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Lead exposure is related to hypercortisolemic profiles and allostatic load in Brazilian older adults

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ABSTRACT

Lead levels (Pb) have been linked to both hyper- and hypo-reactivity of hypothalamic-pituitary-adrenal axis (HPA) axis to acute stress in animals and humans. Similarly, allostatic load (AL), the 'wear and tear' of chronic stress, is associated with inadequate HPA axis activity. We examined whether Pb levels would be associated with altered diurnal cortisol profile, as a primary mediator of AL, during aging. Pb levels were measured from blood samples (BPb) of 126 Brazilian individuals (105 women), between 50 and 82 years old. Six neuroendocrine, metabolic, and anthropometric biomarkers were analyzed and values were transformed into an AL index using clinical reference cut-offs. Salivary samples were collected at home over 2 days at awakening, 30-min after waking, afternoon, and evening periods to determine cortisol levels. A multiple linear regression model showed a positive association between BPb as the independent continuous variable and cortisol awakening response (R^2=0.128; B=0.791; p=0.005) and overall cortisol concentration (R^2=0.266; B=0.889; $p < 0.001$) as the outcomes. Repeated measures ANOVA showed that individuals with high BPb levels showed higher cortisol at 30 min after awakening (p=0.003), and in the afternoon (p=0.002) than those with low BPb values. Regarding AL, regression model showed that BPb was positively associated with AL index (R^2=0.100; B=0.204; p=0.032). Correlation analyzes with individual biomarkers showed that BPb was positively correlated with HDL cholesterol (p=0.02) and negatively correlated with DHEA-S (p=0.049). These findings suggest that Pb exposure, even at levels below the reference blood lead level for adults recommended by the National Institute for Occupational Safety and Health and by the Center for Disease Control and Prevention, may contribute to AL and dysregulated cortisol functioning in older adults. Considering these findings were based on cross-sectional data future research is needed to confirm our exploratory results.

1. Introduction

Environmental contaminants are of increasing interest in relation to stress pathophysiology. Heavy metals exposure, particularly lead (Pb), constitutes one of the main contaminants investigated because of their pernicious consequences over the course of life (Weiss, 2007). Although several public policies have successfully lowered blood lead (BPb) levels in many countries over the last thirty years, exposure to elevated Pb levels still poses a concern especially in emerging and low income countries where environmental regulations are fragile and at the mercy of industrial development (He et al., 2009). Indeed, the general population continues to be exposed to Pb from different resources including solder, gasoline, battery industries and consumer products such as toys, lead-based paint, cosmetic products, crayon, color pencil and even candies (Center for Disease Control and Prevention - CDC, 2009). Elderly are one of the target vulnerable

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populations to the negative effects of Pb exposure mainly because the long lifetime exposure allied to their reduced capacity to compensate for impairment (Weiss, 2007). Furthermore, the skeleton is the site of storage for around 95% of lead in the adult human body, resulting in a release of lead in blood in aging people with bone demineralization, especially during postmenopausal period (Baecklund et al., 1999; Barbosa Jr. et al., 2005).

Pb exposure has been related to several adverse health outcomes in adults including neurological and cardiovascular effects. Consistent evidence demonstrated that low-level Pb exposure as indicated by less than 10 μg/dL is associated with increased blood pressure, increased risk of hypertension, increased incidence of essential tremor and decreased cognitive function (Armstrong et al., 2014; Peters et al., 2010; Power et al., 2014; Weisskopf et al., 2007). Neuroendocrine disrupting effects have also been associated with Pb concentration (Weiss, 2012). Pb can disrupts endocrine systems via steroid hormone dysregulation affecting brain development and functioning (Weiss, 2012). In particular, elevated Pb levels disrupt the hypothalamic-pituitary-adrenal axis (HPA) axis, a stress-sensitive system that culminates in the production and release of glucocorticoid (cortisol in humans) from the adrenal glands following stress exposure (McEwen, 1998).

Studies in animals have shown that maternal Pb exposure, even at low levels, permanently alters the offspring HPA axis function, resulting in basal and stress-induced hypercortisolemism both in the neonatal and post-weaning periods (Cory-Slechta et al., 2004, 2008; Virgolini et al., 2004, 2008). Additionally, Pb exposure during rodent development leads to significant prolonged HPA axis response to acute stress (Rossi-George et al., 2009). In adulthood, rodents and the offspring exposed to Pb (before breeding, during pregnancy and after weaning) exhibit lower glucocorticoid levels than animals with no Pb exposure (Rossi-George et al., 2011). In humans, a few studies have examined the relationship between Pb exposure and stress. Children with high Pb levels exhibit heightened cortisol response following a standard cold pressor task. By contrast, no association was observed between Pb exposure and cortisol changes in basal levels (Gump et al., 2008). More recently, high prenatal BPb exposure (≥10 μg/dL) was associated with low cortisol concentration producing a downshift in the cortisol curve at 12 months-olds children (Tamayo y Ortiz et al., 2016). In adults, Fortin et al. (2012) reported a significant association between lead exposure and higher ACTH/CORT ratio in occupational-Pb exposed participants, suggesting a Pb-induced alteration of the HPA axis (Fortin et al., 2012). In non-occupational adults, pregnant women with higher BPb concentrations showed reduced cortisol awakening response that is an important predictor of adverse health outcomes (Braun et al., 2014). Given that the HPA axis functions within a multi-systemic network, it is critical that future studies of Pb in humans also assess broader physiological dysregulations.

To date, the available evidence vis-a-vis Pb and stress biology in humans is scarce and limited to situations of acute stress (e.g., reactivity). This is problematic since chronic stress promotes widespread dysfunctions in numerous systems and is associated with several disorders and neurological problems (Lupien et al., 2009; Souza-Talarico et al., 2011; Virgolini et al., 2008). Based on the allostatic load (AL) framework of chronic stress (McEwen and Stellar, 1993), prolonged and repeated activation of the stress system leads to frequent recalibrations in basal body functioning in order to adapt to environmental demands. This strain produces a “domino effect” in a non-linear multi-systemic physiological dysregulations that may lead to stress-related disorders over time (McEwen and Stellar, 1993; Juster et al., 2011a, 2011b; Picard et al., 2014).

At the subcellular level, the AL “domino effect” begins with glucocorticoid dysregulations and altered glucose levels (primary mediators) induced by chronic stress exposure, followed by mitochondria damage due to oxidative stress and inflammation (primary effects) and cellular dysfunction (primary outcomes; Picard et al., 2014). Cumulatively, these primary effects perturb metabolic, cardiovascular, immunological and neural systems producing subclinical outcomes such as elevation of blood pressure and body mass index, and dyslipidemia (secondary outcomes) that consequently, throughout organ and systems failure, leads to disease (tertiary outcomes) (McEwen and Stellar, 1993; Lupien et al., 2009; Juster et al., 2011a, 2011b; Picard et al., 2014).

There is a small but growing literature linking Pb exposure to AL. In epidemiological studies, cross-sectional and longitudinal associations have been reported between Pb exposure and elevated blood pressure including development of hypertension (Schwartz, 1988; Cheng et al., 2001; Navas-Acien et al., 2008). More recently, Zota et al. (2013) showed that higher BPb is associated with elevated blood pressure among adult men with high AL those with low AL suggesting that AL may amplify the BPb and blood pressure relationship (Zota et al., 2013). Taken together, most findings linking Pb exposure and AL are based on secondary outcomes (e.g., lipids, blood pressure) as predictors of tertiary outcomes (e.g., cardiovascular disease).

Another critical aspect that weakens the understanding of the relationship between Pb and chronic stress in humans is the lack of evidence during aging. This is a matter of concern since elderly populations are especially vulnerable to the effect of Pb. Given that the world population is growing older, it is imperative to identify environmental factors that influence the aging population in order to prevent loss of life-quality and to ensure the most successful aging possible.

The current study aimed to investigate whether Pb levels are associated with altered diurnal cortisol profiles, a primary mediator of AL, during aging. Given that a failure to shut down the HPA axis is a neuroendocrine profile that characterizes pathophysiological states and AL during aging (McEwen et al., 1998), we hypothesized that older adults with higher BPb levels would exhibit hypercortisolemic profiles and higher rate of subclinical AL biomarkers and AL index.

2. Methods

2.1. Participants recruitment, selection and ethic procedures

One hundred and twenty-six older adults (n=126; 105 women and 21 men) with 65.9 (± 8.1) years of age and 9.8 (± 4.5) years of education from São Paulo, Brazil were included in the study protocol. Individuals were recruited using media advertisements (radio, television and internet) and those who contacted the Department of Psychobiology were initially screened by a standardized telephone interview. Those who did not fulfill the following criteria were excluded: neurological or psychiatric disorder, history of alcohol, drug or tobacco abuse in the last 10 years, Pb occupational exposure, individuals living in geographic areas of known for high Pb contamination, under use of psychoactive drugs, synthetic glucocorticoids or steroids medications. Recent dental treatment was also an exclusion criterion to prevent saliva contamination with blood, which in turn could influence free-cortisol levels. All female participants were post-menopausal.

Selected participants were scheduled for an individual interview at the Department of Psychobiology of UNIFESP for routine laboratory tests, anthropometric measures and neuropsychological assessment. Participants were excluded if they presented hemoglobin alterations (elevated Pb is associated with decreased levels of hematocrit and hemoglobin; Barbosa Jr. et al. 2005), cognitive decline combined with functional impairment analyzed by the Mini-Mental State Examination (MMSE) (Brucki et al., 2003; Folstein et al., 1975) and the Informant Questionnaire on Cognitive Decline (IQCODE) (Bustamante et al., 2003; Jorm and Jacomb, 1989), and if they screened for depression according to the Geriatric Depression Symptoms (Yesavage et al., 1982). Of the 136 participants who contacted the research center, eight refused to participate and two were not eligible because of dental

treatment, which could contaminate salivary sampling for cortisol determination, combined to low cognitive functional performance.

The study was approved by the Ethic Committee of Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil (# 0823/09) and all participants provided informed consent.

2.2. Blood sampling

Morning fasting venous blood samples (30 mL) were drawn from the antecubital vein in vacuum tubes (Vacutainer®) for AL biomarkers collected into 6 mL trace metal-free tube (Vacutainer®, EDTA) for blood Pb (BPb) concentrations. Specimens were centrifuged (800×*g* for 10 min). Plasma fractions were then alliquoted and immediately frozen at −20 °C.

2.3. Blood lead determination

BPb was determined by inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer DRC II; PerkinElmer, Wellesley, MA, USA) (Barbosa Jr. et al. 2005). Samples were diluted 1:50 in 15-mL polypropylene Falcon® tubes (Becton Dickinson) with a solution containing 0.01% (v/v) Triton® X-100, 0.5% (v/v) nitric acid and 10 μgL^{-1} of each one of the internal standards (Rhodium and Iridium). The detection limit was 0.04 μg/dL. The values obtained in this study were in adequate agreement with target values according to Student's t-test at 95% confidence level. The target values and confidence intervals were provided by the L'Institut National de Santé Publique du Quebec, Canada. Between and within-batch precision for BPb were lower than 14%. Participants were classified into two groups according BPb median concentration: BPb ≥2 μg/dL and low BPb levels for those with BPb levels less than the median.

2.4. Allostatic load assessment

2.4.1. Allostatic load index

Based on prior review regarding AL framework and biomarkers (Juster et al., 2010), we analyzed AL by creating a cumulative index of physiologic systems dysregulation following six biological markers: dehydroepiandrosterone-sulfate – DHEA-S (neuroendocrine biomarker that antagonizes cortisol), triglycerides, total cholesterol, high density lipoprotein – HDL – cholesterol, glucose (metabolic biomarkers) and body mass index (anthropometric biomarker). Values of each participant's biomarkers were aggregated into a count-based AL index as traditionally done (Seeman et al., 1997). Therefore, quartile ranges based on the clinical reference ranges distribution determined the risk cut-off for each biomarker. The AL index was calculated by summing the number of values equal or above the 75th percentile for total cholesterol, triglycerides, glucose and body mass index. For HDL and DHEA-S values falling below the 25th percentile were used to compute the AL index, as low levels denote risk. AL index was computed for each subject based on the total number of factors in which the participant's measurement scored in the high-risk category. AL ranged from 0 to 5 and each participant was dichotomized into high (AL≥3) or low (AL < 3) based on the AL median (Crimmins et al., 2003 and Geronimus et al., 2006).

2.4.2. Diurnal cortisol profiles

As previously described, cortisol is a primary mediator and, therefore, a major player in the AL model. Cortisol dysregulation is linked to cumulative physiological "wear and tear" resulting from chronic overactivity of the body's stress-response system (McEwen et al., 1998; Picard et al., 2014). Despite the fact that several studies incorporated a single measure of cortisol as a neuroendocrine biomarker into AL index, recent findings demonstrate that cortisol profiles (i.e., hypocortisolemia) under acute stress or over the course of the day are associated with high AL (Juster et al., 2011a, 2011b). In addition, previous findings have shown that a single cortisol measure does not contribute to AL indexation (Juster et al., 2011a, 2011b). As cortisol is part of the primary mediators of AL dysregulation, it is possible that AL indices are capturing physiological dysregulations downstream of HPA-axis malfunctioning.

The AL theoretical framework of pathophysiology postulates four neuroendocrine profiles that represents altered stress responses that contribute to allostatic states: (1) repeatedly activated responses, (2) non-habituating responses, (3) prolonged responses, and (4) and inadequate responses (McEwen, 1998, 2006). In the aging scenario, hypercortisolemic profiles, related to hippocampal dysregulation of HPA axis negative feedback have been reported (McEwen, 1998, 2006). Altogether, this evidence supports the assessment of cortisol profiles as an indicator of AL manifestation that may be captured in a repeated measures manner more than a single measure. In this manner, our AL index does not incorporate a summary measure of diurnal cortisol as this would be redundant in analyses assessing repeated measures of diurnal cortisol that we view as an outcome representing allostatic states.

Salivary samples were collected with Salivette© (Sarstedt©, code: 51.1534), a polyester-coated cotton swab, that was placed in the participant's mouth for two minutes in order to saturate the cotton and was then placed in a plastic tube and stored in a home freezer until the second visit. Detailed oral and written instructions for proper collection were provided.

Participants were guided to collect the saliva at five time points per day during two non-consecutive workdays (upon awakening, 30 min after wakening, 14:00 h, 16:00 h and at bedtime). Participants' compliance regarding saliva sampling was monitored using the Medication Event Monitoring System (MEMS®). This is an electronic monitoring system composed of a standard plastic vial (where the Salivettes© are placed in) and a screw top that contains a microelectronic circuit that registers times when the screw top is opened and when it is closed. The events stored in the microelectronic circuit in the MEMS® can be transferred to a computer and read using the MEMS® software. Participants were instructed to open the plastic vial and remove a single Salivette© at each designated sampling time. They were also advised that the Salivette© must remain in the container until usage to ensure valid hormone analysis. This compliance technique has been widely used as the gold standard measure of patient compliance (Kudielka et al., 2003). When the participant returned the saliva samples to the research center, the sampling times were immediately verified and in case of non-adherence to the protocol the participant was asked to collect the samples again. Only five participants had to repeat the sampling protocol. This procedure ensured that all the saliva samples were collected in the required time.

The sampling time yielded the assessment of cortisol awakening response (CAR), a reliable biomarker of HPA axis functioning, and is considered a promising sub-clinical outcome of psychosocial status and health (Pruessner et al., 1997; Hellhammer et al., 2007; Clow et al., 2010) and diurnal cortisol rhythm. Under physiological conditions, the diurnal cortisol follows a circadian pattern of secretion characterized by increasing levels in the morning as a response to the awakening demands followed by a progressive decline until a nadir phase at approximately midnight (Pruessner et al., 1997; Stalder et al., 2015). Both heightened and attenuated CAR are associated with several adverse outcomes such as cardiovascular disease and psychopathologies (Fries et al., 2009).

Saliva samples were stored at −20 °C until free cortisol levels were determined using a commercially available enzyme immunoassay kit (Salimetrics©, State College, PA, USA) following the Salimetrics recommendations by a certified research laboratory. The limit of cortisol detection was 0.01 μg/dL, the intra- and interassay variability was 7.4% and 12.4%, respectively (on a range of 0.1–10 μg/dl dose).

2.5. Questionnaires

Participants were administered the Perceived Stress Scale - PSS (Cohen et al., 1983) to assess stress perception in the past thirty days and the Geriatric Depression Symptoms – GDS (Yesavage et al., 1982) to evaluate humor state. Socioeconomic status (SES) was also evaluated according the criteria of Brazilian economic classification that is composed of questions regarding education level of the household head, durable goods and access to public services (Associação Brasileira de Empresas de Pesquisa – ABEP, 2015).

2.6. Statistical analysis

All dependent variables were checked for assumptions of normality. The non-normally distributed dependent variables (cortisol, BPb concentrations and AL index) were log-transformed. Cortisol awakening response (CAR) and overall cortisol levels after CAR were determined using the area under the curve (AUC). Briefly, the calculation of AUC is based on breaking down the AUC in many trapezoids, then, calculating and adding the area of each trapezoid to yield one overall value (Pruessner et al., 2003). Thus, the CAR was calculated using the area of trapezoid 1 from awakening to 30 min after waking (Pruessner et al., 2003; please see Fig. 1). The overall cortisol levels or total cortisol secretory activity after the CAR was calculated adding the area of trapezoid 2, 3 and four from 30 min after waking to bedtime (Pruessner et al., 2003; please see Fig. 1). Differences between groups were analyzed by the chi-square test for categorical data and by Student's-*t*-test for continuous data.

To test whether BPb concentrations were associated with cortisol levels (AUC total using AUC_G and CAR using AUC_I) and with AL index, we used Pearson's correlation coefficients and Multiple Linear Regression (MLR) modeling. In addition to BPb concentration as the continuous independent variable, the model included age, sex, time of awakening, socioeconomic status, PSS scores and GDS scores as determinants of cortisol levels and AL index. BPb by SES interaction term (BPb multiplied by SES) was also inserted in the MLR as an independent variable to test whether the interaction between BPb concentration and SES would predicts cortisol levels (CAR and AUC total) and AL index. Multicollinearity effects were reduced using centered methodology to create BPb by SES centered interaction terms (Howell, 2013).

To analyze whether the diurnal cortisol profile would change as a function of BPb levels, we performed Repeated Measures Analysis of Variance (RM – ANOVA). Dichotomized BPb levels (BPb above the median *x* BPb below the median) were inserted as the between-subject factors and average cortisol concentrations at awakening, 30 min after waking, 14:00 h, 16:00 h and at bedtime as the within-subject factor. BPb above the median group was classified as such when BPb concentration was equal or above the median. The dichotomization of Pb values allows us to explore how BPb and allostatic states (McEwen, 1998) effect and interact to modulate cortisol rhythmicity over the day. Moreover, allostatic load states represent pathophysiological profiles that are best observed using repeated measures design that requiring a grouping variable.

The level of significance was set at 5% ($p < 0.05$, 95% confidence interval) and the statistical calculations were performed with SPSS Statistics software for Windows (SPSS Inc., Chicago, IL, USA, 14.0 version).

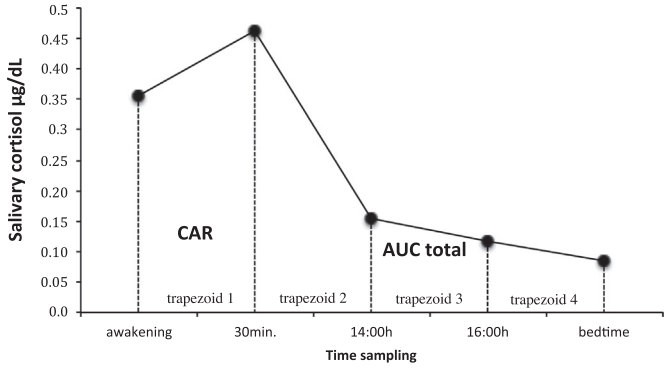

Fig. 1. Schematic representation of the area under the curve for cortisol levels. The area of a trapezoid is calculated as following: Interval between two samples x (cortisol concentration at time 1+ concentration at time 2)/2. **CAR**=area trapezoid 1=30 min×(0.25 µg/dL +0.35 µg/dL)/2=9 µg/dL over the course of the 30 min after awakening **AUC total** = area trapezoid 2+ area trapezoid 3+ area trapezoid 4=420 min×(0.45 µg/dL +0.15 µg/dL)/2+120 min×(0.15 µg/dL +0.13 µg/dL)/2+420 min×(0.13 µg/dL +0.1 µg/dL)/2=126+16.8+48.3=191.1 µg/dL over the course of 16 h.

3. Results

3.1. Blood lead levels, demographics, and neuropsychological characteristics

All the participants exhibited BPb levels below the current reference blood lead level recommended by the National Institute for Occupational and Safety Health (NIOSH), the CDC and by the Adult Blood Lead Epidemiology & Surveillance program (ABLES) that is < 5 µg/dL, ranging from to 0.6–6.1 µg/dL (M=2.1; SD= ± 0.9). No demographic characteristics or psychological differences were observed between participants with BPb above the median, compared to those with lower BPb levels (Table 1).

3.2. Blood lead and diurnal cortisol profiles

Controlling for age, gender, time of awakening, socioeconomic status (SES), GDS and PSS scores the MLR revealed that BPb was positively associated with CAR (p=0.007) and AUC total ($p < 0.001$), explaining 12.8% of the CAR and 26.6% of the AUC total variability (Table 2). BPb×SES interaction term was not associated with CAR or with AUC total (Table 2).

Diurnal cortisol profiles was assessed controlling for age (p=0.561), sex (p=0.613) and time of awakening (p=0.187), a repeated-measure ANOVA analysis showed BPb x Time within-subject effect ($F(4, 492) = 3.5$; p=0.025) on cortisol levels. Participants with BPb above the median exhibited higher cortisol levels than those with lower BPb concentration at the following sampling times: 30 min after waking (p=0.003) and 14:00 h (p=0.002). A marginally significant difference was observed at the 16:00 h sampling time (p=0.073; Fig. 2).

3.3. Blood lead and allostatic load biomarkers

AL index ranged from 0 to 5 points (M=2.24; SD= ± 1.04). Controlling for age, gender, SES, PSS and GDS scores the MLR revealed that BPb was positively associated with AL index (r=0.204; p=0.032), explaining 10.0% of the AL index variability (Table 2).

Supplemental analyses assessed correlations with individual biomarkers comprising the AL index. The percentage of individuals who exhibited high risk for each AL biomarker was higher for glucose followed by BMI and HDL cholesterol (Table 3). An association between BPb and individual AL biomarker was observed (p≤0.049). The higher the BPb the higher the DHEA-S and the lower the HDL concentration (Table 3). AL was not associated with CAR (r =0.083; p=0.365) or AUC total (r =0.083; p=0.766).

4. Discussion

This study investigated whether BPb concentrations is associated with hypercotisolemic diurnal profiles as a primary mediator of AL in a sample of non-occupationally exposed Brazilian older adults.

Table 1
Demographic and neuropsychological characteristics according blood lead levels.

	Total sample		High BPb		Low BPb		
	n=126		n=64		n=62		
Variable	Mean (SD ±) or N (%)	Range	Mean (SD ±) or N (%)	Range	Mean (SD ±) or N (%)	Range	p*
Age	65.9 (8.1)	50–82	65.1 (8.4)	50–82	66.4 (7.5)	50–79	0.062
Years of education	9.8 (4.5)	3–25	9.9 (4.5)	3–20	9.7 (4.5)	3–25	0.447
Socioeconomic status[a]							
Low; N (%)	39 (31.0)		18 (46.2)			21 (53.8)	
Medium; N (%)	76 (60.3)		40 (52.6)			36 (47.4)	0.779
High; N (%)	11 (8.7)		6 (54.5)			5 (45.5)	
Perceived stress (PSS)[b]	23.7 (6.4)	7–45	24.3 (7.3)	7–45	22.9 (5.5)	14–37	0.247
Depression (GDS)[c]	2.9 (2.8)	0–8	3.3 (3.2)	0–8	2.5 (2.3)	0–5	0.108
Cognition (MMSE)[d]	27.6 (1.5)	23–30	27.7 (1.5)	23–30	27.5 (1.5)	23–30	0.154

* Student's *t*-test for continuous data and Chi-square for categorical data, [a]Criteria of Brazilian economic classification (*Associação Brasileira de Empresas de Pesquisa*). [b]Perceived stress scale, [c]Geriatric Depression Scale, [d]Mini-Mental State Examination.

Corroborating our hypothesis, BPb was positively associated with CAR and overall cortisol levels after CAR during the course of the day. Specifically, participants with BPb ≥2 μg/dL showed higher cortisol levels at 30 min after awakening and in the afternoon than those with BPb concentrations below the median. Additionally, high BPb concentrations were correlated with high AL index and with low HDL and high DHEA-S secondary outcomes of AL. Our findings suggest that Pb exposure, even at levels below the NIOSH/CDC/ABLES recommended threshold, may contribute to an AL state represented by inefficient HPA axis negative feedback in older adults.

The cumulative biological damage derived from chronic stress exposure compromise the HPA axis. This pattern of dysfunction may lead to a failure to shut down the activated HPA axis by deficiency of the glucocorticoid negative feedback (McEwen et al., 1998). During the first hours of the day the HPA axis negative feedback, mediated by the corticolimbic system (i.e. prefrontal cortex, amygdala and hippocampus), is essential for the regulation of the CAR and awakening demands (Clow et al., 2010). During aging, a distinctive pattern characterized by large CAR in combination to high diurnal cortisol has been reported in older adults (Kumari et al., 2010). Similarly, elevated HPA axis reactivity to acute psychosocial stressors and to pharmacological challenge reinforces the age-related HPA axis negative feedback deficiency (for a review see Kudielka et al., 2009). It has been postulated that inefficient age-related HPA axis 'shutting down' derives from a diminished ability of the hippocampus and pre-frontal cortex to maintain a sufficient negative feedback (Sapolsky et al., 1986). Interestingly, the corticolimbic system is also a brain target of Pb *via* the dopaminergic and glutamatergic pathways acting mainly in the nucleus accumbens and hippocampus (Cory-Slechta et al., 2004; Rossi-George et al., 2009, 2011; Zuch et al., 1998). Therefore, the fact that a higher CAR and overall cortisol secretion throughout the day were observed among participants with high BPb concentrations may be interpreted as a decreased HPA axis responsiveness to endogenous awakening demands added to a less efficient negative feedback of the HPA axis, leading to higher cortisol levels.

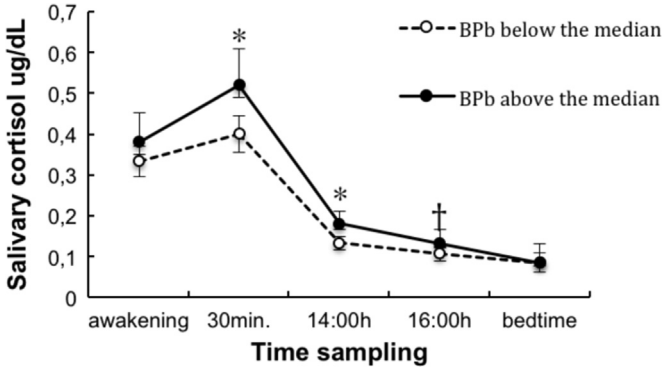

Fig. 2. Mean cortisol concentrations throughout the day according to BPb groups (BPb ≥2 μg/dL x low BPb). Participants with blood lead levels equal or more than 2 μg/dL exhibited higher cortisol concentration in the morning and at 14:00 h. *p < 0.05 and †indicate a marginally significant difference between BPb groups in *post hoc* tests. Values are presented as mean ± SEM.

While animal evidence corroborates our interpretations (Cory-Slechta et al., 2004, 2008; Virgolini et al., 2004, 2008), human findings are quite contradictory. In children, high prenatal and postnatal Pb exposure does not impact basal cortisol levels, albeit children with

Table 2
Multivariate regression results between blood lead, cortisol concentration and allostatic load adjusted for covariates.

Model	CAR					AUC total					AL index				
	B	*P*	R^2	F	*p*	B	*p*	R^2	F	*p*	B	*p*	R^2	F	*p*
			0.128	1.929	0.047*			0.266	4.222	< 0.001*			0.100	3.933	0.011*
BPb	0.791	0.005*				0.889	< 0.001*				0.204	0.032			
Age	−0.008	0.251				−0.006	0.210				−0.105	0.286			
Gender	−0.157	0.279				−0.182	0.140				0.202	0.044*			
Awakening	−0.150	0.112				− 0.355	< 0.001*				–	–			
SES[a]	0.699	0.093				0.331	0.296				0.143	0.142			
BPb x SES	−0.203	0.203				−0.090	0.490				0.071	0.245			
PSS[b]	−0.012	0.343				−0.001	0.911				−0.023	0.863			
GDS[c]	0.010	0.712				−0.012	0.586				0.060	0.652			

* indicates significant association (p≤0.05). [a]Criteria of Brazilian economic classification (*Associação Brasileira de Empresas de Pesquisa*). [b]Perceived stress scale, [c]Geriatric Depression Scale.

Table 3
Correlation between PbB and each AL biomarker and criteria for clinical AL index.

AL biomarker (Unit)	Blood lead (μg/dL) r (p)[a]	Mean (S.E.M.)	Clinical reference	25th percentile	75th percentile	High risk N (%)[b]
DHEA-S (μmol/L)	0.180 (0.049)*	58.5 (4.1)	0 – 18.7	4.68[b]	14.03	0 (0)
Glucose (mmol/L)	0.026 (0.776)	5.6 (0.09)	3.3 – 5.5	3.85	4.95[b]	106 (84.1)
Triglycerides (mmol/L)	0.041 (0.652)	1.2 (0.05)	< 2.26	0.56	1.69[b]	21 (16.7)
Total cholesterol (nmol/L)	−0.022 (0.808)	5.1 (0.09)	3.3 – 5.2	1.55	4.65[b]	14 (11.1)
HDL cholesterol (mmol/L)	−0.210 (0.02)*	1.6 (0.05)	> 1.55	1.16[b]	–	68 (53.9)
Body mass index	0.025 (0.784)	25.9 (0.4)	18.5–25	18.5	25[b]	70 (55.6)

[a]Pearson's correlation coefficient. [b] Frequency of participants who attained scores within the high-risk cut-off for each biomarker. The 25th and 75th percentiles were derived from the clinical reference range and the number of biomarkers reaching these criteria became the individual's AL index, ranging from 0 to 6. * indicates significant association (p≤0.05).

higher Pb showed heightened cortisol responses to the cold pressor task (Gump et al., 2008). Conversely, occupationally exposed middle-age men with high Pb levels (blood Pb levels > 4 μg/dL) exhibit lower baseline cortisol levels and adrenal hyporesponsiveness to a psychological stressor (Fortin et al., 2012). Altogether, this evidence sustains the interpretation that altered cortisol profiles represent an AL related to environmental Pb exposure.

Reinforcing our finding that Pb exposure is related to AL during aging, we also found that high BPb concentrations were correlated with high AL index and with low HDL and high DHEA-S secondary outcomes of AL. As previously described, a temporal and causal sequence of damage due to repeated physiological recalibrations compensate for environmental insults under chronic conditions. After over- or underactivation of primary mediators – such as the hypercortisolemic diurnal profile we observed in high BPb participants – secondary outcomes become manifested. Secondary outcomes represent subclinical patterns of biomarkers commonly used in biomedicine that emerge as a result of the cumulative biological damage of primary mediators like cortisol, catecholamines, and cytokines (Juster et al., 2011a, 2011b). For example, the association between high BPb and low HDL cholesterol concentrations may represent dyslipidemia that is a powerful risk factor for cardiovascular diseases (Yusuf et al., 2004). Interestingly, a recent study demonstrated that AL amplifies the effect of Pb exposure on blood pressure in middle-aged participants (Zota et al., 2013). Lower HDL cholesterol levels are observed in Pb exposed workers compared to controls (Sharma et al., 2012) and non-occupational Pb exposure is also associated with cardiovascular diseases such as hypertension, coronary heart disease, stroke mortality and peripheral artery disease, in some cases at Pb levels < 5 μg/dL (for a review see Navas-Acien et al., 2007).

The correlation between BPb and DHEA-S again reinforces the suggestion that AL load induced by environmental contaminants may compromise health. This correlation may indicate the cumulative physiological effort of the adrenal system to antagonize the negative effects derived from hypercortisolemia related to Pb exposure. However, no evidence exists to support this interpretation. Interestingly, some data showed association between DHEA-S and metabolic syndrome that is in turn associated with AL (Muller et al., 2005; Phillips et al., 2010; Seeman et al., 2001). Although we observed only a trend toward significant associations between BPb and the composed AL biomarkers index, previous findings showed that specific clusters of AL biomarkers predict tertiary AL outcomes differentially (Juster et al., 2010). Additionally, primary mediators may contribute independently to cardiovascular and metabolic risk factors in predicting unhealthy outcomes (Karlamangla et al., 2002).

Some limitations should be considered in the interpretation of the current findings. First, the ratio between women and men in the sample was uneven. While sex was used as a covariate in the analysis, Pb levels interfere with gonadal hormones (Weiss, 2012) and a Pb by sex interaction effect could be present. Second, Pb exposure was determined using blood Pb levels that may reflect both recent and past exposure (Barbosa Jr. et al., 2005). Thus, Pb effects may reveal cumulative or current effects on the HPA axis. Bone lead is the preferable marker of cumulative Pb exposure to more accurately ascertain the biological window of exposure (Barbosa Jr. et al., 2005). Third, other environmental contaminants such as cadmium should be taken into account in future studies since they often interact with Pb (Zota et al., 2015). Finally our results were based on a small size and on a cross-sectional design and no “cause and effect” assumptions could not be addressed.

Despite these limitations, the levels of BPb reported in the current study could be considered in the range of reference values previously determined. A population-based study conducted in the metropolitan area of São Paulo, Brazil (the same city of the current study), reported a BPb mean similar to US population values (Kuno, 2009). Furthermore, the current findings were observed at low levels of exposure, that was < 5 μg/dL, among non-smokers and non-occupationally exposed older adults and thus may be generalized to various populations. Another strength is our contribution to understanding Pb effects on cortisol levels and AL under different socioeconomic contexts. The participants were recruited in an urban center from an emerging economy country known to be more often susceptible to the exposure to multiple combinations of environmental contaminants and to the psychosocial determinants of AL (Sexton, 1997; Juster et al., 2016).

In addition to the previous studies that demonstrated the effects of Pb on the acute stress response, our findings advance the understanding of Pb effects on basal HPA axis functioning by reporting heightened CAR and increased cortisol levels throughout the day in older individuals with high BPb levels. Furthermore, high BPb levels were associated with high AL index. This mechanism is noteworthy when considering that persistent hypercortisolemia is detrimental to health and represents a critical risk factor for several psychopathologies (Juster et al., 2011a, 2011b; McEwen, 2006).

5. Conclusion

The current findings showed that even at very low levels of exposure, BPb concentrations are associated with AL mediators and outcomes such as hypercortisolemic diurnal profile, low HDL cholesterol and high DHEA-S. The diminished functional biological reserve of metabolic substrates during aging may be challenging to numerous physiological systems that overcompensate against the cumulative damage of Pb exposure. Ultimately, this noxious interaction could lead to greater vulnerability to develop stress-related disorders, as individuals get older. Taken together, our results complement previous studies and sustain the idea that Pb reference values for older adults need to be reviewed and that strategies to prevent exposure to heavy metals and stress must be reinforced.

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