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Low-level Pb and Cardiovascular Responses to Acute Stress in Children: The Role of Cardiac Autonomic Regulation

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Abstract

Objective—A number of studies suggest that Pb exposure increases cardiovascular disease risk in humans. As a potential mechanism for this effect, we recently reported a significant association between early childhood Pb levels and cardiovascular response to acute stress. The current study considers the association between current Pb levels and the autonomic nervous system activation pattern underlying the cardiovascular response to stress in a new cohort of children.

Methods—We assessed blood Pb levels as well as cardiovascular responses to acute stress in 9–11 year old children ($N = 140$). Sympathetic activation (measured with pre-ejection period) and parasympathetic activation (measured with high frequency heart rate variability) were also assessed.

Results—In a sample with very low levels of blood Pb ($M = 1.01 \mu\text{g/dL}$), we found that increasing blood Pb was associated with coinhibition of sympathetic and parasympathetic activation in response to acute stress. In addition, increasing Pb levels were associated with the hemodynamic stress response pattern typical of coinhibition – significantly greater vascular resistance and reduced stroke volume and cardiac output.

Conclusions—Blood Pb levels were associated with significant autonomic and cardiovascular dysregulation in response to acute psychological stress in children. Moreover, these effects were significant at Pb levels considered to be very low and notably well below the $10 \mu\text{g/dL}$ the Centers for Disease Control and Prevention definition of an elevated blood Pb level. The potential for

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autonomic dysregulation at levels of Pb typical for many US children would suggest potentially broad public health ramifications.

Keywords

Cardiovascular; children; lead; stress; autonomic balance; vascular

Lead (Pb) exposure is associated with heightened vascular responses to stress in both animals and children [1,2]. In addition, studies have demonstrated that the cardiovascular response to acute stress is a relatively stable individual difference [3] and predicts future hypertension [4–6] as well as heart disease [7]. For example, children that showed heightened cardiovascular responses to a series of acutely stressful laboratory tasks (e.g., hand tracing a pattern using only the mirror image as a guide) were at significantly greater risk of elevated resting blood pressure 6.5 years later [8]. Finally, findings from a number of studies suggest a small but significant positive association between Pb exposure and resting blood pressure [9,10]. Therefore, it is possible that repeated and exaggerated Pb-induced vascular responses to acute stress produce vascular remodeling and subsequent hypertension [11], thereby explaining the association between Pb exposure and baseline blood pressure. In order to increase our understanding of the association between Pb and vascular responses to acute stress, the present study investigates the autonomic activation pattern that may underlie this particular hemodynamic response to acute stress associated with low-level blood Pb.

The autonomic nervous system is comprised of two independent neural systems – the sympathetic and parasympathetic. Pre-ejection period (PEP) is considered a relatively pure measure of sympathetic activation [12] such that a shorter PEP corresponds to greater sympathetic activation. In addition, pharmacological blockade of the parasympathetic (vagal) system suggests that a specific component of heart rate variability (HRV), namely the high frequency (HF) component from spectral analysis of HRV (HF-HRV), represents a relatively pure measure of parasympathetic activation [13,14]. In this case, greater HF-HRV corresponds to greater parasympathetic activation. A number of studies have demonstrated that these systems operate independently both at rest [15] and in response to stress [16]. In addition, individuals demonstrate stereotypic patterns of response over time [17], although some tasks will tend to elicit a particular response pattern [18].

Recent efforts have been directed at quantifying this “autonomic balance” between the sympathetic and parasympathetic systems. For example, Berntson and colleagues [15,17,19] describe two orthogonal dimensions that represent the balance of sympathetic and parasympathetic activation within this autonomic space (see Figure 1, adapted from ref. 15), namely *cardiac autonomic balance* (CAB) and *cardiac autonomic regulation* (CAR). Along the CAB dimension are *reciprocal parasympathetic responders* (PEP increases and HF-HRV increases) and *reciprocal sympathetic responders* (PEP decreases and HF-HRV decreases). Along the CAR dimension are *coinhibitors* (PEP increases and HF-HRV decreases) and *coactivators* (PEP decreases and HF-HRV increases). For the present study, we expect CAR to be associated with blood Pb levels for two reasons. First, our prior research suggests that increasing blood Pb levels are associated with reduced stroke volume and cardiac output and heightened vascular resistance [2]. This hemodynamic pattern is indicative of coinhibitors [16], i.e., those with reduced CAR. Second, CAR but not CAB was negatively associated with a prior history of cardiovascular disease events [15] and therefore represents that dimension most relevant as a potential mechanism for Pb-induced hypertension.

In the present study, we measured current blood Pb levels as well as children's cardiovascular responses within a standard acute stress laboratory protocol in a cohort of 9 – 11 year-old children ($N = 140$). This protocol involves a series of computer tasks that are designed to be challenging for all participants and thereby reliably produce mild stress and a cardiovascular response. These tasks were not designed to assess potential cognitive deficits associated with Pb exposure but rather were designed to serve as reliable “probes” of the children's cardiovascular responses to acute stress. In addition, we measured PEP and HF-HRV responses to acute stress in order to quantify CAR. Consistent with our prior study of early Pb exposure [2], we hypothesized that greater concurrent blood Pb would be associated with significantly reduced cardiac output and stroke volume and heightened vascular resistance during acute stress. In addition, we hypothesized that blood Pb levels would be associated with reduced CAR during stress and thereby provide a potential mechanism for the observed hemodynamic acute stress response pattern.

Methods

Participants

Participants ($N = 140$) were recruited as part of an ongoing study designed to address the effects of low level Pb exposure on cardiovascular responses to acute stress. Recruitment criteria included: 1) being 9–11 years old, 2) reporting no current use of medication that might affect cardiovascular functioning (e.g., Ritalin), and 3) having no significant developmental disorders that might affect task performance. Recruitment occurred through distribution of an informational brochure in local pediatrician's offices and through direct mailing of invitations to those homes having children of appropriate age. Our sample had roughly an equal number of boy and girls (77 and 63, respectively) and the average age was 10.21 years ($SD = 0.75$). A blood draw for measuring nonessential toxic metal levels was followed within 2 weeks by a 2-hr laboratory visit during which cardiovascular responses to acute stress were measured. Children were paid \$100 for their participation in the current study. As described in detail below, the collection of HF-HRV was only possible for 95 children due to an initial lack of appropriate software ($N = 40$) and poor signal quality for some children ($N = 5$).

Child's Blood Pb and Hg Levels

Whole blood specimens (2 mL) were collected into Vacutainer tubes that had been pre-certified by the analyzing laboratory for low-level measurements of Pb. Blood specimens were refrigerated pending shipment to the Trace Elements section of the Laboratory of Inorganic and Nuclear Chemistry at the New York State Department of Health's Wadsworth Center, Albany, NY. This laboratory is New York State's principal reference laboratory for the measurement of trace metals in blood, as well as other fluids and tissues, and is responsible for operating the state's proficiency testing program for blood metals. The analysis for Pb and Hg in whole blood was carried out using a Perkin Elmer Sciex Model ELAN DRC Plus inductively coupled plasma-mass spectrometer equipped with a dynamic reaction cell (DRC-ICP-MS). The method detection limit (MDL) was 0.34 $\mu\text{g/dL}$ (Pb) and 0.24 $\mu\text{g/dL}$ (Hg) for most samples ($N = 100$), and 0.28 $\mu\text{g/dL}$ and 0.14 $\mu\text{g/dL}$, respectively, for some samples ($N = 40$) because of a change in nebulizer method.

Physiological Recording Apparatus

Impedance cardiography and the electrocardiogram (ECG) were used for the measurement of stroke volume and heart rate. An Impedance Cardiograph (Model HIC-2000, Bio-Impedance Technology, Chapel Hill, NC) was used for the generation of the impedance waveforms using a tetrapolar band electrode configuration [20]. The ECG signal was transduced using two disposable silver/silver chloride electrodes (Meditrace 533) placed on

each side of the abdomen below the impedance electrode bands, as well as a ground electrode beside the navel. Calculations of the physiological measures from the impedance cardiography were performed as previously described [21]. Systolic blood pressure and diastolic blood pressure were monitored using the Vasotrac device (APM 205A; Medwave, Danvers, MA); however, this data was unavailable for 1 participant due to signal loss.

After our first 40 children had already participated, we purchased and began using the Nevrokard™ HRV program (Nevrokard Kiauta, k.d., Slovenia). Inter-beat intervals (the times between successive heart beats) are generated and stored from ECG data generated at a sampling rate of 500 Hz, well within the sampling frequency recommended by the Task Force of the European Society of Cardiology that published guidelines for the collection and interpretation of HRV data [22]. Nevrokard HRV software conducts a spectral analysis of this inter-beat interval data and calculates the HF component, among other heart rate variability measures. The HF component of HRV is considered a relatively pure index of parasympathetic activation [22]. Although we attempted to measure HF-HRV for 100 participants, we had insufficient signal quality for 5 participants. In addition, individual inter-beat interval data for our initial 40 participants was not available for analysis of HF-HRV using the Nevrokard software. Therefore, complete HF-HRV data was available for 95 participants.

Experimental Tasks

Mirror Tracing—The mirror image tracing task is a psychomotor task that traditionally requires subjects to trace the outline of a figure while viewing the figure in a mirror. A computerized version of this task was created by slightly modifying an existing E-Prime™ mirror image tracing program [23]. During this E-Prime™ task, participants are asked to trace an image of a star on the computer screen using a cursor directed by a mouse. However, the mouse control is inverted (cursor moves to the right when mouse moves to the left and vice versa) and a loud tone is sounded if the participant's cursor leaves the star. Participants are instructed to perform this task as rapidly and accurately as possible during 1.5 minute trials administered 2 times. We selected the mirror image tracing task because it has reliably produced increases in peripheral vascular resistance in past studies [24]. The number of star segments completed was difficult to measure because the cursor was frequently outside of the star. Therefore, task performance was measured by the percent of time the cursor was outside the star. Due to an initial error in our task program, this performance data was not stored for the first 40 participants.

Reaction Time—Using the E-Prime™ program, an auditory choice reaction time task was developed for this study and run on a Dell Pentium computer. This task is run with 2.5 minute trials administered 2 times, an “easy” version with either a 1000 Hz (target) or 2000 Hz (non-target) tone presented and a second “hard” version with either a 1000 Hz (target) or 1500 Hz (non-target) tone presented. All tones were 1 second in duration. The goal is to respond as quickly as possible to the target tone but not to the non-target tone. Tones occurred on a variable interval schedule of 10 seconds and included 8 targets and 7 non-targets. This task was chosen on the basis of past studies indicating that it produces cardiovascular responses consistent with β -adrenergic activation [25]. Task performance was measured by hit rate (proportion of correct tones with response) and false alarm rate (proportion of incorrect tones with response). In addition, reaction time to hits was used as another measure of task performance.

Continuous Performance Task—E-Prime™ software is used to program five variants of the signal detection task that systematically manipulate demands on response inhibition. In short, each signal detection task employed a series ($N = 100$) of numerical stimuli (0–9)

that were presented in rapid and random sequence (250 ms stimulus interval, 500 ms inter-stimulus interval). The target is the numeral 9. The proportion of targets to non-targets was varied across the five signal detection conditions (target proportions of 10, 30, 50, 70 or 90% at 200 trials per condition). This approach utilizes the principle of Signal Detection Theory that Commission errors are most likely when targets exceed non-targets. In a signal detection task where targets appear rarely (10 or 30% of the time), the predominant requirement for the child is to occasionally initiate a response when the target (infrequently) appears. This methodology is typical of most continuous performance tasks, and places demands on sustained attention [26]. In contrast, in a signal detection task where targets appear very frequently (70 or 90% of the time), the children are continuously responding on almost every trial, and the predominant requirement for the child is to stop responding when the non-target (infrequently) appears. This places demands on response inhibition [27,28]. The five signal detection tasks in the present experiment were given as randomized blocks for each participant. To measure performance on this task, we calculated the signal detection parameters d' , a measure of discriminability calculated from the difference between hit rate and false alarm rate, and β , a measure of bias calculated from the proportion of all stimuli with responses.

Procedure

On the day of testing, participants arrived at the laboratory and first signed an assent form while their parents signed a separate consent form approved by the Institutional Review Board of SUNY Oswego. Height and weight were then measured, followed by the application of electrodes for impedance cardiography and the electrocardiogram (ECG) and the blood pressure cuff was positioned on the nondominant arm. Each experimental session was comprised of the following: 1) an initial rest period (10 minutes), 2) a mirror tracing task (2 minutes, 2 trials), 3) an inter-task rest (8 minutes), 4) a choice reaction time task (2 minutes, 2 trials), 5) an inter-task rest (8 minutes), 6) a mirror tracing task (90 seconds, 3 trials), and 7) a final recovery/rest period (10 minutes). The order for the three acute stress tasks was counterbalanced.

Physiological Data Collection

Blood pressure and impedance-derived variables—Blood pressure measurement was recorded every 30-seconds during the last 3-minutes of the initial rest period and during the entire acute stress tasks. Similarly, data for heart rate and impedance-derived variables were collected using a 15 second inter-sample interval and 14 second ensemble average duration (allowing 1 second for storing data) was collected during the last 3 minutes of the initial rest and during the entire acute stress tasks. For the calculation of HF-HRV, inter-beat interval data was collected continuously for the last 3-minutes of the initial rest and continuously during each acute stress task.

Potential Confounds

Potential confounders were chosen using an *a priori* selection of a limited set of variables shown in prior literature to relate to the outcome [29]. This approach avoids over-fitting a model that occurs when “cherry picking” covariates from a larger set of potential confounds [30]. The following covariates were used in multivariate models: gender, age, race, BMI percentile standing (age and gender adjusted using a SAS program developed by the Centers for Disease Control and Prevention; [31]), socioeconomic status (SES) score based on parents education, occupation, and income, family history of cardiovascular disease (CVD; based on endorsement of history of high blood pressure, stroke, or heart disease for the child’s parent or maternal/paternal grandparents), and Hg levels. Details regarding the measurement of Hg are provided above. Because of the potential effects of Hg exposure on

the cardiovascular system [32], this metal was measured as a potential confound. We conducted analyses of Hg effects using the same approach used below to analyze Pb effects, but Hg was not significantly associated with any of the cardiovascular outcomes. Therefore, we include Hg only as a control variable.

Data Analysis

Cardiovascular responses to acute stress—Change scores for systolic blood pressure, diastolic blood pressure, and heart rate were computed by subtracting baseline levels of a variable from the task means. For impedance-derived variables involving volume measures (cardiac output, stroke volume, and total peripheral resistance), percent change from baseline to task was used because of questions about the accuracy of absolute levels of these variables [33,34]. For all analyses of responses to acute stress, cardiovascular change scores were standardized within the three acute stress tasks and then averaged across tasks (cf. [5]). We took this approach because averaging across tasks improves the reliability of cardiovascular reactivity assessment [35], and averaging responses across these same tasks (with the addition of two other tasks) has been shown to predict future blood pressure in children [5].

Assessment of CAR and CAB—Based on the method outlined by Berntson et al. [15], HF and PEP were normalized by conversion to z-scores to represent indices of increasing parasympathetic activity (HFz) and increasing sympathetic activity (-PEPz). Using these scores, we calculated CAR and CAB: $CAR = HFz + (-PEPz)$ and $CAB = HFz - (-PEPz)$. However, the assessment of CAR and CAB was necessarily restricted to those participants with HF values ($N = 95$). CAR and CAB were calculated for each task and then averaged across tasks for computation of task change scores.

Analytic Models—In all models, the seven covariates outlined above were first entered. Because the distribution of blood Pb levels was not normally distributed and showed a significant positive skew (skewness = 1.74, standard error of skewness = 0.20, $z = 8.71$, $p < 0.05$), we analyzed quartiles that contained a roughly equal number of participants and corresponded to the following blood levels: for Pb, 0.14 – 0.68 µg/dL (1st quartile; $N = 35$), 0.69 – 0.93 µg/dL (2nd quartile; $N = 35$), 0.94 – 1.20 µg/dL (3rd quartile; $N = 34$), and 1.21 – 3.76 µg/dL (4th quartile; $N = 36$). Using these values, all children with levels below the MDL were part of the 1st quartile for Pb. For the analysis of quartiles, SAS PROC GLM was used with a linear contrast to test the effects of increasing blood Pb levels. As an alternative analytic approach throughout, we analyzed Pb as a continuous variable (log transformed and centered). Laboratory values below the MDL were still used in these data analyses, with the assumption that such values constitute the best available estimate of the true value and are preferable to assigning a zero or an arbitrary constant such as ½ the MDL [36,37]. In these analyses, SAS PROC CORR was used to calculate partial correlations that controlled for the seven covariates.

Results

Sample Characteristics

Table 1 presents characteristics of children in our sample, across Pb quartiles. Our sample included 9, 10, and 11 year-olds ($Ns = 55, 74$, and 11, respectively), a roughly equal number of males and females, and was predominantly white (90%). The parents of these children had, on average, “some college” (scale score = 5), a family income between \$45,000 and \$65,000, and an occupational status between “Clerical and Sales” (Hollingshead score = 5) and “Technician or Semiprofessional” (Hollingshead score = 6). The mean BMI percentile for our sample was 72.3 (27.8% were obese) and approximately 75% of the children had a

family history of CVD (at least 1 parent and grandparent with some form of CVD). Table 1 also shows analyses of these characteristics as a function of Pb quartiles. Increasing blood Pb was associated with parents having less education ($F(1, 136) = 5.99, p < 0.05$, for linear trend) and lower income ($F(1, 136) = 21.12, p < 0.0001$, for linear trend).

Task Performance

We also considered the effect of Pb on task performance, after the addition of covariates (data not shown). During the continuous performance task, blood Pb was not significantly associated with either d' or β (all p values > 0.25). For the mirror tracing task, blood Pb was not significantly associated with the percentage of time the participant was outside of the star ($p > 0.25$). Finally, the number of hits and false alarms during the reaction time task and the reaction time for hits were not significantly associated with Pb (p values > 0.25).

Covariates: Relationship to Exposure and Cardiovascular Outcomes

Lower blood Pb levels were associated with significantly higher SES ($r = -0.23, p < 0.01$). In addition, blood Pb levels in males ($M = 1.10 \mu\text{g/dL}$) was significantly higher than in females ($M = 0.91 \mu\text{g/dL}$), $F(1, 138) = 4.78, p < 0.05$. No other covariates were significantly related to blood Pb levels (p values > 0.10). Associations between covariates and cardiovascular outcomes included a significant association between gender and PEP (with females showing greater shortening of PEP in response to stress relative to males, $p < 0.05$) and greater BMI was associated with lower cardiac output responses ($r = -0.21, p < 0.05$) and greater vascular responses ($r = 0.19, p < 0.05$). Collectively, covariates accounted for the following proportion of variance in outcomes: R^2 s = 0.03 (systolic blood pressure), 0.04 (diastolic blood pressure), 0.06 (heart rate), 0.05 (stroke volume), 0.07 (cardiac output), 0.04 (PEP), 0.03 (HF-HRV), 0.20 (CAB), and 0.10 (CAR).

Cardiovascular and Autonomic Response to the Acute Stress Tasks

As shown in Table 2, there was a significant increase during the acute stress tasks in systolic blood pressure, diastolic blood pressure, and heart rate (p values < 0.05), relative to baseline. In addition, there was a significant decline in PEP and HF-HRV (p values < 0.0001) indicating activation of the sympathetic nervous system and withdrawal of the parasympathetic nervous system, respectively, during the acute stress tasks. Based on recommendations for analysis of baseline impedance variables [21], we did not analyze impedance variables (stroke volume, cardiac output, and total peripheral resistance) for this and the following analysis. These results suggest that the tasks did indeed induce a stress response in children.

Pb and Baseline Cardiovascular and Autonomic Levels

After covariate control for potential confounds, we considered the association of blood Pb and children's baseline cardiovascular levels. Blood Pb levels were not significantly associated with baseline systolic blood pressure ($F(1, 129) = 0.44, p > 0.10$), diastolic blood pressure ($F(1, 129) = 0.84, p > 0.10$), heart rate ($F(1, 130) = 2.00, p > 0.10$), PEP ($F(1, 130) = 2.59, p > 0.10$), HF-HRV ($F(1, 84) = 0.32, p > 0.10$), CAR ($F(1, 84) = 3.36, p = 0.07$), and CAB ($F(1, 84) = 0.06, p > 0.10$). All means for these analyses are shown in Table 3. When analyzed with Pb as a continuous variable, only the association with CAR reached significance (see Table 3).

Pb and Cardiovascular Responses to Acute Stress

After controlling for potential confounds, we considered the association of blood Pb levels and children's cardiovascular responses to the acute stress tasks. As shown in Table 4, increasing blood Pb levels were associated with a significantly greater increase in total

peripheral resistance ($F(1, 129) = 4.58, p < 0.05$), a significant reduction in stroke volume ($F(1, 130) = 4.30, p < 0.05$), and a marginally reduced cardiac output ($F(1, 130) = 3.80, p < 0.10$) in response to acute stress. Blood Pb levels were not significantly associated with differing acute stress responses for systolic blood pressure ($F(1, 129) = 1.06, p > 0.10$), diastolic blood pressure ($F(1, 129) = 1.11, p > 0.10$), or HR ($F(1, 130) = 0.04, p > 0.10$). Analysis using Pb as a continuous variable revealed a similar pattern of results. As shown in Table 4, increasing blood Pb levels were associated with a significant increase in total peripheral resistance ($p < 0.05$), and marginally significant reductions in stroke volume and cardiac output (p values < 0.10). In addition, increasing blood Pb levels were associated with a marginally significant increase in diastolic blood pressure ($p < 0.10$), but no significant associations with systolic blood pressure or heart rate (p values > 0.10).

Autonomic Balance

In order to consider Pb effects on sympathetic and parasympathetic activation, we analyzed blood Pb levels in relation to PEP and HF-HRV, respectively. Increasing blood Pb levels were associated with a significantly smaller reduction in PEP ($F(1, 130) = 4.11, p < 0.05$) and a significantly greater reduction in HF-HRV ($F(1, 85) = 4.59, p < 0.05$), as shown in Figure 2, panels A and B. As predicted, blood Pb levels were associated with lower CAR in response to stress ($F(1, 85) = 6.33, p < 0.05$; see Figure 2, panel C). CAB levels in response to stress were not significantly associated with blood Pb levels ($F(1, 84) = 2.31, p > 0.10$). In analyses using continuous Pb levels, these significant effects were observed again for PEP ($r = 0.17, p = 0.05$), HF-HRV ($r = -0.22, p < 0.05$), and CAR ($r = -0.24, p < 0.05$).

CAR Dysregulation as a Potential Mediator for Pb-induced Cardiovascular Effects

First, CAR was tested as predictor of stroke volume, cardiac output, and total peripheral resistance responses to acute stress after entering the standard covariates. In these analyses, CAR was a significant predictor of stroke volume ($F(1, 86) = 11.04, p < 0.01$), only marginally significant predictor of cardiac output ($F(1, 86) = 3.79, p < 0.10$), and not a significant predictor of total peripheral resistance ($F(1, 86) = 2.53, p > 0.10$). Based on these results, we tested CAR as a potential mediator for the effect of Pb on stroke volume responses to acute stress. By entering CAR in the model with Pb predicting stroke volume, the effect of Pb dropped from $F(1, 84) = 3.96, p < 0.05$, to $F(1, 83) = 1.65, p > 0.20$, representing a 34% drop in effect size (from Cohen's $d = 0.43$ to 0.28).

Discussion

The current study provides additional insight into the association between blood Pb and cardiovascular responses to stress in children. First, the current study revealed an association between increasing concurrent blood Pb and a pattern of cardiovascular responses to acute stress characterized by increased total peripheral resistance, reduced stroke volume, and reduced cardiac output. This pattern of associations is consistent with the associations we previously reported for early childhood Pb levels and cardiovascular responses to acute stress in another cohort of 9 ½ year old children [2]. Second, we measured sympathetic (PEP) and parasympathetic (HF-HRV) activation in the present study. Increasing blood Pb was associated with coinhibition of these systems during stress, as demonstrated by a significant negative association with CAR. Finally, there was evidence that reduction in CAR mediated the association between Pb and stroke volume (but not cardiac output or total peripheral resistance).

There are a number of possible explanations for the association between blood Pb levels and autonomic coinhibition during acute stress. First, Pb may exert a direct effect on vasoconstriction [38], thereby increasing total peripheral resistance during stress. This

increase in total peripheral resistance may produce both longer PEP due to cardiac afterload [39] and reduced HF-HRV with longer PEP due to the baroreceptor reflex. In general, it is important to note that hemodynamic outcomes are highly interdependent. For example, an increase in heart rate will be associated with shorter pre-ejection period and typically with greater cardiac output (assuming stroke volume doesn't drop as compensation for the increase in heart rate). Therefore, within this system with multiple compensatory mechanisms seeking to maintain homeostasis [40], it remains a challenge to locate the initial site of Pb action on the cardiovascular system. Second, blood Pb may act directly on the central nervous system and thereby inhibit sympathetic and parasympathetic activation during stress. For example, the brain mesocorticolimbic system is implicated in both behavior effects of Pb exposure as well as the stress response [41]. Although further research is necessary to elucidate the mechanism for these associations with Pb, there is now accumulating evidence that Pb exposure alters the stress response in animals [42–44] and humans [2,45].

The behavioral and health consequences of Pb-induced dysregulation of the stress response in children remain to be determined. Although speculative, autonomic dysregulation during stress may serve as the mechanism accounting for the association between Pb and hypertension [46,47]. In addition, autonomic dysregulation (particularly sympathetic and parasympathetic coinhibition) is associated with a number of behavioral and emotional characteristics in children, such as conflict [16], externalizing problems [48], and maladjustment [49]. As such, perhaps Pb-induced autonomic dysregulation accounts for the observed associations in children between Pb exposure and poor social adjustment [50], conduct disorders [51], and risky behaviors [52,53].

Although our current acute stress protocol involved cognitive tasks, these tasks were not selected to assess potential Pb-induced cognitive deficits but rather to reliably induce a cardiovascular response in order to measure potential Pb effects on this particular physiological response. However, blood Pb levels are associated with cognitive deficits in children [54,55]. Therefore, an intriguing possibility that awaits further research is whether, in addition to the effects on peripheral vascular resistance, Pb increases cerebral vascular resistance and thereby reduces cerebral blood flow. Although studied primarily in clinical populations (e.g., those with sickle cell anemia), reduced cerebral blood flow has been shown to be associated with lower IQ in children [56] and adults [57].

The present study has a few limitations. First, the cross-sectional design has well known weaknesses. It is possible that cardiovascular functioning affects the toxicokinetics of these nonessential toxic metals, or perhaps psychological stress affects toxicant uptake or clearance. One study in monkeys demonstrated greater blood Pb levels following acute psychological stress in monkeys [58]; however, maternal stress in rats did not produce differing levels of blood Pb following consistent dietary exposure [43]. Ideally, a time-series design would provide a test of the temporal relationship between exposure to nonessential toxic metals and changes in cardiovascular and autonomic functioning. Second, the children in our sample were predominantly white. Therefore, we cannot test potential racial differences in the associations of blood Pb with cardiovascular and autonomic functioning. Third, we have no measures of subclinical heart disease in our sample. Therefore, although a Pb-induced reduction in cardiac autonomic regulation may be associated with an increased risk of subsequent hypertension and heart disease, we were unable to test this hypothesis in our sample. Fourth, 28% of our participants had a BMI $\geq 95^{\text{th}}$ percentile, which is higher than the national average of 19.6% for American children within this age group [59]. We are not aware of published data on obesity rates within Oswego County, NY; however, Oswego County is one of the 5 counties in NY State with the highest rates of obesity [60]. As a precaution, BMI was included as a covariate in all analyses. Finally, it is important to

establish potential health risks at all levels of exposure to Pb. Although the present study considered low level Pb exposure that will likely affect a large percentage of the general population, we were unable to investigate these potential effects at moderate or high Pb exposure levels.

Conclusions

The present study demonstrates a significant association between blood Pb levels and increasing total peripheral resistance, reduced cardiac output, and reduced stroke volume in response to acute stress in 9 – 11 year old children. In addition, blood Pb levels were associated with increasing coinhibition of sympathetic and parasympathetic activation during acute psychological stress. Consistent with autonomic coinhibition, CAR was inversely associated with blood Pb levels. Finally, these effects were significant at Pb levels considered to be very low and notably well below 10 µg/dL, the Centers for Disease Control and Prevention definition of an elevated blood Pb level. The present finding of autonomic dysregulation at levels of Pb typical for many US children would suggest potentially broad public health ramifications.

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Abbreviations

BMI	body mass index
CAB	cardiac autonomic balance
CAR	cardiac autonomic regulation
CDC	Centers for Disease Control and Prevention
CVD	cardiovascular diseases
ECG	electrocardiogram
HF-HRV	high frequency component of HRV
Hg	mercury
MDL	method detection limits
Pb	lead
PEP	pre-ejection period
SES	socioeconomic status

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Figure 1. Autonomic space based on independent levels of inhibition-activation of the sympathetic and parasympathetic branches of the autonomic nervous system (adapted from [15]).

Figure 2.
Sympathetic activation (measured with PEP), parasympathetic activation (measured with HF), and cardiac autonomic regulation (CAR) as a function of Pb quartiles.

Table 1

Characteristics of Participants across Pb quartiles (N = 140)

Measure	Full Sample	Pb Quartile				p value ¹
		1	2	3	4	
Child age (yrs)	10.21	10.44	10.12	10.29	10.21	0.380
Gender (% female)	45.00	62.86	45.71	38.24	33.33	0.068
Race (% white)	90.00	91.43	85.71	94.12	88.89	0.980
Socioeconomic Status						
Education (score)	5.34	5.60	5.50	5.17	5.07	0.021
Occupation (score)	5.67	6.17	5.63	5.47	5.41	0.062
Income ²	7.90	8.71	8.00	7.91	7.00	<0.001
Child's BMI (percentile ³)	72.33	70.26	77.70	69.80	71.50	0.830
Family History of CVD (% yes)	76.43	77.14	74.29	73.53	80.56	0.761
Blood Hg (µg/dL)	0.75	0.58	0.99	0.74	0.69	0.930

¹ p value for analysis of linear trends for continuous variables (age, education, occupation, income, BMI, and blood Hg) and from χ^2 for categorical variables (gender, race, and family history of CVD)

² On this income scale, a score of 8 corresponds to “\$45,000 – \$65,000”. As outlined in the methods section, this income was subsequently adjusted for the number of persons living in the household.

³ Percentiles are derived from national age and gender adjusted BMI tables provided by the CDC.

Table 2

Children's Physiological Response to the Acute Stress Tasks.

Hemodynamic Measures	N[/]	Baseline	During Tasks	t	p value
Systolic blood pressure (mmHg)	139	105.91	112.87	12.30	<.0001
Diastolic blood pressure (mmHg)	139	56.54	61.09	11.32	<.0001
Heart rate (beats/minute)	140	85.28	86.07	2.35	<.05
Pre-ejection period (msec)	140	105.55	104.03	4.42	<.0001
HF HRV (ms ²)	95	5002.07	2266.58	5.62	<.0001

[/] Sample size varied across outcomes for reasons outlined in the methods section.

Table 3

Associations (i.e., Main Effects) for Childhood Pb Levels and Children's Baseline Physiological Functioning.

Hemodynamic Measures	N ²	Pb Quartile ¹				p value for Linear Trend Across Quartiles	Partial r
		1	2	3	4		
Systolic blood pressure (mmHg)	139	106.10	102.60	107.34	102.47	0.51	0.02
Diastolic blood pressure (mmHg)	139	56.14	54.28	56.97	53.42	0.36	-0.09
Heart rate (beats/minute)	140	82.47	83.24	83.69	78.55	0.16	-0.20 ^τ
Pre-ejection period (msec)	140	107.75	108.39	105.17	104.45	0.11	-0.13
HF HRV (ms ²)	95	5775.77	5237.09	5014.43	8798.16	0.12	0.16
CAR (HFz + (- PEPz))	95	0.00	-0.09	0.07	0.85	0.07	0.23 [*]
CAB (HFz - (- PEPz))	95	0.27	0.17	-0.06	0.47	0.80	0.05

^τ p < .10;

^{*} p < .05

¹ For quartiles, the ranges (in µg/dL) for Pb were: 0.14 – 0.68 µg/dL (1st quartile), 0.69 – 0.93 µg/dL (2nd quartile), 0.94 – 1.20 µg/dL (3rd quartile), and 1.21 – 3.76 µg/dL (4th quartile).

² Sample size varied across outcomes for reasons outlined in the methods section.

Table 4

Associations of Blood Pb and Children's Cardiovascular Reactivity to Acute Stress (controlling for all covariates).

Hemodynamic Measures	N ²	Pb Quartile ¹				p value for Linear Trend Across Quartiles	Partial r
		1	2	3	4		
Systolic blood pressure (mmHg)	139	5.30	7.33	7.07	7.23	0.31	0.12
Diastolic blood pressure (mmHg)	139	4.02	5.64	5.09	5.53	0.29	0.17 ^τ
Heart rate (beats/minute)	140	0.91	0.19	0.86	0.58	0.85	-0.01
Stroke volume (% change)	140	2.23	0.91	-3.47	-0.89	0.04	-0.16 ^τ
Cardiac output (% change)	140	3.26	1.19	-2.31	-0.20	0.05	-0.15 ^τ
Total peripheral resistance (% change)	139	2.91	8.18	9.55	9.51	0.03	0.20 *

^τ p < .10;

* p < .05

¹ For quartiles, the ranges (in µg/dL) for Pb were: 0.14 – 0.68 µg/dL (1st quartile), 0.69 – 0.93 µg/dL (2nd quartile), 0.94 – 1.20 µg/dL (3rd quartile), and 1.21 – 3.76 µg/dL (4th quartile).

² Sample size varied across outcomes for reasons outlined in the methods section.