Brief Communication: Prenatal and Early Postnatal Stress Exposure Influences Long Bone Length in Adult Rat Offspring

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ABSTRACT Stress during the prenatal and early postnatal periods (perinatal stress, PS) is known to impact offspring cognitive, behavioral, and physical development, but effects on skeletal growth are not clear. Our objective was to analyze effects of variable, mild, daily PS exposure on adult offspring long bone length. Twelve pregnant rat dams were randomly assigned to receive variable stress from gestational days 14–21 (Prenatal group), postpartum days 2–9 (Postnatal), both periods (Pre–Post), or no stress (Control). Differences in adult offspring tibia and femur length were analyzed among treatment groups. Mean tibia length differed among groups for males ($P = 0.016$) and females ($P = 0.009$), and differences for femur length approached significance for males ($P = 0.051$). Long bone length was shorter among PS-exposed offspring, especially those exposed to postnatal stress (Postnatal and Pre–Post groups). Results persisted when controlling for nose–tail length. These differences might reflect early stunting that is maintained in adulthood, or delayed growth among PS-exposed offspring. This study suggests that PS results in shorter long bones in adulthood, independently of effects on overall body size. Stunting and growth retardation are major global health burdens. Our study adds to a growing body of evidence suggesting that PS is a risk factor for poor linear growth. Am J Phys Anthropol 149:307–311, 2012.

A number of human and non-human animal studies suggest that stress exposure during the prenatal and early postnatal periods (perinatal stress, PS) can impact physical growth and development of offspring (Beydoun and Saftlas, 2008). Many of these effects are evident in gross physical measures. For example, PS has been shown to result in decreased birth weight (Hobel et al., 2008; Maric et al., 2010) and length (Dancause et al., 2011), and increased adiposity later in life (Entringer et al., 2010; Li et al., 2010; Dancause et al., 2012). A number of mechanisms might be involved, particularly elevated levels of maternal glucorticoids (GCs), which can cross the placenta or be transmitted via maternal milk and disrupt offspring development (Lazinski et al., 2008). The outcomes observed might reflect effects on central mediators of growth, such as the hypothalamic-pituitary-adrenal axis, or direct effects on the affected tissue, such as bone cells (Swolin-Eide et al., 2002).

Despite mounting evidence of long-term effects of PS on physical characteristics and the plausibility of mechanistic pathways whereby exposure might impact skeletal growth, there are few recent studies on the effects of PS exposure on bone growth. Researchers from the University of Pittsburgh conducted a number of studies on PS and skeletal growth during the 1970s and 80s (Siegel and Doyle, 1975a,b; Doyle et al., 1977; Siegel et al., 1977; Brandt and Siegel, 1978; Mooney et al., 1985; Gest et al., 1986) and observed variable impacts of PS on dental and long bone length, symmetry, and composition. In general, results suggested that some types of PS exposure could decrease long bone length, but findings were inconsistent. These studies thus answered some interesting and important questions, but also raised a great deal more, which have not been explored recently.

Our objective was to assess the effects of PS on long bone length in a rodent model. We analyzed the effects of both prenatal stress, and postnatal stress administered during a period of rat development that is similar to that of third trimester human development. We hypothesized that PS would result in shorter length of long bones.

MATERIALS AND METHODS

All procedures were approved by the Animal Care Committee of McGill University and followed ethical guidelines from the Canadian Council on Animal Care.

Animals

Twelve pregnant Sprague-Dawley rat dams (C. River, St. Constant QC, Canada) were received at gestation day 13 and maintained in the Douglas Hospital Research Center

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TABLE 1. Unadjusted means (SDs) for adult offspring outcomes by treatment group, with P-values for among-group differences tested by nested ANOVA and effect sizes (Cohen's d) compared to controls

	Treatment				
	Control	Prenatal	Postnatal	Pre-post	*P*-value
Males					
Nose–tail length (cm)	49.1 (2.1)	49.8 (1.1)	48.8 (0.8)	49.4 (1.2)	0.743
Weight (g)	565 (43)	506 (16)	505 (27)	524 (40)	0.064
Tibia length (mm)	43.97 (0.40)	43.33 (0.69)	42.59 (0.81)	42.37 (0.64)	0.016
Effect size	1.135	2.160	2.998		
Femur length (mm)	39.44 (0.71)	39.03 (0.48)	38.56 (0.49)	38.05 (0.78)	0.051
Effect size		0.677	1.443	1.864	
Females					
Nose–tail length (cm)	43.4 (1.2)	43.5 (1.4)	42.6 (0.9)	43.0 (1.0)	0.834
Weight (g)	327 (24)	314 (26)	289 (19)	322 (29)	0.255
Tibia length (mm)	39.34 (0.49)	39.06 (0.68)	37.76 (0.78)	37.68 (1.11)	0.009
Effect size	0.472	2.426	1.935		
Femur length (mm)	35.19 (0.67)	34.99 (0.60)	34.23 (0.38)	33.94 (1.07)	0.094
Effect size		0.314	1.763	1.400	

Animal Facility under controlled conditions (lights on 0800–2000 h, 18–25°C, humidity 25–40%) with *ad libitum* access to food and water except during food deprivation stress (described below). The day of birth was considered postnatal day 0. On postnatal day 1, litters were sexed and culled to 10 pups of approximately equal numbers of males and females per dam. Offspring were weaned on postnatal day 21 and housed in same-sex groups of two to three.

Stress procedure

Procedures were developed from a model of chronic mild stress shown in other studies to evoke responses among exposed offspring (Hougaard et al., 2005; Brunton and Russell, 2010). Dams were randomly assigned to one of four groups: daily stress during the last week of gestation (gestation days 14–21) (Prenatal); daily stress during the first week postpartum (postpartum days 2–9) (Postnatal); both prenatal and postpartum stress (Pre–Post); and a non-stressed Control group. The prenatal stress schedule included (in daily order): light from 2000 h–0800 h, housing in a wire mesh floored cage (24 h), food deprivation (12 h), tilting the cage 45° (6 h), exposure to strobe light (1 h), forced swim (10 min), restraint (30 min), and wet bedding (10 h). The postpartum stress schedule was similar, including (in daily order): strobe light, wet bedding, wire mesh floored cage, food deprivation, restraint, male intruder (5 min), forced swim, and housing in a small cage (designed for mice, 24 h). Both dams and offspring were exposed to all stressors with the exception of restraint and swim stress, to which only dams were exposed. Dam weights (g) were obtained every morning. Six offspring per litter (39 males, 33 females, $n = 72$) were sacrificed for this study by rapid decapitation on postnatal day 80.

Outcome measurements

To minimize stress, same-sex pups from each litter were weighed as a group on postnatal day 1. Mean weight of male and female pups was calculated for each litter. Two days before sacrifice, offspring length was measured from the tip of the nose to the tip of the tail to the nearest mm. Offspring weight (g) was measured immediately prior to sacrifice. After decapitation, right and left tibias and femurs were excised. Bones were soaked at room temperature in a meat tenderizer solution for approximately 14 h, manually cleaned of soft tissue, and allowed to dry. Soaking in meat tenderizer solution at up to 90°C is a common maceration procedure used to ensure complete removal of soft tissue for accurate bone measurement (Steadman et al., 2006; Lee et al., 2010). Maximum length of each bone was measured twice by the same person, who was blind to treatment group, to the nearest 0.01 mm using digital calipers. The mean of the two measurements was used for analyses.

Statistical methods

One-way ANOVA with Bonferroni pairwise comparisons was used to test differences in dam weight, litter size, and weight of male and female pups at birth for each treatment group. To control for potential random differences between batches, nested ANOVA (batch nested within treatment) with Bonferroni pairwise comparisons was used to analyze effects of treatment on offspring outcomes at age 80 days. We first tested effects of treatment on adult weight and nose–tail, tibia, and femur lengths. Because we would expect longer bones among larger animals, and PS might impact body size, we then included nose–tail length as a covariate in long bone analyses. Statistical significance was defined as $P < 0.05$. Analyses were completed using PASW 18.0 (IBM SPSS Inc., 2010, Chicago, IL).

RESULTS

Dams and litters

Controlling for litter size, initial (pre-treatment) dam weights differed among treatment groups ($P = 0.016$), with heavier mean weight in the Prenatal and Control groups compared to the Postnatal group, and heavier mean weight in the Control group compared to the Pre–Post group. These differences were ameliorated by gestation day 18. Controlling for initial dam weight, weight of dams did not differ among treatment groups during the prenatal or postnatal stress periods. Litter size averaged 13.25 pups (range 10–16). Litter size and average birth weight of male and female pups did not differ among treatment groups.

Offspring measurements

Table 1 includes means for offspring measurements by treatment group and sex, and *P*-values for among-group differences. Tibia length differed among groups for both

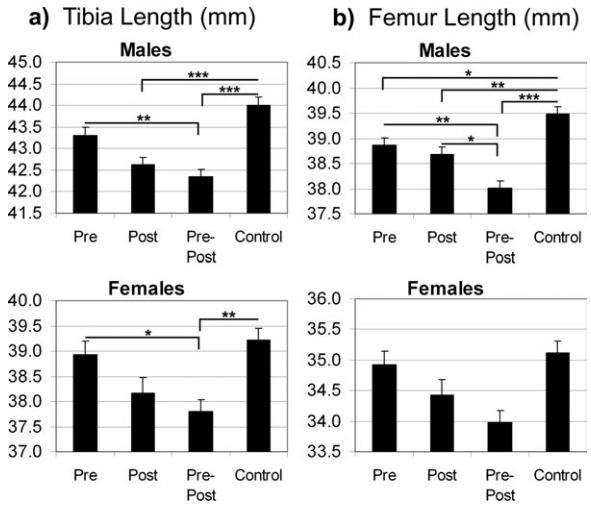

Fig. 1. Estimated marginal means (controlled for nose–tail length) and Bonferroni pairwise comparisons for outcomes by treatment group. Error bars show standard errors; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (Note that for figure clarity, scales differ between measurements and sexes.)

males and females, and femur length showed a strong trend among males ($P = 0.051$). We then analyzed bone length controlling for body size. Controlling for nose–tail length, tibia length (Fig. 1a) differed among groups for males ($P = 0.021$) and females ($P = 0.027$). Among males, Controls had longer tibias compared to both groups exposed to postnatal stress (Postnatal and Pre–Post), and the Prenatal group had longer measurements than Pre–Post. Among females, both the Prenatal and Control groups had longer tibias than the Pre–Post group. These differences persisted when controlling for weight rather than nose–tail length (males, $P = 0.041$; females, $P = 0.010$). Femur length (Fig. 1b) also differed among treatment groups for males ($P = 0.031$) but not for females ($P = 0.121$). Control males displayed longer femur measurements compared to all three PS groups, and the Prenatal and Postnatal groups had longer measurements than the Pre-Post group. Differences did not persist when controlling for weight rather than nose–tail length (males, $P = 0.085$; females, $P = 0.097$).

DISCUSSION

Both human and non-human animal studies provide evidence for programming effects of PS on offspring physical and mental health and a variety of behavioral outcomes (Beydoun and Saftlas, 2008). We sought to clarify the effects of PS exposure on long bone length. Programming effects of the prenatal environment on skeletal growth outcomes have been addressed in past studies, but with inconclusive results. Such programming might have long-term implications for offspring development, and thus warrants further consideration.

We observed shorter long bones among PS-exposed offspring compared to Controls at age 80 days, particularly among those exposed to postnatal stress (Postnatal and Pre–Post). This might result from early growth stunting resulting in permanently shorter bone length in adulthood, or from a slower long bone growth rate. Rats continue to grow beyond 80 days of age, and can experience "catch-up" growth as observed in stunted children (Williams, 1981). Whether the effects of PS on long bone growth are permanent or diminish with age might represent an area for further research.

Our results provide a reminder of an environmental factor that has long-term implications for childhood growth, but that remains understudied. Although researchers increasingly recognize that psychosocial stress impacts growth, data are limited (Batty et al., 2009), especially when focusing on the perinatal environment specifically. We have observed that prenatal stress from a natural disaster affects growth outcomes such as birth length in exposed children (Dancause et al., 2011), and the results of the current study provide support for our observations in humans. Stunting is a major global health burden affecting an estimated 171 million children worldwide (de Onis et al., 2012). Stunting and early growth retardation are associated with long-term cognitive and developmental delays, and represent major factors in the intergenerational transfer of poverty (Grantham-McGregor et al., 2007). The mild stressors in our study were sufficient to result in slightly shorter long bone length, suggesting that PS represents one contributing factor to stunted or delayed growth. Coupled with poor nutrition or infectious diseases, even a modest effect of PS might be relevant from a public health perspective.

Our results mirror those from several rodent studies of PS and long bone growth. For example, prenatal noise stress resulted in shorter long bone length at birth (Gest et al., 1986) and at age 21 days (Doyle et al., 1977), as well as decreased mass per unit length (Doyle et al., 1977). Prenatal heat stress produced similar effects on long bone length (Gest et al., 1986) and cortical thickness (Brandt and Siegel, 1978). However, effects are not evident in all studies, which likely reflects both the type of stressor and the measure being examined (Brandt and Siegel, 1978). For example, Brandt and Siegel (1978) observed no effects of prenatal noise stress on femoral cortical thickness at age 21 days, and Gest et al. (1986) observed no effects of prenatal cold stress on femoral length at birth. Furthermore, Siegel et al. (1977) observed that prenatal heat stress resulted in longer femur length and increased mass per unit length of the ulna, humerus, tibia, and femur at age 21 days. Results might reflect prenatal selection, as stressed dams in that study were more likely to abort their litters, and those that carried to term had fewer pups than controls. The surviving pups might be those that were more resistant to stress and had better growth (Siegel et al., 1977). Furthermore, while dams were randomly selected in all of the above studies, there might be some dam or litter effects that were not controlled in the analyses.

The effect of PS on offspring physical growth is thought largely to reflect the actions of hormones such as GCs. Thus, a number of rodent studies have examined the effects of different perinatal hormonal exposures on bone growth. Inhibited bone growth has been observed after prenatal exposure to dexamethasone (Chagin et al., 2010), leptin (Nilsson et al., 2003), and the cytokine IL-1β (Swolin-Eide et al., 2004). Our results suggest a similar effect of mild, chronic PS, particularly among groups exposed to early postnatal stress. A longer period of PS exposure might contribute to the large effect sizes in the Pre–Post group, but effect sizes are also larger among the Postnatal compared to Prenatal group despite the same length of PS exposure, suggesting other underlying factors besides exposure length.

The timing of PS exposure plays an important role in the outcomes observed (Lazinski et al., 2008; Charil et al., 2010). Perhaps long bone growth is more sensitive to the effects of PS later in offspring development. Even after birth, maternal stress hormones can pass through maternal milk and thus directly impact offspring growth (Catalani et al., 2011). Pregnant and lactating rat dams have similarly blunted hormonal responses to stress compared to virgin rats (da Costa et al., 1996; Hill et al., 2003), which buffers their pups to some extent. However, pups in the Pre–Post and Postnatal groups experienced many of the stressors directly in addition to being exposed to stress hormones through maternal milk, and so might have experienced greater GC exposure. Furthermore, postnatal maternal stress has been shown to decrease milk production (Dewey, 2001; Lau and Simpson, 2004) which, coupled with effects of maternal GCs in maternal milk, might amplify the stunting effects of postnatal stress exposure on long bone growth. In contrast, prenatal stress exposure has been shown to result in increased offspring feeding behavior (Lesage et al., 2004), even among very young pups (Purcell et al., 2011), which might be expected to mitigate long bone stunting that occurred before birth.

Strengths, limitations, and conclusions

The conclusions we can draw from our data about the mechanisms underlying effects of PS on long bone length are limited. In particular, we do not have measurements of maternal GC response to stressors, and we did not measure maternal or offspring food intake, which might be affected by PS. All of these mechanisms likely played a role in the outcomes observed. Furthermore, we had a small group of dams that, although randomly selected, showed differences in initial weights among treatment groups. Our statistical analyses help to correct for these differences, but similar studies would be strengthened by including more dams. Finally, while some studies from the University of Pittsburgh group included both upper and lower limb bones, we measured only lower limb bones and so cannot compare effects of PS on humerus and ulna length between the studies. However, the design of our study contributes to a growing body of literature on perinatal factors that might program physical growth. In particular, our procedure was designed to represent mild chronic stress. This might be expected to provoke different responses than acute stress, synthetic GC administration, or physiological stressors such as heat or cold exposure, but was still sufficient to impact skeletal development. Considering the continued global health burden posed by stunting and growth retardation, the identification of any potentially preventable risk factors is important, and our results suggest that PS represents one of these factors.

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