Author's response to reviews

Title: Allostatic load amplifies the effect of blood lead levels on elevated blood pressure among middle-aged U.S. adults: -a cross-sectional study

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Author's response to reviews: see over
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To the Editors:

On behalf of my co-authors, I am pleased to re-submit our manuscript, entitled, “Allostatic load amplifies the effect of blood lead levels on elevated blood pressure among middle-aged U.S. adults: a cross-sectional study” for review with Environmental Health.

We appreciate the reviewers’ excellent feedback and have revised the manuscript significantly. Our specific responses to their comments appear below. If you require additional information, please do not hesitate to contact me via email: rmf@berkeley.edu or phone: 510-643-6358. I look forward to hearing from you.

Very Truly Yours,

Rachel Morello-Frosch, Ph.D., M.P.H.
Professor, School of Public Health & Department of Environmental Science, Policy, and Management
Minor Essential Revisions
1. Please clarify the sample size discussion. The authors state they use a sample size of 6,511; however, the full model in Table 3 only has n=6069, which is a sizeable decrease. Similarly, were there any differences between the participants not included in the study due to lack of lead and/or blood pressure measurements compared to those who were included in analyses?

Following reviewer recommendations, we added one additional cycle (2007-2008) of NHANES data. We could not add the 2009-2010 cycle because they have not released triglyceride measurements on the entire study population, and this is a core variable in our allostatic load measurement. Our new sample size is 8194. The study sample consists of US adults aged 40 to 65 years that had data on all key variables including blood lead, allostatic load components, and model covariates. In the revised manuscript, all tables have the same sample size except for sensitivity analyses.

After adding the additional data, there were 9918 eligible participants. Of those, 1724 (16%) were not included because they were missing data on one of the covariates or key variables. There were no significant differences in mean levels of blood lead or blood pressure nor in blood pressure medication use between the two groups. However, those not included had a higher prevalence of systolic and diastolic hypertension and a lower mean allostatic load score. Those not included were also more likely to be younger, female, non-Hispanic black or other/mixed race, lower educated, never married, current or former smokers, but less likely to drink alcohol regularly.

We have added information on our sample size in the results section of the main text.

2. Please include some measure of variability for the percentages presented in Tables 1 & 2, such as the standard errors you present for the means/geometric means.

We have added standard errors for the percentages presented in Tables 1 and 2.

Discretionary Revisions
1. Is there a reason why you chose these particular years of NHANES (as data from 2007-2010 are now available)? Also, as noted by the prior reviewer, was there a reason why this specific age group was selected?

As noted above, in the revised analysis we included data from 2007-2008 cycles, but did not included 2009-2010 due to incomplete data on key variable. As stated in the study population description in the methods of the manuscript,

“In this study, we restricted the study population to those who were 40-65 years of age to minimize the effect of confounding by age, which is strongly associated with lead exposure, blood pressure, and AL”

2. Out of curiosity, did you notice any effect of different NHANES cycles on any of these parameters? Blood lead measurements decrease over this period, for example; there may be other differences as well.

There were no significant differences in prevalence of elevated systolic or diastolic blood pressure by NHANES cycle. As anticipated, blood lead levels decreased over the study period. Blood pressure medication use increased slightly over the study period (BP med use was 22.9 (1.6)% in 1999-2000 versus 26.3 (1.2)% in 2007-2008). Mean allostatic load decreased over the study period.

3. Out of curiosity, did you also assess potential effects with prehypertension status?

We are unclear how the reviewer defines “prehypertension status”, but in the revised manuscript we now report results for continuous blood pressure outcomes as well as binary hypertension outcomes.
4. Consider including the numeric concentrations of lead instead of/in addition to the “quintile 1”, etc. headings within Tables 3 & 4. This would help the reader orient themselves to the concentrations without having to go back to the prior tables.

We appreciate the recommendation. Given that Tables 3 and 4 are already very dense, we have chosen to not include numeric concentrations. However, to facilitate interpretation of our results, we have used the same sample size in all of our various tables and analyses.

5. I agree with the previous reviewer that some discussion of the potential for reverse association would be useful here, given the relationships between lead exposure and particularly allostatic load and blood pressure.

We agree with the reviewer that this could be an issue with our analysis, but the cross-sectional design of our study and the inherent limitations of NHANES data make it impossible to examine this issue in this paper. We have raised this limitation in the discussion section which reads:

“In addition, the cross-sectional design of our study precludes a systemic assessment of the temporality of lead exposure and allostatic load, the potential for reverse causation between hypertension and AL, or of temporal trends or the potential effects of cumulative lead exposures throughout the life course, since blood lead (as opposed to bone lead) mostly reflects recent and ongoing exposures as well as lead that has been mobilized from tissue stores such as bone [48].”

6. In the discussion, page 15, paragraph 1, last sentence: given that this directly follows discussion of temporality, it seems that the last sentence implies that the sensitivity analysis addresses all of the study limitations; this could be made clearer.

Thank you. We have revised the language in the discussion section so that it is clear that our manuscript does not address all study limitations.
**Reviewer 2: M. Hicken**

**Major Compulsory Revisions**

1. The major problem that I see with this version of the paper is the operationalization of social stress with a version of the allostatic load score. In the abstract (summarizing the argument of the Background section), the authors state, “We conducted a cross-sectional study to determine whether chronic stress, operationalized as allostatic load (AL), modifies the effect of lead exposure on blood pressure among middle-aged adults.” Then, in the Background section, the authors state that, “Allostasis refers to how the body’s stress response systems regulate internal physiology in response to chronic exposure to physical, social, and environmental stressors.” [emphasis added]. Because of this definition, it is difficult for the reader to isolate the notion of allostatic load to social stress. However, this problem can be circumvented by simply (relatively simply) reframing the Background section so that the focus is not on social stress, but on the notion of vulnerability to the hypertensive effects of lead. This vulnerability may stem from numerous sources to result in the physiologic dysregulation that can be operationalized by their version of the allostatic load score. For example, others have examined the notion that diabetes, for example, increases vulnerability to the health effects of environmental hazards. In the Discussion section, then, the authors can broaden their scope to link this work to the literature on the notion that social stress may increase vulnerability to the health effects of environmental hazards and the literature linking social stress to the notion of allostatic load.

   We agree with this important issue and have rewritten the background section of the abstract to address this feedback. It now reads:

   “Scientists and regulators have sought to understand whether and how physiologic dysregulation due to chronic stress exposure may enhance vulnerability to the adverse health effects of toxicant exposures. We conducted a cross-sectional study to determine whether allostatic load (AL), a composite measure of stress response in the body, amplifies the effect of lead exposure on blood pressure among middle-aged adults.”

   We also added a sentence on page 6 in the background section of the main text that reads:

   “Therefore, allostatic load is a composite biomarker of the cumulative biological burden exacted by ongoing disruption of the body’s stress-response system [33] that may increase vulnerability to the adverse health effects of toxicants, such as lead [8].”

2. There have been discussions in the epidemiology literature about the problems of endogeneity when adjusting for medication use. The authors were well-advised to run sensitivity analyses on the medication-free sample. However, this introduces considerable bias as well. Rather, statisticians argue that less bias is introduced when adding 15mmHg to the SBP of those taking anti-hypertensive medication (Tobin, Sheehan et al. 2005). Does this measure of SBP alter the results?

   We ran models for systolic blood pressure effects using the 15mm/Hg adjustment for medication users suggested by the reviewer and saw that our effects were mostly the same. In these new models, the association between lead exposure and systolic blood pressure (both modeled continuously and dichotomously) remained null in the entire study population. We also saw no significant effects of lead in either low or high AL in stratified models. In comparison to the results presented in the manuscript, effect estimates in the sensitivity analysis were closer to the null, and there was no longer a significant linear dose-response between lead exposure and odds of elevated systolic blood pressure (p = 0.97).

3. How did the authors come to use their version of the allostatic load measure?

   In a recent review (Juster, McEwen et al. 2010), McEwen and colleagues listed the different permutations of the measure and the one used in this manuscript is different from all of those in the review. Is there a reason? Similarly, why was the median used as the threshold for "high" versus "low"? Can the authors report on sensitivity analyses using alternate thresholds?
Thank you for the suggestion. In the revised manuscript, we conducted additional sensitivity analyses with high allostatic load defined as the top 20 percent of the distribution. These results are now presented in a revised Figure 1 so that the reader can compare them to the results when high allostatic load is defined as the top 50th percentile. In general, effect estimates for lead are larger with the 20 percentile cut-off, but with wider confidence intervals.

Minor essential revisions
1. Why did the authors use a dichotomous outcome rather than continuous blood pressure? There is considerable loss of information that may have been helpful, particularly when running interactions.

Thank you for the suggestion. In the revised manuscript, we now present results with the outcomes modeled both continuously as well as dichotomously. We prioritize the dichotomous outcome models since these employ a clinically relevant cutoff.

Discretionary revisions
1. In the first sentence of the Background section, the authors state that "high blood pressure is a major risk factor for hypertension. . . ". According to their JNC-VII citation, high blood pressure is hypertension in the sense that they are using it. If, for example, they were saying that a high blood pressure reading at a certain clinic visit may signify hypertension (because it may or may not), then I would agree. But in the more general sense, high blood pressure is hypertension.

We have modified the first sentence in the background section by removing the word 'hypertension'. It now reads:

“High blood pressure is a common condition among U.S. adults and a major risk factor for hypertension, strokes, heart attacks, congestive heart failure, and kidney disease.”
Reviewer 3: J. Peters

A. Major Compulsory Revisions

1. The key conceptual concern is that the outcome - elevated blood pressure (systolic and diastolic blood pressure) - is one of the parameters used to operationalize allostatic load (McEwen 2000; Seeman et al. 1997; Sebbah et al. 2008; Allsworth et al. 2005). In reviewing the papers cited, blood pressure was included in the definitions of allostatic load. Allostatic load is based on a number of parameters that reflect activity across a range of regulatory systems (Seeman, 1997; McEwen 2000). For example, blood pressure is thought to index cardiovascular activity. Not sure then how blood pressure relates to the other physiological measures of allostatic load as an outcome modified by these components.

A comprehensive review by Justier et al. (Justier, McKwen and Lupien. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neuroscience and Biobehavioral Reviews 35 (2010) 2–16) examined several papers that analyzed the health effects of allostatic load, some of which looked at CVD outcomes and many of which utilized NHANES data. Our operationalization of AL closely matches the summary measures used in several of these papers. In addition, we followed the method of Mattei et al (Mattei J, Demissie S, Falcon LM, Ordovas JM, Tucker K. Allostatic load is associated with chronic conditions in the Boston Puerto Rican Health Study. Social Science & Medicine 70 (2010) 1988-1996) who applied a similar AL metric to examine its effects on risk of hypertension, and cardiovascular disease. Mattei et al. constructed similar AL scores as ours to examine associations with hypertension by removing SBP and DBP from the AL metric. Citations to these papers are in the manuscript.

2. Should consider allostatic load as a measure of cumulative psychological dysregulation or biological risk? Allostatic load reflects more than chronic stress also encompassing many aspects of a person life that can affect regulations of physiological systems including genetics and lifestyle choices (such as smoking, diet, etc.) (McEwen & Seeman (Annals of New York Academy of Science. 2006, Vol. 896, Issue 1). Thus allostatic load could be thought of as a mediator of chronic stress (along the pathway) and not necessarily a measure of stress per se.

We agree and this issue was also raised by another reviewer. We have reworked our background section in the abstract and in the main text (see page 5-6) to reflect the fact that AL should be conceptualized as an indicator of physiological dysregulation due to chronic stress exposure, which can potentially enhance vulnerability to the toxic effects of compounds, such as lead.

3. Some studies use either a specific cut-point (Sebbah et al. 2008 or Allsworth et al., 2005) whether based on clinical or other criteria while others use the top quintile versus the other quintiles (Seeman et al., 2001). What was the rationale behind using the median of the scores as opposed to these methods (any references?). One benefit of identifying specific cut points by whatever method is the ability to compare the cut-offs in this study with those in other studies (here the median and range were presented). Additionally, are the results sensitive to the cut-offs/method used (are results similar over various definitions of high allostatic load). At minimum, the Discussion section could include the pros and cons of the method chosen for this paper. It may also help to state what sensitivity tests were conducted (pg. 15, para 1).

Informed by prior studies that we discussed in our paper, as well as data availability within NHANES, we operationalized AL by creating a cumulative index of physiologic dysregulation of the cardiovascular, inflammatory, and endocrine systems using seven biological markers: serum triglycerides, HDL serum cholesterol, waist circumference, urinary C-reactive proteins, urinary creatinine, serum albumin, and plasma levels of glycosolated hemoglobin. Cut-points were empirically defined using sex-specific quintiles for each of the seven components as done by Allsworth et al 2005 and others. This method has been termed the “group AL index” approach where the summary measure represents the number of biomarkers falling within a high risk percentile (e.g., upper or lower 25th percentile) based on the sample’s distribution of biomarker values. Because each biomarker is dichotomized as 0 or 1 depending on cut-offs, each biomarker is allotted an equal weight in the index. This is the traditional count-based formulation that is mostly commonly used in studies of AL effects on health outcomes (Justier et al. 2010).
In response to this reviewer’s important question regarding the sensitivity of our results to the “high versus low” AL cut-offs used for the cumulative scores, we conducted additional sensitivity analyses with high allostatic load re-defined as the top 20th percentile of the distribution of the AL score. These results now appear in our revised Figure 1 to facilitate comparison to the results when high allostatic load is defined as the top 50th percentile. In general, effect estimates for lead become larger with the 20th percentile cut-off, but these results also have wider confidence intervals.

4. Was there truly a modifying affect [sic] (two groups – high versus low allostatic load - significantly different)? Would be informative to see the results for the interaction terms particularly the interaction between lead and allostatic load (from pg. 9, para 2). Also, as an added curiosity, were any interactions observed using lead and allostatic load as linear (as opposed to categorical) variables?

In response to this important question, we conducted several sensitivity analyses to assess the statistical significance of a multiplicative interaction term between lead exposure and allostatic load. We also ran additional models examining the main effect estimates when modeling lead and AL as linear terms. These tables appear in the Supplement. Although our main models suggest stronger effects of lead for elevated diastolic blood pressure among those with high allostatic load, the interaction terms for both systolic and diastolic results were not statistically significant, indicating that there was no evidence of a synergistic effect. We have revised our methods and results sections to reflect these additional sensitivity analyses.

On pg. 6 the age range of 40-65 was chosen to ‘minimize the effect of confounding by age.’ What was the rationale behind choosing middle-aged versus older adults.

We chose this age range because lead levels tend to be lower in this population and because we would be able to have a larger sample size for our analysis. In addition, we believed that the effects of “aging” on blood pressure on older adults would overwhelm the effects of AL and lead.

2. A ‘better’ way to test for trend could be to use the median value for each quintile.

We thank the reviewer for the suggestion and compared the suggested approach for evaluating trends to our original method and results are generally the same. We have decided to use ordinal integers to test for trend since median lead concentrations for quintiles may vary between the low and high AL groups; thus, we believe that our original approach is more suitable for this analysis.

3. It would be great for an environmental health audience to say something about what physiological system the different components of allostatic load correspond to. Also what is ‘bad’ for each – some may not be as obvious. Also the reader may not be familiar with or get the implications of ‘primary’ verses ‘secondary’ mediators of chronic stress response (may want to say something about that in the Introduction and state which is being measured here).

In the section explaining our allostatic load measure, we added descriptors in parenthesis to delineate what categories of biomarkers each of these AL components corresponds to.

4. Given the interrelation of stress, hypertension and allostatic load may want to address the possibility that there may be reverse association - higher blood pressure increasing allostatic load (not sure how it would relate based on #1 of the Major Concerns).

We have added mention of this issue in the discussion section of our paper, although given the cross-sectional nature of our analysis we cannot examine this important question in a systemic way in our study.
Reviewer #4: J. Clougherty

1) The central hypothesis needs better motivation in the abstract and introduction. It should be easy to enrich this background, given ample recent EPA workshops on the topic, growing interest among the cumulative risk community, and a few very good reviews in recent years.

2) More attention to plausible mechanistic pathways for combined effects would be useful in the Introduction (perhaps from the toxicological evidence on combined effects).

In response to points 1 and 2 we added the following paragraph to the beginning of the introduction of the main text:

“Regulatory agencies and environmental health scientists are beginning to examine whether and how chronic stress exposure potentially amplifies human vulnerability to the adverse health effects of toxicant exposures [1]. Emerging evidence suggests that cumulative physiological “wear and tear” resulting from chronic over-activity of the body’s stress-response system may impair immune functioning and increase vulnerability to environmental stressors [2] by increasing the absorption of toxicants into the body through increased respiration, perspiration, and consumption [3]; compromising the body’s defense systems against toxicants; affecting the same physiological processes as environmental agents; and directly causing illness [4, 5].”

We also reworked the text in the background section of the abstract to read:

“Scientists and regulators have sought to understand whether and how physiologic dysregulation due to chronic stress exposure may enhance vulnerability to the adverse health effects of toxicant exposures.”

3) A mean BP was used except where only one was available – suggesting greater misclassification in these individuals. A nice sensitivity test is to re-run the main analyses excluding these individuals with greater expected misclassification, to observe whether EM magnifies in expected direction.

In response to this question, we re-ran our models excluding these individuals and our effect estimates did not substantially change.

4) Using blood (rather than bone) Pb may be a problem in regards to short retention time and consequent problems in the relative temporality of exposures. The authors need to somehow establish that stressor exposures plausibly occurred prior to physical exposures, so as to have heightened susceptibility to subsequent Pb exposures – else the directionality is quite difficult to interpret, and observed effect modification (EM) may be due to other factors. This issue is discussed in the Clougherty & Kubzansky review.

The cross-sectional nature of our study design and the NHANES data generally precludes a systematic assessment of the relative temporality of our exposures of interest (lead and allostatic load). We have mentioned this limitation of our analysis in the discussion section on page 17.

“However, residual confounding remains possible. In addition, the cross-sectional design of our study precludes a systemic assessment of the temporality of lead exposure and allostatic load, the potential for reverse causation between hypertension and AL, or the potential effects of cumulative lead exposures throughout the life course, since blood lead (as opposed to bone lead) mostly reflects recent and ongoing exposures as well as lead that has been mobilized from tissue stores such as bone [48].”

Moreover bone lead measurements are not available in NHANES data with which to compare effect estimates with blood lead levels. However, research indicates that lead levels in blood generally reflect both recent and ongoing exogenous exposures and the mobilization of lead from the skeleton back into the circulation [ See: Hu H, Shih R, Rothenberg S, Schwartz BS. The epidemiology of lead toxicity in adults: measuring dose and consideration of other methodologic issues. Environ Health Perspect. 2007;115:455–462. ; Hu H, Rabinowitz M, Smith D. Bone lead as a biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms. Environ Health Perspect. 1998;106:1–8.]
5) The allostatic load model has been criticized in that it is actually a composite measure of metabolic and systemic dysregulation – it is not, inherently, a measure of stress. As such, using AL as a marker of stress, and showing an association with a systemic illness (e.g., hypertension) is, well, ... expected. The ‘exposure’ marker here is actually inherently more similar to the outcome under study (hypertension) than to the true exposure from which it is posited to arise (stress). (Indeed, one might wonder why BP was not an original component of the AL model…) For this reason, the authors really need to find some way of establishing that AL is actually associated with some measures of ‘stress’ (a construct of perception which requires questionnaire data or similar) or, less convincingly, with some measure of ‘stressors’ (e.g., census data on poverty, SEP). The latter is much less satisfying (and spatial misclassification rampant), but may be all that is currently possible with the existing data.

We agree that this is an important question and a similar question was raised by another reviewer. We have changed our text in the background section as well as in the introduction of the main text [See our response #1 to Reviewer #2 (Hicken)]. Also, based on our review of the AL literature, there is precedent for applying AL metrics as an indicator of physiologic dysregulation of the body’s stress response due to chronic stress exposure [See the review by Justier et al.] to examine CVD outcomes such as elevated blood pressure. We followed a similar approach employed by Mattei et al (Mattei J, Demissie S, Falcon LM, Ordovas JM, Tucker K. Allostatic load is associated with chronic conditions in the Boston Puerto Rican Health Study. Social Science & Medicine 70 (2010) 1988-1996) who constructed an AL metric to examine its effect on the risk of hypertension, and cardiovascular disease by removing SBP and DBP from the AL scores.

6) Relatedly, once the authors have established that AL is associated with some important ‘stressors,’ they must also somehow justify their assumption that is it the stress associated with say, living in a low-income census tract - - and not the myriad facets of material deprivation (e.g., poor diet, poor housing …) – which is responsible for the observed EM.

While this is an important question, answering it with this study is beyond the purview of this paper and the data we used for the analysis. Unfortunately, the limitations of NHANES data do not enable us to compare neighborhood level measures of material deprivation, (due to the lack of geocoding specificity available for our sample), with more specific information on variables such as housing and diet metrics. Moreover, the AL literature indicates that either category of stressor could elicit a similar stress response as discussed by Justier et al. 2010.

7) I wonder what other, more distal, outcomes the authors may have chosen from NHANES? are corroboratory analyses possible with other outcomes?)

This is a great question, but additional analyses with another health outcome are beyond the main objective and purview of this paper.

8) There is ample risk here of over-controlling by including individual-level covariates on sex, race/ethnicity, education, marital status…. There is a need for more sensitivity testing to establish the robustness of the central EM, and some indication of variance in this effect.

In response to this comment, we reran some of our primary models, first taking out education and race/ethnicity separately and then together. In this sensitivity analysis, effect estimates increase slightly for both AL groups, but the p-value for the interaction term is very similar to what we report in the paper.

9) Why is the “High AL” group larger than the “Low AL” group? Can this really be interpreted as “High AL” when many below the median are included? Again the construct of “high stress” needs be better conceptualized & applied. (In general, better conceptualization and activation of the stress chain would be useful in the manuscript.)

We conducted a sensitivity analysis based on a similar question from reviewer #3 (Peters) examining the effects of a different cut-off for high versus low AL, where high was defined as above the 20th percentile for the cumulative score. Overall, effect estimates increased a bit for the high AL group, but the confidence intervals were slightly larger with this new cut-off. Results can be seen in our revised Figure 1. Our description of how AL
functions as a mechanism that leads to physiologic dysregulation of the stress-response and can lead to vulnerability to the adverse effects of toxicant exposures such as lead are now described in more detail in the introduction.