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ORIGINAL CONTRIBUTION

Prenatal stress during the 1999 bombing associated with lower birth weight—a study of 3,815 births from Belgrade

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Abstract During the 3-month bombing of Serbia (March 24–June 9, 1999), the entire population, including pregnant females as an especially vulnerable group, was exposed to a high degree of stress. This is the first study to explore the effects of prenatal stress during the 1999 bombing of Belgrade on the main obstetric characteristics of newborns. The design of the study was retrospective cross-sectional. The total number of birth records in the sample was 3,815, namely, 1,198 from the group exposed to prenatal stress and 1,251 and 1,366 from the respective control periods, years 1996 and 2003, when no stressful events affected the city. We found that exposed mothers gave birth to infants with statistically significantly lower birth weight (BW; mean difference=86 g, 95% confidence interval=67 to 104; $F_{(1,\ 3{,}349)}=80.8$, $p<0.001$, $\eta_p{}^2=0.024$), when controlling for confounding effects of body length and head circumference. There was no specific relation between the trimester of stress exposure and BW in infants born in 1999. Neither increased frequency of preterm deliveries nor more complications of pregnancy and delivery were found in the given sample. Possible consequences of lower BW on psychosocial and somatic functioning should be evaluated through the lifetime.

Keywords Prenatal stress · Low birth weight · Newborns · Bombing

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Introduction

During the 3-month bombing of Serbia (March 24–June 9, 1999), the entire population, including pregnant females as an especially vulnerable group, was exposed to a high degree of stress because of life-threatening situations on a daily basis. The psychological and endocrinological effects of the prolonged stressful situation on healthy adults from Belgrade were investigated by Zarkovic et al. (2003). During the bombing, the authors found elevated scores on the Beck Inventory of Depression and Hamilton Anxiety Rating Scale as well as suppression of the hypothalamic–pituitary–adrenal axis (manifested by low morning cortisol and reduced cortisol response to adrenocorticotropic hormone), while 2 1/2 years later, neither psychiatric nor endocrinological dysfunctions were observed among the subjects.

A body of evidence suggests that increased maternal anxiety and prenatal stress are associated with changes observable at birth such as: low birth weight (BW), decreased length and head circumference, as well as more obstetric complications (preterm delivery, malformations, and preeclampsia; Copper et al. 1996; Hansen et al. 2000; Hedegaard et al. 1996; Lederman et al. 2004; Leeners et al. 2007). During the World Trade Center (WTC) disaster (Lederman et al. 2004), exposure to stress of mothers living within a 2-mile radius of the WTC was associated with significantly lower BW and length (149 g and 0.82 cm, respectively) of full-term infants, while after the exposure of pregnant women to hurricane Katrina, the frequency of low BW was almost three times higher than in those who were not exposed (14.0% vs. 4.7%, respectively; Xiong et al. 2008).

Literature is inconsistent about which gestational phase is the most vulnerable and when the fetus is most susceptible to adverse effects of stress. Stressful events in early gestation could affect physical outcomes, while stress during late gestation could result in behavioral and emotional problems in adulthood (Glover and O'Connor 2002).

In Belgrade, the capital of Serbia, with a population of approximately 1,600,000, there are about 14,000 births annually (Statisticki Godisnjak Beograd 2007). The territory is divided into 17 municipalities, and the city covers 3.6% of the territory of Serbia while 24% of the country's population lives in the city. The prolonged exposure to various stressors during the bombing of Belgrade may have induced certain neuropsychoendocrinological alterations in pregnant females. The effects of such exposure can be evaluated through obstetric parameters (BW, birth length, head circumference, labor complications, etc.). Our pilot study (Maric et al. 2006) showed lower BW in babies born during 1999 in comparison to samples from 3 years before and after, but due to methodological limitations, this finding needed further examination and additional obstetric parameters. The current study analyzes the effects of prenatal stress during the 1999 bombing of Belgrade on the main obstetric characteristics of a large sample of newborns.

Subjects and methods

Study design and setting

The design of the study was retrospective cross-sectional. The data about mothers, deliveries, and infants were obtained from the Medical Birth Registry, which contained records on all births at the Institute of Gynecology and Obstetrics, Clinical Center of Serbia in Belgrade, with permission from the head of the institute. The Institute of Gynecology and Obstetrics is one of five obstetric hospitals in the city of Belgrade. During the observed periods, the institute was performing about 36% of the total number of deliveries in Belgrade.

Subjects, sampling methods, and exposure definition

The random systematic sampling method was used to collect data. From the chronological registry, every fifth medical record of birth was included in three selected periods.

Infants born between March 24, 1999 and March 23, 2000 were the population of interest. Mothers who gave birth in this period were subjected to a high level of existential stress in various points of their pregnancies due to the 77 days of bombing which occurred between March 24 and June 9, 1999. The population was regularly exposed to impending air raids, which would usually commence in the evening and cease the next morning. Both the beginning and the end of the air threat were announced by air-raid sirens. The location of the delivery hospital was in the 500-m radius from several bombing sites.

The two control populations were infants born 3 years before and 3 years after, i.e., between March 24, 1996 and March 23, 1997 and between March 24, 2003 and March 23, 2004. The control periods were chosen in order to eliminate the influence of possible carry-over effects and/or trends. No stressful events affected the city in these years. These two sets of data were then combined into a single control group, since no considerable differences in risk factors were observed in the two control periods.

The bombing of Belgrade was well circumscribed in terms of time; therefore, we addressed another important question: susceptibility for adverse effects of prenatal stress on BW in relation to gestational phase of exposure. For these analyzes, we divided the sample into four subgroups: infants born from March to June 1999 were born during the bombing (n=312), those born from July to September had late gestation exposure (n=258), infants born from October to December 1999 were exposed to stress during midgestation (n=262), while infants born from January to March 2000 were exposed to stress in the early gestation (n=231). The two control populations were infants born 3 years before and 3 years after, when no stressful events affected the city.

Variables and measurement

Maternal sociodemographic variables:

1. age (in ranges 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, and 45–49 years),
2. marital status (married/unmarried),
3. education (university/high school/other, nonspecified),
4. place of residence (Belgrade/out of Belgrade).

Pregnancy-related variables:

1. number of deliveries (primiparae/multiparae),
2. gestational age (preterm, i.e., <38 gestational weeks/full term, i.e., 38–42 gestational weeks/post-term, i.e., >42 gestational weeks),
3. type of delivery (vaginal/cesarean delivery/forceps),
4. complications of pregnancy and delivery (stillbirth, malformations, breech and other atypical presentations, umbilical cord around the neck, fetal asphyxia, premature amniotic rupture, preeclampsia, twin pregnancy, and Rh incompatibility).

Infant-related (outcome) variables:

1. body weight (in grams),
2. body length (in centimeters),

3. head circumference (in centimeters),
4. body mass index (BMI; in kilograms per square meter).

Statistical methods

We used SPSS 16.0 for Windows (SPSS, Chicago, IL, USA) to obtain descriptive statistics and make comparisons. The normality of distribution was assessed using histograms and *Q–Q* plots. The means from two independent samples were compared using Student's *t* test with Cohen's *d* for effect size. Mann–Whitney's *U* test was used to test the difference in medians. Pearson's chi-square test was used for categorical variables, while phi (Φ) and odds ratio (OR) with 95% confidence interval (95%CI) were calculated to assess the effect. Furthermore, we conducted one-way analysis of covariance (ANCOVA) to assess the effect of maternal stress during pregnancy on body weights of live, full-term, singleton infants, while controlling for their body length and head circumference. Partial eta-squared (η_p^2) was the measure of effect size. The level of statistical significance was set to 0.05, after applying Bonferroni adjustment for multiple comparisons.

Results

The total number of birth records in the sample was 3,815. The group with exposure to prenatal stress consisted of 1,198 records, while 2,617 records were obtained from the respective control periods (1996–1997 and 2003–2004).

Maternal sociodemographic variables

Mothers in both exposed and control group were mostly married (90.2% and 91.6%), Belgrade residents (87.5% and 80.0%), and with high school education (51.2% and 48.8%). Detailed sociodemographic description of mothers is given in Table 1.

Main features of births and infants

Of the 3,815 infants, three were excluded from the sample due to missing data on head circumference. Furthermore, 54 infants were stillborn (34 or 1.3% in the control group and 20 or 1.7% in the group with exposure; $\chi_{(1)}^2=0.808$, $p=0.369$). Subsequent analyses refer to the remaining 3,758 records.

Sex distribution of infants was equal across the two groups. In the group with exposure to stress, natural (vaginal) birth was significantly more frequent in comparison with the control group (88.4% and 83.7%, respectively; $\Phi=0.06$, small effect). Furthermore, mean head circumference was larger in the exposed group ($d=0.30$, medium effect), while mean BMI was significantly lower ($d=0.09$, small effect). The mean difference in body weight was 37 g and it has not reached statistical significance (95%CI=−5 to 78). There were no statistically significant differences in body length, incidence of prematurity, or multiple births (Table 2).

The occurrence of preeclampsia was significantly less frequent in the group with exposure (OR=0.592, 95%CI=0.407 to 0.863), while malformations were more frequent but statistically insignificant (OR=3.288, 95%CI=0.926 to 11.675). The remaining complications were uniformly distributed (Table 3).

The exposure and birth weight

In order to avoid confounding effects of preterm and post-term deliveries and multiple pregnancies on infant-related variables, a one-way independent ANCOVA was conducted

Table 1 Sociodemographic description of mothers

Variable	Control group (n=2,617)	Group with exposure (n=1,198)
Marital status, n (%)		
Married	2,351 (91.6)	1,058 (90.2)
Unmarried	216 (8.4)	115 (9.8)
Place of residence, n (%)		
Belgrade	2,060 (80.0)	1,030 (87.5)
Out of Belgrade	514 (20.0)	147 (12.5)
Education, n (%)		
Other nonspecified	862 (34.8)	388 (34.9)
High school degree	1,208 (48.8)	569 (51.2)
University degree	407 (16.4)	154 (13.9)
Parity, n (%)		
Primiparae	1,287 (49.9)	633 (53.8)
Multiparae	1,292 (50.1)	544 (46.2)
Age (years), mean (SD)	31.8 (5.6)	28.8 (5.4)

Table 2 Descriptive data and statistics about births and infants

Variable	Control group (n=2,581)	Group with exposure (n=1,177)	Statistical tests	p value
Sex of the newborn, n (%)			$\chi^2_{(k-1)}$=1.081	0.299
Male	1,330 (51.5)	585 (49.7)		
Female	1,251 (48.5)	592 (50.3)		
Term of the newborn, n (%)			$\chi^2_{(k-1)}$=3.201	0.197
Preterm	225 (8.7)	87 (7.4)		
Full term	2,346 (90.9)	1,082 (91.9)		
Post-term	10 (0.4)	8 (0.7)		
Childbirth procedure, n (%)			$\chi^2_{(k-1)}$=15.333	<0.001
Vaginal	2,161 (83.7)	1,041 (88.4)		
Cesarean	394 (15.3)	131 (11.1)		
Forceps	26 (1.0)	5 (0.4)		
Multiple births, n (%)			$\chi^2_{(k-1)}$=1.693	0.193
Single birth	2,475 (95.9)	1,139 (96.8)		
Twin birth	106 (4.1)	38 (3.2)		
Body weight (grams), mean (SD)	3,259 (583)	3,222 (596)	$t_{(3,756)}$=1.773	0.076
Body length (cm), mean (SD)	49.8 (2.9)	49.8 (3.0)	$t_{(3,756)}$=0.568	0.570
Head circumference (cm), mean (SD)	34.8 (1.8)	35.4 (2.0)	$t_{(3,756)}$=−9.373	<0.001
BMI (kg/m^2), mean (SD)	13.0 (1.4)	12.9 (1.4)	$t_{(3,756)}$=2.655	0.008
Childbirth in order, median (range)	2 (8)	1 (6)	Z_U=−1.684	0.092

on 3,353 live full-term and singleton infants, analyzing body weight at birth as outcome variable, while body length and head circumference served as covariates. The main factor was pregnancy and/or birth during the 1999 bombing.

The covariates, body length and head circumference, were significantly related to the body weight: $F_{(1,\ 3,349)}$= 2,997.8, p<0.001, η_p^2=0.472 and $F_{(1,\ 3,349)}$=513.7, p< 0.001, η_p^2=0.133, respectively. The two covariates explained 60.5% variance of body weight in the model, and their influence on body weight was eliminated by keeping them constant at 50.4 cm for body length and 35.3 cm for head circumference. When controlling for the confounding effects of body length and head circumference, we found that the exposed mothers got infants with statistically significant lower BW (mean difference=86 g, 95%CI=67 to 104; $F_{(1,\ 3,349)}$=80.8, p<0.001, η_p^2=0.024; see Fig. 1).

Table 3 Complications and malformations

Variable		Control group (n=2,581)		Group with exposure (n=1,177)		Pearson's χ^2 test	
		n	%	n	%	$\chi^2_{(1)}$	p value
Breech and other atypical presentations	No	2,316	89.7	1,073	91.2	1.87	0.171
	Yes	265	10.3	104	8.8		
Cord around the neck	No	2,222	86.1	1,016	86.3	0.036	0.849
	Yes	359	13.9	161	13.7		
Premature amniotic rupture	No	2,526	97.9	1,153	98.0	0.033	0.856
	Yes	55	2.1	24	2.0		
Rh incompatibility	No	2,462	95.4	1,132	96.2	1.201	0.273
	Yes	119	4.6	45	3.8		
Imminent fetal asphyxia	No	2,513	97.4	1,157	98.3	3.093	0.079
	Yes	68	2.6	20	1.7		
Preeclampsia	No	2,458	95.2	1,143	97.1	7.113	0.008
	Yes	123	4.8	34	2.9		
Malformations[a]	No	2,580	100.0	1,176	99.9	–	0.528
	Yes	1	0.0	1	0.1		

[a]Pearson's chi-square test not applicable due to expected cell counts less than one. Fisher's exact probability test performed instead

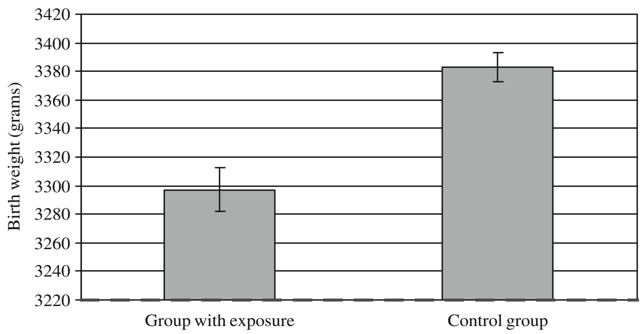

Fig. 1 Estimated mean body weight and 95%CI of live, full-term, singleton infants (n=3,353) while holding covariates constant

The timing of stress exposure and birth weight

For these analyzes, we divided the sample into four subgroups: infants born during the bombing and infants with late gestation, midgestation, and early gestation exposure (see methods). A one-way independent ANCOVA was conducted on 1,063 live full-term and singleton infants, analyzing body weight at birth as outcome variable, while body length and head circumference served as covariates. The control group consisted of infants born during the corresponding periods of 1996–1997 and 2003–2004 (n=2,290).

Infants from all subgroups exposed to stress had significantly lower BW in comparison to the respective control groups. On the other hand, there was no significant difference in BW among infants exposed to stress in different gestational phases (Table 4).

Discussion and conclusion

The principal finding of our study is that exposure of pregnant females to the 1999 bombing of Belgrade was associated with lower BMI of infants, but it was neither connected with increased frequency of preterm deliveries nor more complications of pregnancy and delivery. We also found the association between exposure to prenatal and increased head circumference. Exposed mothers had more vaginal deliveries when compared to control periods. Infants' BWs were statistically significantly lower (86 g, on average) in the exposed group after controlling for confounding effects of body length and head circumference. There was no specific relation between the trimester of stress exposure and BW in infants born in 1999.

Anthropometric measurements, such as BW, are a summary measure of the intrauterine environment as well as an indirect measurement of adverse prenatal influences (Phillips 2007). Possible explanation of the observed phenomena includes two mechanisms. The first one (Van den Bergh et al. 2005) assumes fetal exposure to increased concentrations of maternal glucocorticoids. The exposure is facilitated by alterations in permeability of the placental barrier due to the reduction of protective enzyme 11β-HSD2 in stress conditions. BW reduction presumably reflects the catabolic action of stress steroids, particularly noticeable during the period of maximal fetal somatic growth (late gestation). In animal experiments, studies demonstrated that prenatal glucocorticoid administration retards brain weight at birth (Huang et al. 1999), delays neuronal maturation and myelination, reduces glia and vasculature (Huang et al. 2001a, b), has effects upon synapse formation (Antonow-Schlorke et al. 2003), and may permanently alter brain structure (Matthews 2000). The persistence of glucocorticoid effects was shown in rhesus monkeys treated with dexamethasone antenatally, which had neuronal degeneration of hippocampal neurons and reduced hippocampal volume at 20 months of age (Uno et al. 1990). Gestational stress also increases corticotropin-releasing hormone (CRH) activity in the amygdala and the incidence of anxiogenic and depressive-like behavior in rats and nonhuman primates, which can be ameliorated by CRH antagonists (Weinstock 2005).

The second possible explanation of adverse prenatal influences includes stress-related changes that occur on the vessels and result in decreased blood supply (i.e., oxygenation). Stress-induced increased level of norepinephrine provokes vasoconstriction and hypoperfusion of tissues, including placenta. According to Teixeira et al. (1999), pregnant females who were more anxious had significantly lower perfusion through the uterine artery in comparison with pregnant females who were less anxious. In addition,

Table 4 The timing of stress exposure and BW

Exposure to stress	Mid gestation		Late gestation		Birth during the bombing		Control group	
	Mean BW difference (g)	p value	Mean BW difference (g)	p value	Mean BW difference (g)	p value	Mean BW difference (g)	p value
Early gestation	5	0.809	29	0.192	23	0.296	−95	<0.001
Mid gestation	–	–	24	0.273	17	0.413	−82	<0.001
Late gestation	–	–	–	–	−7	0.748	−90	<0.001
Birth during the bombing	–	–	–	–	–	–	−75	<0.001

the same authors found stronger associations for state anxiety than for trait anxiety.

The described phenomenon in life-threatening situations might as well be adaptive, in terms of evolution. One hypothesis (Teixeira et al. 1999) speculates that a mother has evolved such mechanisms to protect herself in times of stress at the expense of her fetus.

Low BW (defined as weight below 2,500 g) is considered as a risk factor for various somatic diseases (diabetes mellitus, cardiovascular diseases, etc.) and mental disorders such as schizophrenia, affective disorders (especially depression), and attention deficit hyperactivity disorder (Breslau 1995; Fall et al. 2002; Gale and Martyn 2004; Gunther et al. 2005; Wiles et al. 2005). Moreover, lower BW was more relevant than the twin status as a risk factor for child problem behavior (Van Os et al. 2001) or metabolic syndrome (Bo et al. 2001).

All these findings fit well into Baker's hypothesis about the fetal origin of adult diseases. The hypothesis presumes that the in utero environment could be altered by various factors (e.g., maternal anxiety) and eventually leads to modification in fetus development and subsequent phenotype in adulthood (Barker 1995; Van den Bergh et al. 2005; Evans et al. 2008). Altered physiological, neuroendocrine, and metabolic conditions contribute to the constant reprogramming of the developmental model, resulting in changes of function of tissues and organs and may have pathological consequences in adulthood (Barker 1995; Phillips 2002; Van den Bergh et al. 2005).

It is still disputed which trimester of gestation is the most vulnerable, in terms of the fetus being especially susceptible to unfavorable environmental effects. Overall, research points to an increase in vulnerability to stress during early, rather than mid to late, gestation in both animal species and humans (Lazinski et al. 2008), but a large cohort study found that mid-to-late gestational distress was associated with low BW and preterm birth in humans (Rondó et al. 2003). Our analysis did not identify a trimester that would be particularly vulnerable to stress in terms of lower birth weight. Nevertheless, we think that the question of vulnerability to stress regarding trimester of exposure is still open and very important. For more decisive answers, we need a prospective study design controlling for confounding effects of various factors: gestational age, parity, socioeconomic status, mother's age, morbidity, anthropometry, etc., as well as precise definition of stress (the event of bombing) vs. distress (personal experience).

We obtained given data from the Institute of Gynecology and Obstetrics, one of five obstetric hospitals on the territory of Belgrade. As the biggest one, it admits about a third of Belgrade region population. Precise data referring to the municipality of residence of pregnant women was not recorded but it might have been of certain importance because municipalities were unevenly exposed to the bombing.

Furthermore, the Medical Birth Records neither included data on maternal anthropometry, maternal habits (smoking, alcohol consumption, drug use and abuse, nutrition, sleep, etc.), maternal health status, nor information about some confounders during bombing, apart from stress. All aforementioned data should be included in prospective study.

Despite these limitations, our study had its strengths as it included a large sample, targeted a poorly understood, yet important topic, and conducted several levels of analyses. This is the first study to specifically explore the effects of prenatal stress during the 1999 bombing of Belgrade on the main obstetric characteristics of newborns.

The exposure of pregnant females to the 1999 bombing of Belgrade was associated with lower BW, but not with increased frequency of preterm deliveries or complications of pregnancy and delivery. Altogether, two important concerns arise: could this moderate reduction of BW found in our sample have unfavorable effects on psychosocial and somatic functioning and which trimester of stress exposure would have a particular impact on postnatal development? Identifying the period of greatest vulnerability to prenatal stress might help us understand the underlying mechanisms for prenatal stress effects and would lead to more successful early intervention. Follow-up studies of the exposed population through the lifetime are of vital importance.

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