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Intrauterine Exposure to Cigarette Smoke Is Associated with Increased Ghrelin Concentrations in Adulthood

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Key Words
Cigarette smoke · Depression · Energy metabolism · Epigenetics · Ghrelin · Intrauterine exposure · Nicotine

Abstract
Background: The appetite-stimulating hormone ghrelin is a fundamental regulator of human energy metabolism. A series of studies support the notion that long-term appetite and weight regulation may be already programmed in early life and it could be demonstrated that the intrauterine environment affects the ghrelin system of the offspring. Animal studies have also shown that intrauterine programming of orexigenic systems persists even until adolescence/adulthood. ***Methods:*** We hypothesized that plasma ghrelin concentrations in adulthood may be associated with the intrauterine exposure to cigarette smoke. We examined this hypothesis in a sample of 19-year-olds followed up since birth in the framework of the Mannheim Study of Children at Risk, an ongoing epidemiological cohort study of the long-term outcome of early risk factors. ***Results:*** As a main finding, we found that ghrelin plasma concentrations in young adults who had been exposed to cigarette smoke in utero were significantly higher than in those without prenatal smoke exposure. Moreover, individuals with intrauterine nicotine exposure showed a significantly higher prevalence of own smoking habits and lower educational status compared to those in the group without exposure. ***Conclusion:*** Smoking during pregnancy may be considered as an adverse intrauterine influence that may alter the endocrine-metabolic status of the offspring even until early adulthood.

Introduction

The gut-derived peptide hormone ghrelin stimulates appetite by providing signals to hypothalamic growth hormone secretagogue receptors [1–5]. Plasma ghrelin is

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increased in states of negative energy balance, such as starvation, and decreased during states of positive energy balance, such as feeding [6]. Ghrelin has thus been found to be inversely related to the body mass index (BMI) in patients with eating disorders, e.g. morbidly obese patients [7–9] and patients with anorexia and bulimia nervosa [10–12].

A series of studies support the notion that long-term appetite and weight regulation may be already programmed in early life [13–15]. A high weight gain in infancy has been associated with both body fat and concentrations of ghrelin in adolescence [16]. In addition, it could be demonstrated that the intrauterine environment affects the ghrelin system of the offspring. A study by Smith et al. [17] revealed that offspring (aged 2.5–25 years) born before an anti-obesity surgery (biliopancreatic diversion bariatric surgery) of their mother had lower ghrelin concentrations than their siblings born after the mother's anti-obesity surgery. The profound effect of intrauterine growth retardation induced by methyl-donor deficiency on ghrelin function [18] suggests the involvement of transgenerationally mediated epigenetic mechanisms. In utero exposure to cigarette smoke, a known factor causing epigenetic modifications [19, 20], led to significantly higher serum ghrelin concentrations in adult male rats compared to a control group of rats born to mothers without cigarette smoke exposure during gestation [21]. In humans, the newborns of smoking mothers were found to exhibit significantly higher cord blood levels of ghrelin than those of nonsmoking mothers [22].

Further animal studies have also shown that intrauterine programming of obesity-related orexigenic systems persists even to adolescence/adulthood. Using a rat model of intrauterine caloric growth restriction, Nagata et al. [23] found that pups of undernourished mothers were hyperphagic and heavier, and had higher ghrelin levels until the adult age of 7 months compared to rats of mothers that had been fed ad libitum during gestation. Confirming these results, Yousheng et al. [24] provided an electrophysiological basis for altered ingestive behavior by studying the postmortem brain tissue of adult rats that had been previously subjected to maternal food restriction during gestation/lactation.

Based on the observations described above, we hypothesized that plasma ghrelin concentrations in adulthood may be associated with the intrauterine exposure to cigarette smoke. Here, we examined this hypothesis in a sample of 19-year-olds from a long-term, prospective, epidemiological cohort study followed up since birth.

Materials and Methods

Subjects

This investigation was conducted in the framework of the Mannheim Study of Children at Risk [25, 26], an ongoing epidemiological cohort study of the long-term outcome of early risk factors. The initial sample consisted of 384 children of predominantly (>99.0%) European descent born between 1986 and 1988. Infants were recruited from two obstetric and six children's hospitals of the Rhine Neckar Region (Germany) and were included consecutively into the sample according to a two-factorial design intended to enrich and control the risk status of the sample (factor 1 varying the degree of obstetric complications and factor 2 the degree of psychosocial adversity [25, 26]). As a result, approximately one third of the study sample had experienced severe obstetric complications, while about one third of the families suffered from severe psychosocial adversities, such as a parental psychiatric disorder, marital discord or young parenthood. Only firstborn children with singleton births and German-speaking parents were enrolled in the study. Furthermore, children with severe physical handicaps, obvious genetic defects or metabolic diseases were excluded. Assessments were conducted at the age of 3 months and at regular intervals throughout development. The present analysis comprises data from 266 young adults (123 males and 143 females) who agreed to participate in the follow-up examination at the age of 19 years including blood sampling and for whom data on all relevant variables were complete. The study was approved by the Ethics Committee of the University of Heidelberg and written informed consent was obtained from all participants.

Assessments

The variable *obstetric risk status* mirrored an obstetric risk score obtained by counting the presence of nine adverse conditions during pregnancy, delivery and the postnatal period [25]. Early *psychosocial risk status* was assessed according to an 'enriched' family adversity index measuring the presence of 11 adverse family factors covering characteristics of the parents, the partnership and the family environment during a 1-year period prior to birth. The participants' *birth weight* and *gestational age* were extracted from infant neonatal records. The sample also comprised infants suffering from obstetric complications: 72 (27.1%) participants were born by premature birth (gestational age <37 weeks) and 36 (13.5%) were small for gestational age (defined as birth weight below the 10th percentile for the gestational age). The mothers of 38 (14.3%) participants suffered from *preeclampsia*.

Maternal smoking during pregnancy was determined by a standardized interview conducted with the mother at the 3-month assessment. Mothers were asked whether they did not smoke (a) or smoked up to 5 (b), 10 (c), 20 (d), 40 (e) or more than 40 cigarettes/day (f) during pregnancy. To avoid power problems due to small sample sizes, the latter groups (c–f) were pooled, resulting in a three-level variable [0 = nonsmoking: 74.4% (n = 198), 1 = smoking 1–5 cigarettes/day: 10.2% (n = 27) and 2 = smoking >5 cigarettes/day: 15.4% (n = 41)].

Postnatal smoking of the parents was recorded within the framework of a standardized parent interview conducted at all assessments until the participants were aged 15 years. Smoking was defined as having smoked at least 5 cigarettes/day in any of the assessments (0 = parents did not smoke, 1 = at least one parent smoked until the offspring's age of 15 years).

At the age of 19 years, the *anthropometric assessment* included weight and height. We calculated the BMI dividing the weight (kg) by the height (m^2). For the assessment of young adults' cigarette use, participants completed a smoking inventory which is part of the substance use questionnaire designed by Müller and Abbet [27] in collaboration with the World Health Organization. The *current smoking status* was determined using a four-level variable reflecting both the frequency and the quantity of smoking: 1 = never smoked, 2 = experimental use (smoked up to once per month), 3 = regular use (at least weekly) and 4 = dependent use (>10 cigarettes/day). In addition, participants were asked for their highest level of education at the age of 19 years. Derived from this, the dichotomous variable *high school graduation* was set to value 1 for participants achieving the diploma from German secondary school qualifying for university admission and 0 in the remainder.

Laboratory Methods

At the age of 19 years, a laboratory stress procedure was conducted, which comprised repeated blood sampling (for more details see Buchmann et al. [28]). Participants reported to the laboratory at 13.00 h. Until their arrival, smoking and food intake were allowed ad libitum, but all subjects were asked for the time of their last meal. During the study procedure, they needed to abstain from nicotine and caloric intake throughout the experiment. At 17.15 h, blood samples were collected in tubes pretreated with EDTA and aprotinin (2,000 KIU per 7 ml blood), immediately stored on ice and spun at 4°C within 10 min. The plasma was immediately frozen and stored at –80°C. Total ghrelin concentrations were determined using a commercially available radioimmunoassay kit (Mediagnost, Reutlingen, Germany). Intra- and interassay coefficients of variation were 2.6–5.3 and 4.8–8.2%, respectively. Three participants (1 male and 2 females) were considered as outliers due to deviation of more than 2.5 standard deviations from the group mean and were excluded from the sample.

Data Analysis

ANOVA and correlation analyses were conducted in order to explore the effects of potentially confounding variables on plasma ghrelin levels at the age of 19 years. ANCOVA (analysis of covariance) was used to investigate the effect of tobacco exposure during pregnancy on plasma ghrelin concentrations at the age of 19 years controlling for potentially confounding factors. All analyses were conducted using PASW 18.

Results

Total Cohort

In the total study cohort, *gender* revealed a significant effect on young adults' ghrelin levels, with females showing higher ghrelin concentrations than males [females (n = 143): 0.88 ± 0.25 ng/ml vs. males (n = 123): 0.79 ± 0.21 ng/ml; p = 0.002]. There was also a significant effect of the *current smoking status* on ghrelin levels, indicating higher ghrelin concentrations in smokers compared to nonsmokers [dependent user (n = 43): 0.94 ± 0.26 ng/ml; regular user (n = 58): 0.85 ± 0.23 ng/ml; experimental user (n = 104): 0.82 ± 0.22 ng/ml; never user (n = 61): 0.80 ± 0.24 ng/ml; $F_{3, 262} = 3.53$, p = 0.015)]. In addition, significant differences emerged for the level of education *(high school graduation)*. Compared to participants with high school graduation, those without showed significantly elevated ghrelin concentrations [no high school graduation (n = 166): 0.87 ± 0.24 ng/ml vs. high school graduation (n = 100): 0.79 ± 0.23 ng/ml; p = 0.012].

Higher plasma ghrelin levels at the age of 19 years were negatively related to *BMI* (r = –0.158; p = 0.010). Furthermore, ghrelin showed a tendency to increase with the *time since the last meal* (r = 0.119; p = 0.052). There was no significant association of plasma ghrelin with *birth weight* (r = –0.084; p = 0.174), *small for gestational age* (r = –0.006; p = 0.921) or preeclampsia (r = –0.035; p = 0.575).

Intrauterine Exposure to Cigarette Smoking

In our cohort, we identified 41 (15.4%) subjects who had been exposed to the highest level of intrauterine cigarette smoke (>5 cigarettes/day), while 198 (74.4%) subjects were assigned to the lowest level of prenatal smoke exposure (nonsmoking mothers). The remaining 27 subjects (10.2%) pertained to the moderate exposure group, with mothers smoking 1–5 cigarettes/day (table 1). The *psychosocial risk status* was significantly different in the exposed groups compared to the nonexposed group (p < 0.001) in terms of higher psychosocial adversity associated with the degree of cigarette exposure during pregnancy (table 1). The groups with moderate and high exposure also showed a significantly higher *current smoking status* compared to the nonexposed group (p < 0.001) and a lower prevalence of *high school graduation* (p < 0.001). These groups also differed in their *plasma ghrelin* levels: young adults exposed to maternal smoking during pregnancy showed increased ghrelin levels depending on their degree of exposure (high intrauterine exposure: 0.94 ± 0.27 ng/ml; moderate 0.83 ± 0.24 ng/ml, and no exposure: 0.82 ± 0.23 ng/ml; p = 0.016; table 1). Comparisons between these groups with regard to a number of factors known to influence ghrelin concentrations are also presented in table 1. Maternal smoking during pregnancy remained a significant predictor of higher ghrelin levels at the age of 19 years in ANCOVA even after controlling for *current smoking status* and *high school graduation*, as well as *BMI*, *time since the last meal*, *postnatal parental smoking*, *gender*, and participants' *psychosocial* and *obstetric risk status* ($F_{2, 255} = 3.16$, p = 0.043).

Table 1. Sample characteristics

	Cigarette exposure during pregnancy			
	none (n = 198)	moderate (n = 27)	high (n = 41)	p value
Males, n (%)	94 (47.5)	10 (37.0)	19 (46.3)	0.594*
Birth weight (mean ± SD), g	2,863±797.25	2,841±883.05	2,650±724.37	0.298
Small for gestational age, n (%)	23 (11.6)	4 (14.8)	9 (22.0)	0.208*
Preeclampsia, n (%)	30 (15.2)	3 (11.1)	5 (12.2)	0.783*
Psychosocial risk status (mean ± SD)	1.42±1.68	2.33±2.13	3.85±2.06	<0.001
Obstetric risk status (mean ± SD)	1.10±1.04	0.89±1.01	1.07±1.01	0.621
Postnatal parental smoking, n (%)	103 (52.0)	26 (96.3)	40 (97.6)	<0.001
BMI (mean ± SD)	23.41±4.09	23.69±3.78	23.66±4.40	0.902
Current smoking status, n (%)				<0.001*
Never used	56 (28.3)	1 (3.7)	4 (9.8)	
Experimental use	81 (40.9)	11 (40.7)	12 (29.3)	
Regular use	39 (19.7)	6 (22.2)	13 (31.7)	
Dependent use	22 (11.1)	9 (33.3)	12 (29.3)	
High school graduation, n (%)	90 (45.5)	4 (14.8)	6 (14.6)	<0.001*
Time since last meal (mean ± SD), h	5.37±1.10	5.42±1.04	5.81±1.52	0.651
Plasma ghrelin (mean ± SD), ng/ml	0.82±0.23	0.83±0.24	0.94±0.27	0.016

Moderate = Maternal prenatal smoking ≤5 cigarettes/day; high = maternal prenatal smoking >5 cigarettes/day; none = no maternal prenatal smoking. p values: the group with no exposure is compared to the exposed groups (moderate and high exposure). *χ^2 test.

Discussion

In this study, we investigated plasma ghrelin concentrations in 19-year-old participants of an epidemiological cohort study followed up since birth and tested the hypothesis that ghrelin levels in early adulthood may be associated with intrauterine exposure to cigarette smoking. According to our findings, tobacco exposure during pregnancy was associated with higher ghrelin levels at the age of 19 years. Moreover, individuals with intrauterine nicotine exposure were found to show a significantly higher prevalence of own smoking habits and a lower educational status compared to those in the nonexposed group.

Our findings correspond well with evidence indicating that early-life circumstances may lead to individual differences in cognitive, social and emotional development – and even in mental health [29]. Numerous studies have indicated that exposure to adversity already in utero is associated with various structural, physiologic and metabolic changes reaching into the adulthood [30]. Entringer et al. [31] examined telomere length in healthy young adults and found that persons whose mothers had experienced a severe stressor during the index pregnancy had significantly shorter telomeres compared to controls whose mothers had an uneventful index pregnancy. As shown in an increasing number of studies, the prenatal and early perinatal environment (e.g. undernutrition and consecutive low birth weight, but also nutritional excess or preterm birth for example) may contribute to the risk to develop metabolic (hyperinsulinemia, diabetes type 2 or obesity), cardiovascular (hypertension or coronary heart disease), respiratory (asthma or chronic obstructive airway disease) and psychiatric complications, including addictive disorders in adult life [32–41]. The exact underlying pathogenetic mechanisms are not fully explored, but epigenetic modifications are likely to be involved in creating susceptibility to those diseases in later life [42–45]. Importantly, such modifications are preventable and even potentially reversible [39, 46].

Saad and Abbas [21] studied serum and testicular ghrelin levels in adult male offspring exposed to cigarette smoking in utero and found not only higher ghrelin levels in serum and testicular homogenates of exposed male rats compared to controls but also significant decreases in testicular weight and testosterone levels, as well as serum testosterone, LH and FSH levels. Thus, hyperghrelinemia following maternal smoking during pregnancy may con-

tribute to the suppression of male reproductive function in rats [21]. Ghrelin has been repeatedly shown to attenuate the activity of the hypothalamic-pituitary-gonadal axis in animals and humans; it suppressed the secretion of LH and FSH in male and female rats [47, 48], female rhesus monkeys [49] and female sheep [50], but also in human males and females [51–54]. Moreover, ghrelin appears to be related to memory processes and to be involved in the pathophysiology of psychiatric disorders, particularly depression [55].

Our results are in accordance with previous studies showing higher ghrelin levels in females than males among both healthy individuals [8, 56, 57] and patients with psychiatric disorders, e.g. depression [58]. In addition, ghrelin has also been found to be inversely related to BMI [7–9]. However, increased ghrelin levels due to a low BMI were not consistently reported [10–12, 59].

As far as the impact of the smoking status per se on plasma ghrelin concentrations is concerned, we obtained a significant effect of the *current daily smoking status* on peripheral ghrelin levels. This finding is in contrast to that of a study by Pöykkö et al. [60], who found no correlation between ghrelin and smoking. Likewise, no significant differences in baseline plasma ghrelin concentrations between habitual smokers and nonsmokers were reported by Kokkinos et al. [61] and Schanze et al. [62]. However, while ghrelin was unchanged in smoking mothers compared to nonsmoking mothers, their newborns showed 71% higher cord blood ghrelin concentrations than the offspring of nonsmoking mothers [22]. According to Bouros et al. [63], smoking appeared to acutely increase plasma ghrelin concentrations, while a suppression following short-term smoking was observed among nonsmokers [61]. On the other hand, Lee et al. [64] showed ghrelin levels to decrease after cessation of smoking and concluded that other mechanisms than the change in plasma ghrelin may lead to weight gain after smoking cessation.

Numerous studies have shown that active maternal smoking during pregnancy is associated with reduced birth weight due to direct causal intrauterine effects [65, 66]. In fact, each cigarette smoked per day during the third trimester was estimated to decrease birth weight of the neonate by 27 g [67]. On the other hand, intrauterine exposure to smoking was associated with obesity in children up to school age, and specific effects of cigarette smoke have been discussed as being causal to such a finding [68, 69]. In our cohort, birth weight did not differ significantly between infants with and without cigarette smoke exposure. At the age of 19 years, again, BMI did not differ significantly between both groups (exposure vs. no exposure).

In order to assess the extent of confounding, we controlled for a wide range of variables, including *psychosocial* and *obstetric risk status*, as well as *current smoking status*, *BMI* and *high school graduation*. As food intake until 1 p.m. was not strictly standardized, we controlled for the *time since the last meal*. After a meal, ghrelin is suppressed for about 2–6 h [70, 71]. Therefore, we assume that in all subjects ghrelin plasma concentrations reached the fasting state at the time of blood sampling. For the unlikely case that a preceding meal suppressed ghrelin for a longer period of time, we controlled for fasting duration in our analyses. Even after controlling for all these factors, our main result of ghrelin levels depending on the intrauterine exposure to cigarette smoking remained significant. Thus, our finding supports a strong association between fetal exposure to cigarette smoking and lasting alterations in ghrelin concentrations. However, as several other parameters (e.g. genetic factors) were not assessed, our finding cannot imply a causal intrauterine effect of maternal smoking.

The risks of smoking during pregnancy are well established for both mother and child and include sudden infant death, fetal growth restriction, low birth weight, placental abruption, preterm delivery and respiratory distress, for example [72]. Yet, smoking prevalence during pregnancy still reaches up to 24% [73]. In our cohort, we identified 41 mothers (15%) reporting that they had smoked >5 cigarettes/day during pregnancy. Current investigations suggest that up to 25% of pregnant smokers are missed when self-reporting is relied on and routinely collected data in prenatal care may thus not be accurate [74, 75]. Nevertheless, the fact remains that smoking is one of the most important modifiable adverse risk factors in Western countries.

Conclusion

We found that in young adults who had been exposed to cigarette smoking in utero, ghrelin plasma concentrations were significantly higher than in those without prenatal smoke exposure. Therefore, smoking during pregnancy may be considered as an adverse intrauterine effect that may alter the endocrine-metabolic status of the offspring even until early adulthood, thus predisposing to the development of metabolic disorders.

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