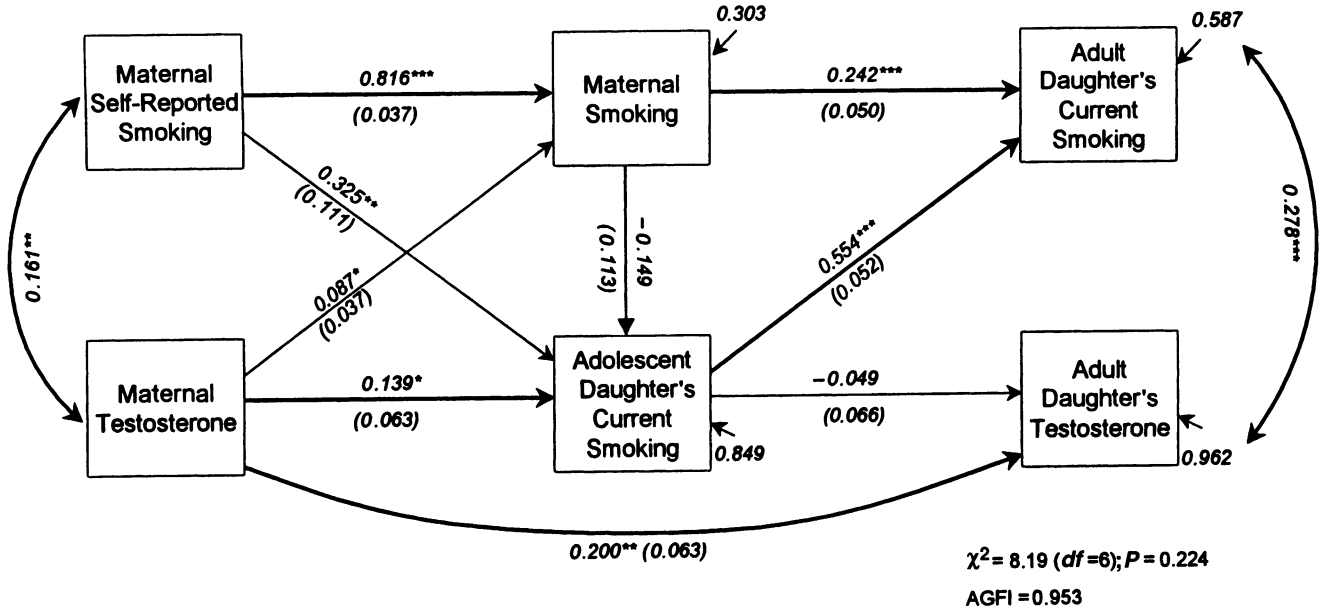
Nonsaturated causal models were estimated to identify the direct and indirect effects of fetal exposure to maternal nicotine and testosterone on daughters' smoking in adolescence and adulthood. We assumed that maternal nicotine and testosterone directly affected daughters' smoking in adolescence but had only indirect effects on their smoking in adulthood (through maternal smoking and daughters' smoking in adolescence). A 3-category measure of maternal smoking when the daughter was an adolescent was entered to control for the modeling effect of mothers by daughters.

Adult daughters' testosterone was assumed to be affected by prenatal exposure to maternal testosterone and to be correlated with their adult smoking. One model included cotinine levels as a measure of prenatal nicotine exposure (Figure 1); another included mothers' self-reported smoking during their pregnancy (Figure 2). The models were reestimated to include a correlated error suggested by modification indexes. For simplification, the paths from maternal education and the correlated errors other than that between adult daughters' current smoking and testosterone are not shown.

Exposure to prenatal maternal cotinine had no direct effect on daughters' smoking (Figure 1). Prenatal maternal testosterone had a direct effect on daughters' smoking in adolescence, an indirect effect on daughters' smoking in adulthood (through its effects on adolescent smoking), and a direct effect on adult daughters' testosterone. There was a highly significant path from prenatal maternal cotinine level to maternal smoking during the daughters' adolescence, which was a significant predictor of the daughters' smoking in adolescence and adulthood. The strongest predictor of an adult daughter's current smoking was her smoking in adolescence. For daughters, as for mothers, there was strong continuity of smoking over time.

Maternal prenatal cotinine and testosterone were positively correlated. Similarly, there was a significant correlation (P<.01) between adult daughters' smoking and testosterone. There was a significant negative effect of maternal education on daughters' smoking in adolescence (standardized path coefficient=–0.276, P<.001) but no effect on mothers' own smoking during their daughters' adolescence over and beyond the correlation between smoking and education observed in pregnancy. Including a correlation between the error terms for mothers' smoking during daughters' adolescence and daughters' smoking in adulthood improved the fit of the model (data not shown).

In contrast to prenatal cotinine, maternal self-reported prenatal smoking had a significant direct positive effect on daughters' smoking in adolescence (Figure 2). In addition, maternal smoking during daughters' adolescence no longer had an effect on daughters' smoking in adolescence. Surprisingly,



Note. Mother's education is controlled for but not shown. Coefficients are standardized; standard errors are shown in parentheses. Correlated errors between mother's smoking and adult daughter's smoking are estimated but not shown. AGFI = adjusted goodness-of-fit index.
*$P<.05$; **$P<.01$; ***$P<.001$.

FIGURE 2—Predictors of daughters' smoking in adolescence and adulthood (n = 227): model including maternal self-reported smoking in pregnancy.

the time-lagged effect of maternal smoking on daughters' smoking in adulthood was stronger than the contemporaneous effect in adolescence. The effects of prenatal testosterone on daughters' smoking in adolescence and on testosterone levels in adulthood remained unchanged. However, the path from prenatal maternal testosterone to maternal smoking during the daughters' adolescence became significant.

Discussion

This study replicated previous findings of an association between self-reported prenatal maternal smoking and smoking by adolescent daughters.[1] However, the hypothesis that fetal exposure to maternal cotinine during pregnancy increases the liability of female offspring to smoking was not confirmed with biological assays of cotinine concentration in prenatal sera. (Despite the length of time that elapsed from collection to assays of the sera, the evidence suggests that the quality of the samples was good.) Prenatal exposure to maternal testosterone emerged as a significant predictor of daughters' smoking in adolescence and their testosterone levels in adulthood. Among both mothers and daughters, testosterone was significantly correlated with smoking. Maternal smoking during the daughters' adolescence was a consistently strong predictor of daughters' smoking in adolescence and adulthood. Daughters' adult smoking was also predicted by their adolescent smoking.

Results regarding the effect of prenatal maternal smoking on offspring smoking appear to depend on the measure of maternal smoking. We speculate that the seemingly stronger effect of self-reported smoking than of cotinine serum levels may result in part from a lack of exact correspondence between cotinine levels in sera and in the brain. Furthermore, nicotine metabolites other than cotinine may contribute to the differential associations of cotinine levels and self-reported smoking. Most studies of the behavioral consequences of fetal exposure to maternal smoking are based on maternal self-reports[39,40] and may exaggerate the effects of prenatal cotinine exposure.

The present findings also suggest that the risk factor indexed by maternal smoking in pregnancy may not be cotinine but testosterone in mothers and offspring. The mechanisms underlying the relationships of prenatal testosterone with mothers' and daughters' smoking remain to be elucidated. Nicotine and cotinine have been reported (with an exception[41]) to affect hormone synthesis and metabolism and to decrease testosterone levels in animals.[42–45] Thus, the relationship between testosterone and nicotine is somewhat paradoxical. Studies of general population samples show, as found here, that at any particular point in time smokers tend to have higher testosterone levels than nonsmokers.[14,25,45] However, inconsistent findings have been reported in studies based on clinical or selected samples.[46] Whether intrauterine fetal exposure to nicotine has any effect on offspring through testosterone in the mother and child remains to be determined for both female and male offspring.

The persistence of the effect of maternal smoking during daughters' adolescence on daughters' smoking in adulthood (after control for adolescent daughters' smoking) and its stronger effect on daughters' smoking in adulthood than in adolescence (when prenatal smoking was indexed by maternal self-reports) were unexpected. We would expect the maternal role modeling effect to be

stronger contemporaneously than 12 years later, especially when adolescents reside with their parents and initiation into smoking takes place. Maternal smoking during daughters' adolescence may index genetic and socialization effects. However, our data do not allow us to examine to what extent and by what mechanism mother-to-child transmission of smoking is genetically mediated.

This study has shown a novel association between fetal prenatal exposure to maternal testosterone and subsequent smoking by adolescent female offspring. Data based on biological assays of prenatal cotinine do not provide support for the initial hypothesis, based on self-reports of smoking, that exposure to intrauterine maternal nicotine directly increases the risk of smoking among female offspring. Correspondence between cotinine levels in blood and in the brain needs to be better established, and the effects of nicotine or cotinine on testosterone and the role of smoke compounds and nicotine metabolites other than cotinine need to be better understood, before the hypothesis of an effect of exposure to prenatal maternal nicotine on offspring smoking is discarded. □

Contributors

Both D. B. Kandel and J. R. Udry planned, supervised, and participated in the data analysis and wrote the paper. Prenatal maternal and paternal blood sera had earlier been obtained by J. R. Udry from the Child Health and Development Study, which also provided the prenatal and adolescent interview data for mothers and adolescent daughters. J. R. Udry, who reinterviewed the daughters as adults, had assayed the prenatal maternal and adult daughters' blood samples for testosterone. D. B. Kandel arranged for the cotinine assays of the prenatal sera.

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References

1. Kandel DB, Wu P, Davies M. Maternal smoking during pregnancy and smoking by adolescent daughters. *Am J Public Health*. 1994;84: 1407–1413.
2. Cornelius MD, Leech SL, Zuo Y, Day NL. Substance use risk factors: a birth cohort of preadolescents. In: *Problems on Drug Dependence, 1997: Proceedings of the 59th Annual Scientific Meeting,* Nashville, Tenn, June 14–19, 1997: Bethesda, Md: National Institute on Drug Abuse and College on Problems of Drug Dependence Inc; 1998:136. NIDA Research Monograph 178.
3. Donnenfeld AE, Pulkkinen A, Palomaki GE, Knight GJ, Haddow JE. Simultaneous fetal and maternal cotinine levels in pregnant women smokers. *Am J Obstet Gynecol*. 1993;168: 781–782.
4. Lambers DS, Clark KE. The maternal and fetal physiologic effects of nicotine. *Semin Perinatol*. 1996;20:115–126.
5. Benwell ME, Balfour DJ, Anderson JM. Evidence that tobacco smoking increases the density of (–)-[3H]nicotine binding sites in human brain. *J Neurochem*. 1988;50:1243–1247.
6. Collins AC. Genetic influences on tobacco use: a review of human and animal studies. *Int J Addict*. 1990–1991;25:35–55.
7. Marks MJ, Pauly JR, Gross SD, et al. Nicotine binding and nicotine receptor subunit RNA after chronic nicotine treatment. *J Neurosci*. 1992;12:2765–2784.
8. Mereu G, Yoon KW, Boi V, Gessa GL, Naes L, Westfall TC. Preferential stimulation of ventral tegmental area dopaminergic neurons by nicotine. *Eur J Pharmacol*. 1987;141:395–399.
9. Corrigall WA. Understanding brain mechanisms in nicotine reinforcement. *Br J Addict*. 1991;86:507–510.
10. Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol Sci*. 1992;13:177–184.
11. Nestler EJ. Molecular mechanisms of drug addiction. *J Neurosci*. 1992;12:2439–2450.
12. Collins AC, Marks MJ. Chronic nicotine exposure and brain nicotine receptors—influence of genetic factors. *Prog Brain Res*. 1989;79: 137–146.
13. Heath AC, Madden AF. Genetic influences on smoking behavior. In: Turner JR, Cardon LR, Hewitt JK, eds. *Behavior Genetic Approaches in Behavioral Medicine*. New York, NY: Plenum Press; 1995:45–66.
14. Bauman KE, Foshee VA, Koch GG, Haley NJ, Downton MI. Testosterone and cigarette smoking in early adolescence. *J Behav Med*. 1989; 12:425–433.
15. Dai WS, Gutai JD, Kuller LH, Cauley JA. Cigarette smoking and serum sex hormones in men. *Am J Epidemiol*. 1988;128:796–805.
16. Matzkin H, Soloway MS. Cigarette smoking: a review of possible associations with benign prostatic hyperplasia and prostate cancer. *Prostate*. 1993;22:277–290.
17. Daniel M, Martin AD, Faiman C. Sex hormones and adipose tissue distribution in premenopausal cigarette smokers. *Int J Obes Relat Metab Disord*. 1992;16:245–254.
18. Thomas EJ, Edridge W, Weddell A, McGill A, McGarrigle HG. The impact of cigarette smoking on the plasma concentrations of gonadotrophins, ovarian steroids and the androgens and upon the metabolism of oestrogens in the human female. *Hum Reprod*. 1993;8:1187–1193.
19. Baron JA, La Vecchia C, Levi F. The antiestrogenic effect of cigarette smoking in women. *Am J Obstet Gynecol*. 1990;162:502–514.
20. McEwen BS. How do sex and stress hormones affect nerve cells? *Ann N Y Acad Sci*. 1994;743: 1–16.
21. Udry JR, Morris NM, Kovenock J. Androgen effects on women's gendered behavior. *J Biosoc Sci*. 1995;27:359–368.
22. Daitzman R, Zuckerman M. Disinhibitory sensation seeking, personality and gonadal hormones. *Pers Individual Differences*. 1980;1: 103–110.
23. Zuckerman M. Good and bad humors: biochemical bases of personality and its disorders. *Psychol Sci*. 1995;6:325–332.
24. Piazza PV, Deroche V, Deminière JM, Maccari S, Le Moal M, Simon H. Corticosterone in the range of stress-induced levels possesses reinforcing properties: implications for sensation-seeking behaviors. *Proc Natl Acad Sci U S A*. 1993;90:11738–11742.
25. Bauman KE, Foshee VA, Haley NJ. The interaction of sociological and biological factors in adolescent cigarette smoking. *Addict Behav*. 1992;17:459–467.
26. van den Berg BJ, Christianson RE, Oechsli FW. The California Child Health and Development Studies of the School of Public Health, University of California at Berkeley. *Pediatr Perinat Epidemiol*. 1988;2:265–282.
27. Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. *Epidemiol Rev*. 1996;18:188–204.
28. Jacob P, Benowitz NL, Shulgin AT. Recent studies of nicotine metabolism in humans. *Pharmacol Biochem Behav*. 1988;30:249–253.
29. Jarvis MJ, Tunstall-Pedoe H, Feyerabend C, Vesey C, Saloojee Y. Comparison of tests used to distinguish smokers from nonsmokers. *Am J Public Health*. 1987;77:1435–1438.
30. Haley NJ, Axelrad CM, Tilton KA. Validation of self-reported smoking behavior: biochemical analyses of cotinine and thiocyanate. *Am J Public Health*. 1983;73:1204–1207.
31. Langone JJ, Gjika HB, Van Vunakis H. Nicotine and its metabolites: radioimmunoassays for nicotine and cotinine. *Biochemistry*. 1973;12: 5025–5030.
32. Cummings SR, Richard RJ. Optimum cutoff points for biochemical validation of smoking status. *Am J Public Health*. 1988;78:574–575.
33. English PB, Eskenazi B, Christianson RE. Black-White differences in serum cotinine levels among pregnant women and subsequent effects on infant birthweight. *Am J Public Health*. 1994;84:1439–1443.
34. Woodward M, Tunstall-Pedoe H, Smith WC, Tavendale R. Smoking characteristics and inhalation biochemistry in the Scottish population. *J Clin Epidemiol*. 1991;44:1405–1410.
35. Wagenknecht LE, Burke GL, Perkins LL, Haley NJ, Friedman GD. Misclassification of smoking status in the CARDIA study: a comparison of self-report with serum cotinine levels. *Am J Public Health*. 1992;82:33–36.
36. Haddow JE, Knight GJ, Palomaki GE, Kloza EM, Wald NJ. Cigarette consumption and serum cotinine in relation to birthweight. *Br J Obstet Gynaecol*. 1987;94:678–681.
37. Jöreskog KG, Sörbom D. *LISREL 7 User's Reference Guide*. Mooresville, Ind: Scientific Software Inc; 1989.
38. Pilgrim C, Reisert I. Differences between male and female brains—developmental mecha-

nisms and implications. *Horm Metab Res.* 1992;24:353–359.
39. Milberger S, Biederman J, Faraone SV, Chen L, Jones J. Is maternal smoking during pregnancy a risk factor for attention deficit hyperactivity disorder in children? *Am J Psychiatry.* 1996; 153:1138–1142.
40. Wakschlag LS, Lahey BB, Loeber R, Green SM, Gordon RA, Leventhal BL. Maternal smoking during pregnancy and the risk of conduct disorder in boys. *Arch Gen Psychiatry.* 1997;54:670–676.
41. Itsvan JA, Buist AS, Hess DL, Voelker H. Relationship of smoking cessation and nicotine gum use to salivary androstenedione and testosterone in middle-aged men. *Metabolism.* 1995; 44:90–95.
42. Meikle AW, Liu XH, Taylor GN, Stringham JD. Nicotine and cotinine effects on 3 alpha hydroxysteroid dehydrogenase in canine prostate. *Life Sci.* 1988;43:1845–1850.
43. Patterson TR, Stringham JD, Meikle AW. Nicotine and cotinine inhibit steroidogenesis in mouse Leydig cells. *Life Sci.* 1990;46:265–272.
44. Yeh J, Barbieri RL, Friedman AJ. Nicotine and cotinine inhibit rat testis androgen biosynthesis in vitro. *J Steroid Biochem.* 1989;33:627–630.
45. Yardimci S, Atan A, Delibasi T, Sunguroglu K, Guven MC. Long-term effects of cigarette-smoke exposure on plasma testosterone, luteinizing hormone and follicle-stimulating hormone levels in male rats. *Br J Urol.* 1997; 79:66–69.
46. Attia AM, el-Dakhly MR, Halawa FA, Ragab NF, Mossa MM. Cigarette smoking and male reproduction. *Arch Androl.* 1989;23:45–49.