Stressors over the life course and physiological dysregulation in Costa Rica

Omer Gersten[1], Will Dow[2], Luis Rosero-Bixby[3]

Draft: Submitted to the *Journal of Gerontology: Social Sciences* on October 11, 2008.

Acknowledgements: This research was generously supported by a grant from the MacArthur Research Network on Socioeconomic Status and Health. We are also thankful for comments from participants of the 2008 Population Association of America (PAA) conference in New Orleans, Louisiana and anonymous reviewers.

[1] Author for correspondence. Institute of Sociology, Academia Sinica, Number 128, Section 2, Academia Road, Nankang, Taipei, Taiwan, 11529. E-mail: omer@gate.sinica.edu.tw. Phone: 886-2-2652-5127. Fax: 886-2-2652-5050. Web: http://idv.sinica.edu.tw/omer.
[2] School of Public Health, University of California at Berkeley.
[3] Central American Population Center (CCP), University of Costa Rica.

Abstract

Objectives: Allostatic load (AL) theory purports that stress experienced over the life course exacts a cumulative, physiological toll on the body which eventually contributes to poor health. Although mounting evidence indicates that elevated levels of AL (as measured by dysregulated physiological systems) is a risk factor for poor health later in life, it is not yet clear whether those same elevated levels are due to stressor exposure. Thus, the paper here attempts to better understand the connection between stressor exposure and AL levels.
Methods: We analyze the CRELES, a new, nationally representative survey of older Costa Rican men and women (ages 60-109), conducted in 2004-2006. This paper focuses on the relation between a variety of stressors experienced over the life course (e.g., economic deprivation early in life, death of children, and years widowed) and four neuroendocrine markers analyzed in an index. These biomarkers are cortisol, dehydroepiandrosterone sulfate (DHEAS), epinephrine, and norepinephrine, and the index of which they are part is termed neuroendocrine allostatic load (NAL).
Results: None of the stressors examined were associated with riskier neuroendocrine biomarker profiles.
Discussion: The result here suggests that neuroendocrine system dysregulation stems from sources other than stressor exposure.

Word count (abstract): 194
Word count (limit): 200

Keywords

stress, biomarkers, neuroendocrine allostatic load (NAL), Costa Rica, aging

Running head: Stressors and physiological dysregulation

Word count main text (excluding title page/abstract/figures/tables/references): 4,602
Word count main text limit: ~5,000
Word count figure/tables: 934

Introduction

At least two important testable hypotheses stem from the allostatic framework, a framework that has grown markedly in popularity and has emphasized the role of stress in illness (Gersten, 2008a; McEwen, 2004). One hypothesis is that allostatic load (AL), a measure of physiological dysregulation, is the result, over extended periods of time, of repeated activation of the body's adaptive processes in response to challenge. Another hypothesis is that AL is a risk factor for morbidity and mortality. Of these two hypotheses, far more support has been found for the latter. Using the MacArthur studies, for instance, Seeman and others (1997) found that high AL at baseline predicted greater cognitive and physical declines and earlier mortality over the study period. In addition, work by Goldman and others (2006) and Turra and others (2005) found that various measures of physiological dysregulation in a Taiwanese population predicted health outcomes such as depression, cognitive and physical function, and survival. In contrast to these findings, using the same Taiwanese data set, both Gersten (2008b) and Glei and others (2007) were largely unsuccessful in linking various measures of stress experienced over the life course (e.g., widowhood, living alone, financial strain, subjective reports of chronic stress) to riskier AL levels. Such negative findings have also been found by others (Kubzansky et al., 1998; Powell et al., 2002). Thus, in an attempt to further investigate the level of supportive evidence for the more questionable hypothesis that markers of life history stress are correlated with higher levels of AL, the paper here will analyze a new, nationally representative data set from Costa Rica.

The data that will be analyzed in this paper comes from the CRELES, which obtained information from older Costa Rican men and women in 2004-2006 (in the first wave of data collection). Much of the data was meant to be comparable to other studies that have investigated

AL, such as the MacArthur studies and the Taiwanese SEBAS, and thus the CRELES has obtained many of the same biomarkers as these surveys. One drawback of the CRELES is the lack of questions that probe subjective levels of stress (e.g., "Do you feel stressed about..."). A strength of the survey, however, is its collection of a number of indicators of stressful life events, especially those occurring in early childhood. Many other surveys investigating the impact of life stressors on AL have only examined stressors that have occurred in middle and later life (Goldman et al., 2005; Seeman et al., 2004), even though the allostatic framework is quite clear about the importance of using a life course approach in analyses (Crimmins & Seeman, 2004; McEwen, 2004).

As suggested earlier, AL is the idea that the body experiences a cost, or "wear and tear," from responding to myriad acute and chronic challenges/stressors over the life course (McEwen, 1988; Timiras and Gersten, 2007). AL is also thought to develop in a number of different and important physiological systems, including the metabolic, cardiovascular, and neuroendocrine ones (McEwen, 1988; Timiras & Gersten, 2007). The paper here will focus on the neuroendocrine markers of the AL construct for a number of reasons. First, in population-level studies that have been conducted to date, the neuroendocrine markers have been some of the most recently added and hence least studied (compared to, say, those markers indicative of cardiovascular and metabolic function). Biomarkers of neuroendocrine system function have been little studied even though they are critical to the stress response and form a core component of the AL measure. Second, despite the recent inclusion of neuroendocrine markers in large-scale studies, there is convincing evidence that certain levels of the markers predict a number of health problems, including more rapid decline in physical and cognitive function, greater incidence of cardiovascular disease, and earlier mortality (Goldman et al., 2006, Karlamangla et al., 2005;

Seeman et al., 2001). In other words, the neuroendocrine markers yield an important contribution in predicting worse health. Third, although one of the strengths of the AL construct has been measurement of different physiological systems in one index in an attempt to gauge health more holistically, such an approach is also one of the construct's weaknesses. That is, from a physiological perspective, it can be difficult to interpret a score from the measure that includes such vastly different markers. Relatedly, it is often unclear which system, if any, is driving an overall pattern of the construct. Thus, for the above reasons and others, this paper will focus on analyzing four neuroendocrine biomarkers (i.e., cortisol, dehydroepiandrosterone sulfate (DHEAS), epinephrine, and norepinephrine) that represent function at a similar level of biological abstraction.

Study hypotheses

Based on the general literature and that specific to Costa Rica, and following in the tradition of the "environmental stress perspective" that focuses on potentially stressful life events (Cohen, Kessler, & Gordon, 1995), we hypothesize that a number of states and experiences have likely lead to greater stress exposure and hence greater AL. Since greater age can only bring about greater exposure to stressors and AL is thought to be cumulative, we hypothesize that greater age is positively correlated with greater AL. We also expect a similar relationship between female sex and AL, since women generally report greater distress and depression than men (Thoits, 1995). Indicators of lesser material resources, such as lower education, lower current household wealth, and economic deprivation early in life, are all expected to be associated with higher AL, and so too are indicators of lesser emotional resources, such as

growing up without a biological father and earlier maternal age at death. (It is worth noting that these latter two variables are also likely indicators of greater economic disadvantage.) Markers of poor health early in life, like having had malaria or asthma, might also very well indicate greater stress (and hence greater AL) that comes with dealing with illness.

Given the suggestion in the literature that some of the negative health effects of social deprivation are due to increased levels of stress (Cacioppo & Hawkley, 2003), we expect that measures of such deprivation (which in our study are whether the respondents are unmarried, live alone, and attend church infrequently or not at all) should also be associated with higher AL. Further, we expect a similar relationship between AL and measures of personal loss, which in this study are the experience of a death of a child and length of widowhood. Status as an immigrant (in comparison to the native born) is included as a variable in our analysis since such status is potentially important, though we remain neutral in hypothesizing its directionality. On the one hand, immigrants are more likely to be disconnected from family, experience more difficult working and living situations, and experience discrimination (Bolaños et al., 2008; Sandoval-García, 2004), but on the other hand they may be healthier and more robust to these sorts of stressors than the native population (Herring et al., in press). Moreover, we suspect that rural residents of Costa Rica, who compare less favorably than their urban counterparts on a number of indicators of welfare (e.g., employment rate, infant mortality, and levels of malnutrition (Bähr & Wehrhahn, 1993; Hall, 1984)), will have higher ALs. Lastly, in analysis of only the currently married, we predict that those who report a spouse in poor health will themselves have higher AL in part because of the possibility of stress caused by caregiving responsibilities (Epel et al., 2004).

Data and Methods

Overview of the data set

We analyze the Costa Rican Study on Longevity and Healthy Aging (CRELES), a population survey conducted in Costa Rica in 2004-2006 (for a more detailed description of the study consult Rosero-Bixby (2007)). The survey is nationally representative of those 60 and older in the non-institutionalized population, and the CRELES drew its sub-sample of respondents from the 2000 census database. Among other things, the interview portion of the CRELES included questions about cognitive and physical functioning, health care utilization, nutrition and other health behaviors, social support, employment history and pensions, and a variety of life stressors. The in-home interviews averaged nearly an hour and a half and during the same visit mobility tests were performed and blood pressure measurements were taken. With the respondents' additional consent, they were enrolled in the more invasive aspect of the survey's data collection efforts. After receiving relevant instructions and materials, participants collected urine and began fasting on the same day as the in-home interview and on the next day the survey staff picked up the urine, drew blood samples, and took anthropometric (e.g., height and weight) measures. The blood and urine samples were used to determine traditional health indicators such as total and HDL cholesterol and less traditional indicators such as epinephrine and cortisol.

Of survivors who could be located and were initially contacted for inclusion in the 2004-2006 CRELES, 96% gave interviews, yielding a sample of 2,827 participants. Of these, 95 and 92% gave blood and urine samples, respectively, and in about 25% of all cases a proxy (most

often the respondent's son or daughter) helped answer some questions for the respondents. The survey over-sampled those over 95 years old.

Dependent variable

The neuroendocrine biomarkers

In this paper we focus on cortisol, DHEAS, epinephrine, and norepinephrine, a physiologically coherent class of markers indicative of neuroendocrine system function (Sapolsky, 2004; Cohen et al., 1995; Crimmins & Seeman, 2001). The measure used here based on these markers is called NAL, for neuroendocrine allostatic load, and has been discussed in more detail elsewhere (Gersten, 2008b). Among NAL's greatest advantages is its interpretability that stems from grouping markers of a similar level of biological abstraction. NAL includes markers related to two neuroendocrine systems: the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). The HPA axis is key in regulating homeostatic processes in the body, and environmental stressors can lead it as well other regulatory systems to react (Sapolsky, 2004; Cohen et al., 1995; Crimmins and Seeman, 2001). Cortisol and DHEAS are indicators of HPA axis activity. The body's "fight or flight" response is in part mobilized by the SNS, and its activity can be measured by norepinephrine and epinephrine levels (Sapolsky, 2004; Cohen et al., 1995; Crimmins & Seeman, 2001).

Measurement of biomarkers

The CRELES attempted to capture basal levels of the neuroendocrine biomarkers and to this end the blood and urine samples were collected in the participants' homes under resting conditions. Three of the four markers were collected in urine samples and when collected in this way the samples represent integrated, in contrast to point-in-time, measures. For cortisol, norepinephrine, and epinephrine, respondents were asked to void urine at 6pm, which was discarded, and to collect all subsequent samples until 6am the following day. In part because dissimilar body size leads to differential concentration of the neuroendocrine markers in the urine, total urine was standardized using grams of creatinine. The subjects also fasted from 6 p.m. onwards on the day they began urine collection until a study affiliate came to their home to collect the urine sample and draw blood. The amount of DHEAS in the body was determined through blood samples.

The blood samples for each respondent were drawn by venipuncture by a phlebotomist and put into three tubes, one tube with EDTA (which acts as an anticoagulant) and two serum-separating tubes (SST) with clot activators. The tubes with the clot activator were centrifuged in the field to separate the serum from the other elements and to prevent hemolysis (the breaking open of red blood cells and the release of hemoglobin into the surrounding fluid). From the point of initial blood collection, the tubes were kept in coolers for no more than six hours until they were separated in various nearby labs into aliquots of 0.5 or 1.0 mL and stored at -40°C. The serum was later used to analyze DHEAS levels and levels of some other markers. Regarding the urine samples, after they were picked up from respondents' homes they were kept in coolers and also taken to nearby labs where their volume was measured and separated into five aliquots (of either 1.0 or 2.0 mL). These aliquots of urine were also stored at -40°C.

From the storage labs, the urine samples were initially sent to Neuroscience Laboratories at the University of Costa Rica for analysis and then to the Central American Center for the Analysis of Hormones (CENHACE), a private laboratory in San Jose, Costa Rica. Both these laboratories were certified by a national reference center of clinical chemistry, an agency under the Ministry of Health. The catecholamines were analyzed by high performance liquid chromatography (HPLC) at Neuroscience Laboratories and cortisol and DHEAS were analyzed by chemiluminescence immunoassay at the CENHACE laboratory. Unfortunately, because of the difficulty involved with acidifying the urine samples properly (and hence some degradation in the samples), for some respondents epinephrine and norepinephrine values could not be determined with a high enough degree of confidence in their validity and were thus excluded from the analysis.

Independent variables

Most of the independent variables used are straightforward to interpret, although the following require some explanation. Household wealth is determined by first creating an index based on whether the respondent's home has a kitchen, electricity or gas as cooking fuel, potable water, indoor toilet, a refrigerator or freezer, television, a phone (either a cell or landline), and washing machine, and whether the respondent owns a car. This index is then coded into high, medium, and low household wealth categories. Economic problems early in life were determined by asking respondents whether or not in childhood and adolescence they lived in a home that had a bathroom or latrine, lived in a home that had electricity, slept on the floor or with others in a bed, and regularly wore shoes. Health problems early in life were determined by whether or not

the respondent reported having in childhood and adolescence tuberculosis, rheumatic fever, poliomyelitis, malaria, or asthma/chronic bronchitis.

In another finance-related question, respondents were asked to describe their present economic situation, to which they could respond "excellent," "very good," "good," "average/normal," or "bad." Respondents were also asked to provide their total monthly income stemming from work and pensions. Respondents who did not give a precise figure, but gave a range (e.g., 80,000-170,000 colones/month) were given the mean income of those reporting an exact amount within the same range. Income from pensions and work were added to that from transfers to produce the final variable of total monthly income.

Lastly, we created a measure of cumulative adversity in which respondents received one point toward their score if they could be characterized by any of the following: less than six years of education, rural residence, lower household wealth, "bad" self-assessed economic situation, monthly income less than or equal to the lower 25th percentile of incomes, being currently unmarried, living alone, death of at least one child, less than weekly religious attendance, mother with no formal education, mother who died before fifty years old, growing up without a biological father, having reported one or more health problems early in life, and having reported three or more economic problems early in life.

Other independent variables serve as controls. Since levels of the neuroendocrine biomarkers can be influenced by a wide variety of factors independent of stress (Cohen, Kessler, & Gordon, 1995; James & Brown, 1997), all models control for smoking, alcohol consumption, and medication use.

Methods

Regarding extreme values, one outlier was removed for norepinephrine, two for epinephrine, and four for cortisol. These outliers were at least 11 standard deviations above the mean for their respective distributions.

The most popular approach to operationalizing AL has been to create a score that gives one point for every biomarker for which the subject can be considered at higher risk (i.e., the elevated risk zone approach). The literature most often represents high risk by greater values for cortisol, epinephrine, and norepinephrine, and lower values for DHEAS; this convention is followed here. Since there is no agreed upon standard for what biomarker values represent different risk levels, it has been most common to define risk as above or below distribution percentiles (e.g., the 10^{th}, 25^{th}, 75^{th}, and 90^{th}). See Table 1 for descriptive statistics and cut-points for the neuroendocrine biomarkers. Since subjects can be assigned 1 point on each of the four biomarkers if they have high risk values, NAL scores can range from 0–4; these scores serve as the dependent variables in OLS regressions.

In addition to the cut .method of scoring, a summed z-score is created for respondents, which is the total number of standard deviations from the mean in the direction of high risk for each biomarker. Unlike the cut-off approach, an index using the z-score method allows for unequal weighting of the biomarkers (e.g., a combined z-score of 3 could stem from being 2 SDs above the mean for cortisol, 1 SD above the mean for epinephrine, and the mean for the other two markers). The combined z-score is again the dependent variable in OLS regressions and can range from 0 to no pre-determined upper limit. Lastly, all analysis is carried out using STATA version 9.0 (StataCorp, 2005) and the multivariate analysis uses weighted data which corrects for the oversampling of the oldest-old and for some non-response by demographics.

Results

Table 2 depicts descriptive statistics (of the entire, unweighted sample) for variables that are used in this analysis. One of the things to note in the table is the relatively low levels of education of those in this sample, with 70% not having completed their primary education (i.e., having less than six years of schooling). Also striking is the percent of respondents who have had at least one of their children die and the percent of respondents who have grown up without a biological father (45% and 22%, respectively). Table 2 also reveals that religion is important in the lives of many older persons in Costa Rica, as nearly 45% of the sample reported going to church one or more times a week. Lastly, it is also worth observing that 39% of those with a spouse reported that the spouse has a serious health problem, suggesting that a fair amount of married older persons provide caregiving services to their husband or wife.

Table 3 presents estimated regression results for different combinations of independent variables, with NAL as the dependent variable. A key finding from this table is the consistency and strength of the relationship between NAL and both age and female sex. Surprisingly, practically every stressor examined was not associated with NAL in the expected way. Most congruent with expectation was the positive correlation between having at least one child who had died (controlling for number of children born) and higher NAL, although this relationship was not statistically significant (p-value = 0.112).

Table 4 is similar to that of Table 3, except that Table 4 only includes widowed respondents and focuses on results for the length of widowhood variables. As can be observed from the table, length of widowhood is not correlated with NAL levels, again a surprising,

negative finding. We also examined only the currently married respondents to uncover whether having a spouse with low education or poor health was linked to higher NAL levels. Results (not shown) reveal no association between these levels and these variables.

In addition to the results already presented and described, we ran a variety of additional analyses. These included using NAL cutpoints at the 10th and 90th percentiles instead of at the 25th and 75th, and also creating a NAL measure based on a summed z-score approach as described earlier. Further, because of the possibility of important sex-specific interaction effects and the possibility of the relevance of biomarker cutpoints based on men only and women only, we reran all the analyses separately by sex and with sex-specific biomarker cutpoints. Results from these models were generally similar to those already discussed, but the relationship between NAL levels and both low education and children who have died essentially disappeared. Immigrant status in these same models was routinely no longer significant at the .05 level, but still retained a negative coefficient[1].

Lastly, we analyzed our measure of cumulative adversity -- in contrast to the stressors singly -- in relation to NAL values. Although the coefficient was positive (i.e., more stressors were correlated with greater NAL values), p-values ranged widely and were not lower than the .05 significance threshold (i.e., p-values ranged from 0.074 to 0.986).

Discussion

This paper investigated stressors throughout the life course -- in early, middle, and later ages -- in relationship to riskier neuroendocrine biomarker profiles in a new, nationally-

[1] For example, duplicating the regression in Table 3, Model 5, but using cutpoints at the 10th and 90th percentiles resulted in a coefficient for immigrant status of -0.07 (p-value = 0.347).

representative study of older Costa Rican men and women. The main finding is that, unexpectedly, these stressors were not associated with levels of NAL, a measure of neuroendocrine system dysregulation. Greater age and female sex, though, were linked to higher NAL values.

Especially considering that respondents in the sample were 60 and older, one limitation of the present study is respondents' ability to remember events early in life. This limitation may be most relevant for the questions inquiring about health problems in childhood and adolescence. It may be the case that respondents did experience the health problems asked about by surveyors, but did not know or did not remember the names of those problems. Imprecise recall seems less of an issue, however, for questions probing economic deprivation early in life, since it seems likely that respondents would be able to remember everyday events such as whether they grew up in a house with electricity, an indoor toilet, and whether they slept in a bed with others.

As mentioned in the introduction, another limitation of the present study is that (except for a question regarding self-assessed economic situation) the study does not probe respondents' subjective interpretations of their life history. Although we assume, for instance, that living alone is likely to be more stressful than not for most of the participants living alone, this may not indeed be the case. Nevertheless, the emotional response to certain human experiences, like the grieving involved in the loss of a child or spouse, seem close to being "universal," and so it is still surprising that a number of variables that we investigated were not associated with our measure of physiological dysregulation. In the case of losing a spouse, not only is the loss itself psychologically difficult to deal with, but the loss could very well also result in future reduced instrumental and emotional support, thereby increasing stress levels for the widow or widower further.

As far as aspects of sample collection are concerned, others have mentioned the benefit to a study like the one analyzed here in having more measures of the neuroendocrine biomarkers (Loucks, Juster, & Pruessner, 2008). For instance, it would be useful to have more than one urine collection and it would also be useful to have a dynamic measure of cortisol levels over the day -- for instance, through salivary samples (Loucks, Juster, & Pruessner, 2008).

As mentioned earlier, age and sex were the two lone characteristics in our study correlated with our measure of neuroendocrine system dysregulation. These findings are not surprising. In the case of female sex, women tend to have lower levels of DHEAS than men (Goldman et al., 2004; Worthman, 2002), although evidence is mixed on whether they have higher resting levels than men on the other markers (Goldman et al., 2004; Hinojosa-Laborde et al., 1999; Van Cauter, Leproult, & Kupfer, 1996; Worthman, 2002). To the extent that women's levels do differ, it seems due to some combination of greater stressor exposure, greater reactivity, and other predisposing pyschological and biological factors (Goldberg, 2006; Kajantie & Phillips, 2006; Piccinelli & Wilkinson, 2000).

In the case of age, since greater age can only bring about greater exposure to stressors, and since the allostatic framework theorizes that the costs to the body in dealing with challenge are cumulative, we would expect a positive relationship between age and NAL. In other words, that age and NAL are correlated with one another is only a necessary, but not sufficient condition for allostatic theory to hold. The challenge for the allostatic framework, then, is to demonstrate that when holding age constant, measures of a stressful life course are correlated with greater load.

In order to reconcile the findings here with the wider literature, we undertook an extensive literature search for articles related to linking to stressors to levels of cortisol, DHEAS,

epinephrine, norepinephrine, and AL. We focused on those articles which collected urinary samples to measure the catecholamines, and cortisol and blood samples to measure DHEAS. It is difficult to compare the findings in this paper with this wider literature because the latter seldom set out to test the hypothesis that stressors over the life course altar baseline levels of the neuroendocrine markers. Indeed, most of the studies that have attempted to link stressors to neuroendocrine marker levels have only examined one source (or few sources) of chronic stress. For instance, a study by Babisch and others (2001) tried to link traffic noise outside of respondents' homes to catecholamine levels and another representative study examined whether women currently undergoing a divorce or separation had higher levels of the catecholamines and urinary free cortisol (Powell, et al. 2001). In addition to the paucity of studies using multiple measures of stress over the life course, many did not include as many indicators of neuroendocrine system function as in the paper here and most made use of considerably smaller, non-population-based samples. Although it is difficult to summarize these varied papers, on the whole results appear mixed, with some supporting (Evans, 2003; Janicki-Deverts et al., 2007; Lemieux & Coe, 1995; Yehuda et al., 1995), some not supporting (Kubzansky et al., 1998; Powell et al., 2002) and others providing evidence for and against (Babisch, 2003; Luecken et al., 1997; Olff et al., 2006; Wheler et al., 2006) the connection between life stress and dysregulated neuroendocrine biomarker levels.

In addition to the studies already described, one stands out for its more thorough operationalization of chronic stress and ready comparability to the study here. This study is the Social Environment and Biomarkers of Aging Study (SEBAS) and it was carried out in Taiwan in 2000. Like the CRELES, the SEBAS is a large study (>1000 participants), is nationally representative, focuses on older persons (aged 54 and older), and has collected the

catecholamines and cortisol through overnight urine samples and DHEAS through blood samples. In one study of the SEBAS by Gersten (2008b), he operationalizes the experience of stress over the lifecourse in part through the experience of such events as being widowed, living alone, and lack of group participation, as well as through respondents' report of stress over their family's work situation, health situation, marital situation, and other domains. Gersten fails to find a link between these stressors and the neuroendocrine markers analyzed in an index. The main findings in his study are strengthened by results in papers by Glei and others (2007) and Dowd and Goldman (2005) who also failed to find links in the SEBAS between their measures of stress and physiological dysregulation.

To conclude, this is the first paper to use data from the CRELES, a nationally representative survey of older persons in Costa Rica, to attempt to link measures of emotional, social, and material resources (as well as negative life events and demands) to measures of physiological dysregulation. The degree of the negative findings in this paper (and in some other high quality studies) raise serious doubts about a key assumption of the allostatic framework – that baseline levels of the neuroendocrine markers become dysregulated through stress experienced over the life course.

References

Babisch, W. (2003). Stress hormones in the research on cardiovascular effects of noise. *Noise and Health, 5*(18), 1-11.

Babisch, W., Fromme, H., Beyer, A., & Ising, H. (2001). Increased catecholamine levels in urine in subjects exposed to road traffic noise. The role of stress hormones in noise research. *Environment International, 26*(7-8), 475-81.

Bähr, J., & Wehrhahn, R. (1993). Life expectancy and infant mortality in Latin America. *Social Science & Medicine, 36*(10), 1373-1382.

Bolaños, R. L., Partanen, T., Berrocal, M., Alvárez, B., & Córdova, L. (2008). Determinants of health in seasonal migrants: Coffee harvesters in Los Santos, Costa Rica. *International Journal of Occupational and Environmental Health, 14*, 129-137.

Cacioppo, J. T., & Hawkley, L. C. (2003). Social isolation and health, with an emphasis on underlying mechanisms. *Perspectives in Biology and Medicine, 46*(3 Suppl.), S39-52.

Cohen, S., Kessler, R. C., & Gordon, L. U. (1995). Strategies for measuring stress in studies of psychiatric and physical disorders. In S. Cohen, R. C. Kessler, & L. U. Gordon (Eds.), *Measuring stress: A guide for health and social scientists* (pp. 3-26). New York: Oxford University Press.

Crimmins, E., & Seeman, T. (2004). Integrating biology into the study of health disparities. In L. J. Waite (Ed.), Aging, health, and public policy: Demographic and economic perspectives. *Population and Development Review, 30,* 89-107.

Crimmins, E. M., & Seeman, T. (2001). Integrating biology into demographic research on health and aging (with a focus on the MacArthur Study of Successful Aging). In C. E. Finch, J. W. Vaupel, & K. Kinsella (Eds.), *Cells and surveys: Should biological measures be included in social science research?* (pp. 9-41). Washington, DC: National Academy Press.

Day, T. A. (2005). Defining stress as a prelude to mapping its neurocircuitry: No help from allostasis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 29*, 1195-1200.

Dowd J. B., & Goldman N. (2006). Do biomarkers of stress mediate the relation between socioeconomic status and health? *Journal of Epidemiology and Community Health*, *60*(7), 633-9.

Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D., & Cawthon, R. M. (2004). Accelerated telomere shortening in response to life stress. *Proceedings of the National Academy of Sciences of the United States of America, 101*(49): 17312-5.

Evans, G. W. (2003). A multimethodological analysis of cumulative risk and allostatic load among rural children. *Developmental Psychology, 39*(5), 924-33.

Gersten, O. (2008a). Neuroendocrine biomarkers, social relations, and the cumulative costs of stress in Taiwan. *Social Science & Medicine, 66*(3), 507-519.

Gersten, O. (2008b). The path traveled and the path ahead for the allostatic framework: A rejoinder on the framework's importance and the need for further work related to theory, data, and measurement. *Social Science & Medicine, 66*(3), 531-535.

Glei, D. A., Goldman, N., Chuang, Y.-L., & Weinstein, M. (2007). Do chronic stressors lead to physiological dysregulation? Testing the theory of allostatic load. *Psychosomatic Medicine, 69*(8), 769-776.

Goldberg, D. (2006). The aetiology of depression. *Psychological Medicine, 36*(10), 1341-7.

Goldman, N., Glei, D. A., Seplaki, C., Liu, I-W., & Weinstein, M. (2005). Perceived stress and physiological dysregulation in older adults. *Stress, 8*(2), 95-105.

Goldman, N., Turra, C. M., Glei, D. A., Lin, Y.-H., & Weinstein, M. (2006). Physiological dysregulation and changes in health in an older population. *Experimental Gerontology, 41*(9), 862-870.

Goldman, N., Weinstein, M., Cornman, J., Singer, B., Seeman, T., & Chang, M-C. (2004). Sex differentials in biological risk factors for chronic disease: estimates from population-based surveys. *Journal of Women's Health, 13*(4), 393-403.

Hall, C. (1984). Regional inequalities and well-being in Costa Rica. *Geographical Review, 74*(1), 48-62.

Herring, A. A., Bonilla-Carrión, R. E., Borland, R. M., & Hill, K. H. (In press). Differential mortality patterns between Nicaraguan immigrants and native-born residents of Costa Rica. *Journal of Immigrant and Minority Health*.

Hinojosa-Laborde, C., Chapa, I., Lange, D., & Haywood, J. R. (1999). Gender differences in sympathetic nervous system regulation. *Clinical and Experimental Pharmacology and Physiology, 26*(2), 122-6.

James, G. D., & Brown, D. E. (1997). The biological stress response and lifestyle: catecholamines and blood pressure. *Annual Review of Anthropology, 26*, 313-335.

Kajantie, E., & Phillips, D. I. (2006). The effects of sex and hormonal status on the physiological response to acute psychosocial stress. *Psychoneuroendocrinology, 31*(2), 151-78.

Karlamangla, A. S., Singer, B. H., Greendale, G. A., & Seeman T. E. (2005). Increase in epinephrine excretion is associated with cognitive decline in elderly men: MacArthur studies of successful aging. *Psychoneuroendocrinology*, *30*(5), 453-60.

Kubzansky, L. D., Berkman, L. F., Glass, T. A., & Seeman, T. E. (1998). Is educational attainment associated with shared determinants of health in the elderly? Findings from the MacArthur Studies of Successful Aging. *Psychosomatic Medicine, 60*(5):578-85.

Lemieux, A. M., & Coe, C. L. (1995). Abuse-related posttraumatic stress disorder: Evidence for chronic neuroendocrine activation in women. Psychosomatic Medicine, 57(2), 105-15.

Loucks, E. B., Juster, R. P., & Pruessner, J. C. (2008). Neuroendocrine biomarkers, allostatic load, and the challenge of measurement. *Social Science & Medicine, 66*(3), 525-530.

McEwen, B. S. (2004). Protective and damaging effects of the mediators of stress and adaptation: allostasis and allostatic load. In: J. Schulkin (Ed.), *Allostasis, homeostasis, and the costs of physiological adaptation*. Cambridge: Cambridge University Press.

Olff, M., Güzelcan, Y., de Vries, G. J., Assies, J., Gersons, B. P. (2006). HPA- and HPT-axis alterations in chronic posttraumatic stress disorder. *Psychoneuroendocrinology, 31*(10), 1220-1230.

Piccinelli, M., & Wilkinson, G. (2000). Gender differences in depression. Critical review. *British Journal of Psychiatry, 177*, 486-92.

Powell, L. H., Lovallo, W. R., Matthews, K. A., Meyer, P., Midgley, A. R., Baum, A., Stone, A. A., Underwood, L., McCann, J. J., Herro, K. J., & Ory, M. G. (2002). Physiologic markers of what chronic stress in premenopausal, middle-aged women. *Psychosomatic Medicine, 64*(3), 502-9.

Rosero-Bixby, L. (2007). *Biomarkers in a survey on aging in Costa Rica*. Oral presentation at the International Union on the Scientific Study of Population (IUSSP) seminar on "Aging in developing countries: Building bridges for integrated research agendas." Santiago, Chile (April 23-24, 2007).

Sandoval-García, C. (2004). *Threatening others: Nicaraguans and the formation of national identities in Costa Rica*. Athens: Ohio University Press.

Sapolsky, R. M. (2004). *Why zebras don't get ulcers* (3rd ed.). New York: Henry Holt & Co.

Seeman, T. E., Singer, B. H., Rowe, J. W., Horwitz, R. I., & McEwen, B. S. (1997). Price of adaptation - allostatic load and its health consequences. MacArthur studies of successful aging. *Archives of Internal Medicine, 157*(19), 2259-68.

Seeman, T. E., McEwen, B. S., Rowe, J. W., & Singer, B. H. (2001). Allostatic load as a marker of cumulative biological risk: MacArthur studies of successful aging. *Proceedings of the National Academy of Sciences of the United States of America, 98*(8), 4770-5.

Seeman, T. E., Glei, D., Goldman, N., Weinstein, M., Singer, B., & Lin, Y.-H. (2004). Social relationships and allostatic load in Taiwanese elderly and near elderly. *Social Science & Medicine, 59*(11), 2245-57.

StataCorp. (2005). Stata statistical software: Release 9.0. College Station, TX: StataCorp.

Thoits, T. (1995). Stress, coping, and social support processes: Where are we? What next? *Journal of Health and Social Behavior*(Extra Issue), 53-79.

Timiras, P. S., & Gersten, O. (2007). The adrenals and pituitary -- stress, adaptation, and longevity. In P. S. Timiras (Ed.), *Physiological basis of aging and geriatrics* (4th ed., pp. 137-57). New York: Informa Healthcare.

Turra, C. M., Goldman, N., Seplaki, C. L., Glei, D. A., Lin, Y.-H., & Weinstein, M. (2005). Determinants of mortality at older ages: The role of biological markers of chronic disease. *Population and Development Review, 31*(4), 675–698.

Van Cauter, E., Leproult, R., & Kupfer, D. J. (1996). Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *Journal of Clinical Endocrinology and Metabolism, 81*(7), 2468-73.

Wheler, G. H., Brandon, D., Clemons, A., Riley, C., Kendall, J., Loriaux, D. L., & Kinzie, J. D. (2006). Cortisol production rate in posttraumatic stress disorder. *The Journal of Clinical Endocrinology & Metabolism, 91*(9), 3486-9.

Worthman, C. M. (2000). Endocrine pathways in differential well-being across the life course. In D. Kuh, & R. Hardy (Eds.), *A life course approach to women's health* (pp. 197-232). Oxford: Oxford University Press.

Yehuda, R., Kahana, B., Binder-Brynes, K., Southwick, S. M., Mason, J. W., & Giller, E. L. (1995). Low urinary cortisol excretion in Holocaust survivors with posttraumatic stress disorder. *The American Journal of Psychiatry, 152*(7), 982-6.

Table 1 Descriptive statistics and cut-points for the neuroendocrine biomarkers – sample population, Costa Rica (ages 60 to 109, both sexes combined, years 2004-2006)[a]

				Percentile cutoffs			
	Mean	SD	N	10th	25th	75th	90th
System							
HPA axis							
Cortisol (µg/g creatinine)	29.5	35.5	2252	--	--	34.0	52.5
DHEAS (µg/dl)	49.1	42.1	2621	10.4	19.7	--	--
SNS							
Epinephrine (µg/g creatinine)	8.6	12.4	1520	--	--	10.2	18.9
Norepinephrine (µg/g creatinine)	43.3	41.1	1571	--	--	52.3	78.2

[a] The tabulations are based on unweighted survey data. The literature most often represents high risk by greater values for cortisol, epinephrine, norepinephrine, and lower values for DHEAS, a convention which is followed in this paper.
Source: Authors' tabulations based on the 2004-2006 CRELES (Rosero-Bixby, 2007).

Table 2 Descriptive statistics for the dependent and independent variables used in the analysis – sample population, Costa Rica (ages 60 to 109, both sexes combined, years 2004-2006)[a]

Variables	% or Mean (SD)	Range	N
Dependent			
Neuroendocrine allostatic load (NAL)[b]	0.99 (1.01)	0-4	1332
Independent			
Demographic			
Age	76.4 (10.2)	60-109	2827
Female sex	54%	--	2827
Low education (< 6 years)	70%	--	2827
Rural residence (v. urban)	40%	--	2827
Immigrant (v. native born)	6%	--	2817
Economic resources			
Household wealth[c]	2.02 (0.62)	1-3	2780
Monthly income (in thousands)	145.3 (358.4)	0-10,548	2738
Self-assessed economic situation[d]	3.6 (0.93)	1-5	2811
Spousal characteristics			
Low education (< 6 years)	68%	--	2827
Serious health problem	39%	--	1402
Social deprivation			
Currently unmarried (v. curr. married)	50%	--	2817
Lives alone	12%	--	2823
Low church attendance (< weekly)	56%	--	2822
Loss			
No. of children who have died (>= 1)	45%	--	2818
Length of widowhood (years)[e]	15.9 (13.4)	0-70	785
Early childhood conditions			
Maternal age at death	73.9 (18.1)	17-115	2302
Low maternal education (no education)	36%	--	2245
Lived without biological father	22%	--	2114
Poor health (>= 1 health problems)	23%	--	2090
Economic deprivation index[f]	2.2 (1.3)	0-4	2103
Cumulative adversity			
Overall stressor index	4.4 (2.4)	0-13	1764

Note: [a] Tabulations based on unweighted data.
[b] Respondents received one point toward their neuroendocrine allostatic load (NAL) score for each biomarker which had a "high-risk" value (i.e., a value below the 25th or above the 75th percentiles).
[c] High wealth is coded as three and low wealth is coded as one.
[d] "Excellent" is coded as one and "bad" as five.
[e] Only includes the widowed respondents.
[f] More severe economic deprivation is represented by higher values on this index.
Source: Authors' tabulations based on the 2004-2006 CRELES (Rosero-Bixby, 2007).

Table 3 Estimated regression results from different models with neuroendocrine allostatic load (NAL) as the dependent variable, Costa Rica (ages 60 to 109, both sexes combined, years 2004-2006)[a]

Dependent variable: NAL[b]	Model 1	Model 2	Model 3	Model 4	Model 5
Independent variables					
Demographic					
Age	**0.02 (0.000)**	**0.02 (0.000)**	**0.02 (0.000)**	**0.02 (0.000)**	**0.02 (0.000)**
Female sex	**0.41 (0.000)**	**0.37 (0.000)**	**0.40 (0.000)**	**0.37 (0.000)**	**0.36 (0.000)**
Low education (< 6 years)	-0.09 (0.116)	--	--	--	-0.13 (0.103)
Rural residence (v. urban)	-0.01 (0.841)	--	--	--	-0.06 (0.507)
Immigrant (v. native born)	-0.22 (0.043)	--	--	--	-0.29 (0.036)
Economic resources					
Household wealth	0.03 (0.588)	--	--	--	0.04 (0.556)
Monthly income (in thousands)	-0.00 (0.215)	--	--	--	-0.00 (0.193)
Self-assessed economic situation	0.02 (0.504)	--	--	--	-0.01 (0.851)
Social deprivation					
Currently unmarried (v. curr. married)	--	0.09 (0.217)	--	--	-0.00 (0.969)
Lives alone	--	0.02 (0.872)	--	--	0.13 (0.311)
Low church attendance (< weekly)	--	0.03 (0.603)	--	--	-0.03 (0.733)
Loss					
No. of children who have died (>= 1)[c]	--	--	0.10 (0.184)	--	0.13 (0.112)
Early childhood conditions					
Maternal age at death (years)	--	--	--	0.00 (0.432)	0.00 (0.630)
Low maternal education (no education)	--	--	--	-0.05 (0.517)	-0.09 (0.286)
Lived without biological father	--	--	--	-0.06 (0.535)	-0.03 (0.785)
Poor health (>= 1 health problems)	--	--	--	-0.12 (0.093)	-0.13 (0.092)
Economic deprivation index	--	--	--	0.01 (0.713)	0.00 (0.944)
Constant	**-1.08 (0.002)**	**-0.85 (0.001)**	**-0.94 (0.000)**	**-0.90 (0.016)**	-0.79 (0.100)
N	1280	1324	1322	833	803
R^2	0.099	0.093	0.096	0.085	0.104

Note: [a] The regression coefficients are unstandardized and p-values are inside the parentheses. All regressions control for alcohol consumption, smoking, and medication use.

[b] NAL ranges from 0 to 4, with 4 representing highest risk. Results presented with NAL cutpoints at the 25th and 75th percentiles.

[c] Regressions with this variable in the model also control for total number of children ever born.

Source: Authors' calculations based on the 2004-2006 CRELES (Rosero-Bixby, 2007).

Table 4 Estimated regression results with neuroendocrine allostatic load (NAL) as the dependent variable and widowhood as the key independent variable, Costa Rica (ages 60 to 109, both sexes combined, years 2004-2006)[a]

Dependent variable: NAL[b] **Independent variables**	Model 1	Model 2	Model 3	Model 4
Widowhood (years)	-0.001 (0.273)	-0.002 (0.863)	-0.008 (0.196)	0.008 (0.633)
Widowhood (years)^2	--	-0.000 (0.798)	--	-0.000 (0.276)
Demographic[c]	included	included	included	included
Economic resources[c]	--	--	included	included
Social deprivation[c]	--	--	included	included
Loss[c]	--	--	included	included
Early childhood conditions[c]	--	--	included	included
F-test (years + years^2)	--	(0.464)	--	(0.140)
N	362	362	183	183
R^2	0.154	0.154	0.245	0.249

Note: [a] The regression coefficients are unstandardized and p-values are inside the parentheses. All regressions control for alcohol consumption, smoking, and medication use.
[b] NAL ranges from 0 to 4, with 4 representing highest risk. Results presented with NAL cutpoints at the 25th and 75th percentiles.
[c] Includes the subset of variables listed in Table 3.
Source: Authors' calculations based on the 2004-2006 CRELES (Rosero-Bixby, 2007).