EVALUATING ALLOSTATIC LOAD: A NEW APPROACH TO MEASURING LONG-TERM STRESS IN WILDLIFE

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Abstract: Animal welfare, conservation, and stress assessment are all critical components of species survival. As organisms experience stressors, they accumulate physiologic dysregulation, leading to multiple negative health outcomes. This brief review suggests measuring the degree of stress-induced damage, known as allostatic load, and then using allostatic load to evaluate changes implemented to improve animal welfare and conservation efforts. Over the past two decades, human clinical research has developed multiple allostatic load indices constructed from composites of neuroendocrine, cardiovascular, metabolic, and immune biomarkers. These indices are designed to estimate allostatic load in hopes of ameliorating or even negating damaging effects of stress. Among humans, allostatic load is associated with a variety of factors such as age, sex, stressful experiences, personality, social position, and early life history. Despite conservation of stress responses throughout mammalian species, reported allostatic load indices for animals are rare. Because many zoo researchers and field scientists already collect data on multiple biomarkers, constructing allostatic load indices may be a relatively affordable, easily implemented, and powerful tool for assessing relative risks of morbidity and mortality within wildlife. As an example, in a study among zoo-housed gorillas, an allostatic load index constructed using seven biomarkers was associated significantly with age, sex, stressful experiences, rearing history, markers of poor health, and mortality risk. Such results evidence that allostatic load is as applicable to animal populations as it is to humans. By using allostatic load as a predictive tool, human caretakers will be better informed of individuals at greatest risk for health declines. Most importantly, allostatic load may provide earlier opportunity for preemptive care while contributing a transformational tool to animal welfare research. Additionally, allostatic load may be compared between individuals and groups within the same population and allow comparisons of health between and across populations, consequently informing habitat and population protection efforts.

Key words: Conservation, morbidity, mortality, preventative care, stressors, welfare.

INTRODUCTION

Vertebrate stress responses are promoted by neuroendocrine and metabolic mechanisms by which the body adapts behaviorally and physiologically to temporary stressors for improved survival. This adaptation is referred to as allostasis. Repeated or chronic allostatic activity can cause physiologic dysregulation, leading to poor health outcomes. In humans, chronic stress response activation is associated with asthma, diabetes, hypertension, hypercholesterolemia, gastrointestinal disorders, cardiovascular disease (CVD), viral infections, cognitive decline, neoplasia, frailty, psychological disorders, immunosuppression, and autoimmune dysfunction.

Even allostatic responses after minor physical, biological, behavioral, or social stimuli may damage cells, tissues, and organs, resulting in measurable physiologic change. Although stress responses and stress-induced pathology are multisystemic, most vertebrate stress research has been limited to reporting responses of a few physiologic biomarkers, such as adrenal glucocorticoids. In humans, an expanding area of research is applying integrated composites of biomarkers from multiple somatic systems to measure stress-induced physiologic dysregulation, or allostatic load. When compared between individuals, higher allostatic load reflects greater subclinical risk for future development of chronic degenerative conditions and shortened lifespan. Applications of allostatic load to nonhuman species are rare. However, a comprehensive measure of allostatic load in wild species may allow us to better care for wild animals in captivity and inform conservation measures by facilitating identification and mitigation of stressors that may be associated with poor health outcomes. In this paper, literature on allostatic load in humans is briefly reviewed, how allostatic
load is determined is explained, methodologic variability is addressed, and results of the first application of an allostatic load index to a nonhuman species are reviewed.

THE ALLOSTATIC LOAD MODEL

Allostatic load accumulates as the soma perceives and deals with stressors across the lifespan (Fig. 1). Multiple predisposing factors, such as developmental, social, and environmental conditions, influence how organisms perceive events. Once stressors are perceived, allostatic responses occur via fluctuations in primary mediators (e.g., stress hormones and inflammatory markers), which promote additional change throughout the soma in response to current stressors. This allostatic activity normally ceases once the perceived stressor is eliminated.\(^5\) However, when allostatic stress responses occur repeatedly over the lifespan, dysregulation in primary mediators (e.g., metabolic and cardiovascular markers) can occur,\(^5\) reflecting long-term exposure to stressors. Systemic dysregulation may then lead to tertiary outcomes associated with morbidity and mortality, such as development of chronic degenerative diseases (Fig. 1).\(^5\) The purpose of constructing allostatic load indices is to estimate the extent of physiologic dysregulation (ie, allostatic load) in members of a sample, which serves as an early, subclinical warning sign of future poor health outcomes.

The first human allostatic load index included 10 biomarkers representing the neuroendocrine, cardiovascular, and metabolic systems, including systolic blood pressure, diastolic blood pressure, waist–hip ratio, total cholesterol : high-density lipoprotein (HDL) ratio, glycosylated hemoglobin (HbA1c), urinary cortisol, urinary epinephrine, urinary norepinephrine, HDL cholesterol, and dehydroepiandrosterone sulfate (DHEA-S).\(^7\) To estimate allostatic load, each biomarker was divided into quartiles of risk (high risk being the highest quartile for all but DHEA-S and HDL, which are considered high risk in the lowest quartile). These biomarkers were then incorporated into a single, comprehensive value by summing the number of biomarkers in the high-risk quartile for each individual.

Because allostatic load accumulates through dysregulation of the entire soma, measuring its effects is not limited to specific biomarkers. As applications of allostatic load to assessing human stress increase, so does the number of biomarkers potentially useful in its estimation,\(^6\)\(^,\)\(^14\)\(^,\)\(^35\) with a 2010 review counting 51 different biomarkers in 58 studies.\(^3\) While allostatic load incorporates traditional stress-activated hormones, such as...
glucocorticoids and catecholamines, additional biomarkers of physiologic dysregulation include inflammatory markers (interleukin 6 [IL-6], fibrinogen), neurotransmitters (serotonin, dopamine), physioactive proteins (creatinine, albumin, C-reactive protein), and triglycerides.6,18,35,45 Because the stress response is highly conserved across vertebrates,63 dozens of biomarkers validated for assessing allostatic load in humans likely will be similarly useful in many other species, although some will not. For example, glucocorticoids, catecholamines, inflammatory markers, and physioactive proteins are used throughout research on humans6,14,35 and are likely to be useful in animal allostatic load indices. Because allostatic load indices typically are constructed by combining biomarkers reflecting acute stress responses (e.g., glucocorticoids) and those reflecting long-term physiologic dysregulation (e.g., albumin), allostatic load estimates both current physiologic stress and long-term stress-induced somatic damage.

**Estimating allostatic load**

Allostatic load is a soma-wide process affecting multiple integrated systems, so providing a comprehensive measure requires an allostatic load index composed of a variety of neuroendocrine, cardiovascular, metabolic, and immune biomarkers. Most allostatic load indices include between eight and 14 biomarkers.6,14,35 Importantly, allostatic load may be estimated using previously obtained data, thereby limiting invasiveness among animals living in frequently studied groups. All biomarkers incorporated into an allostatic load index should be assessed at the same time, although multiple tissue types can be used (e.g., saliva, urine, feces, hair, serum).

Figure 2. How to calculate allostatic load using quartile cut-points. Q1 indicates first quartile (lowest values); Q2, second quartile; Q3, third quartile; Q4, fourth quartile (highest values).
clinical standards are used to determine cut-points for BMI,\textsuperscript{14} most Samoan women will receive a 1 toward their allostatic load, and most Yanomami women will not. This artificially inflates risk in each population, both of which have faced different environments and conditions across the lifespan.\textsuperscript{14} Clinically based constructs directly counteract the usefulness of allostatic load indices in determining those individuals in a group who are at greatest underlying and sub-clinical risk for future health issues and shortened lifespan.

Depending on the biomarker, either hypo- or hypersecretion (hormones) or low or high levels (physiologic measures) may indicate greater risk of early morbidity and mortality (Fig. 2). For example, elevated IL-6 is maladaptive, so the top 25\% of the distribution would be considered high risk and individuals with values in that quartile would receive a 1 toward their allostatic load score, whereas those with values outside the highest quartile would receive a 0. Albumin, on the other hand, is considered dysregulated when it is low, so the bottom 25\% of the distribution would be considered high risk and those with values in that quartile would receive a 1 toward their allostatic load score, whereas individuals with values outside the lowest quartile would receive a 0. Allostatic load is then determined by summing the number of biomarkers within the high-risk quartile for each individual (Fig. 2). Each animal’s allostatic load will lie between 0 (no biomarkers in the high-risk quartile) and the number of biomarkers included (all biomarkers in the high-risk quartile).

Since its initial implementation, other methods of calculating allostatic load have been developed in human research, such as using clinical cut-points when available, calculating scores for individual systems (e.g., cardiovascular, metabolic), demarcating allostatic load into “high” versus “low” categories, using two-tailed cut-points for biomarkers considered dysregulated at both extremes, using sex-specific cut-points, weighting different biomarkers, and adjusting for medications affecting biomarkers of interest.\textsuperscript{14} Some of these modifications may improve allostatic load estimation. Using sex-specific cut-points is beneficial when titer of biomarkers are significantly different between males and females, because that biomarker may differentially associate with age or other predisposing factors based on sex. Differentially weighting biomarkers also may improve predictive value,\textsuperscript{17} although equally weighted constructs adequately reflect allostatic load and predict future outcomes just as accurately.\textsuperscript{19}

Other modifications may be less beneficial, such as adjusting allostatic load to account for medication use. The original allostatic load index and many subsequent reports consider medicinal interventions that control some biomarkers (e.g., blood pressure, cholesterol) to beneficially reduce known risk factors, subsequent wear-and-tear, and physiologic dysregulation, thus lowering allostatic load.\textsuperscript{47,68,78} Other studies account for medications by somehow adjusting the allostatic load index (e.g., adding a point for every biomarker directly affected by a medication regardless of the biomarker’s current level; specific adjustments vary by study).\textsuperscript{1,2,13,19,25,50,67} Following this logic, appropriate medical intervention leads to increased allostatic load. However, it does not follow that when an obese person loses weight by increasing their activity or a hypertensive individual reduces their blood pressure using medication, that their overall allostatic load would be increased. Thus, penalizing a subject’s allostatic load because these activities result in reductions in biomarkers is counterintuitive. Allostatic load was designed to assess subclinical risks for current and future health losses and to allow both increases and decreases in biomarkers to alter its level. When biomarkers are controlled via pharmacologic or behavioral interventions and subsequently lowered to subclinical values, overall risk is reduced, and therefore one’s allostatic load also is reduced. Medication is intended to benefit health and well-being, thereby reducing allostatic load. Reduction in risk factors does not contribute to increased risk of morbidity or mortality, but rather the opposite. When constructing allostatic load indices, researchers must decide which, if any, modifications to the original methodology are necessary for adequately assessing allostatic load in their sample.

Within any sample and no matter the method, higher allostatic load relative to conspecifics corresponds to reduced health and poorer future outcomes, and vice versa. Quantitative analyses allow researchers examining stressors to explore relationships between allostatic load and multiple independent variables, such as sex, age, number of known stressors experienced (e.g., anesthetic events, wounding), and behavioral characteristics. Additionally, researchers can use quantitative analyses to explore relationships of allostatic load with a variety of dependent variables, for example morbidity (e.g., incidences
of illness, biomarkers of health, immune system activity) and lifespan.

**ALLOSTATIC LOAD IN HUMANS**

Despite using different biomarkers for construction, studies incorporating allostatic load indices in humans report similar results. For example, there are multiple predictors of higher allostatic load. Corresponding to natural age-related declines in many biomarkers, allostatic load in humans is positively associated with age. Just as early life experiences can affect stress response activity, they also can influence adult allostatic load. For example, low birth weight and childhood abuse or neglect are associated with higher allostatic load in adulthood. Personality and sociality also affect allostatic load. Hostility, poor coping skills, and higher perceived stress are associated with higher allostatic load, whereas strong social networks are associated with lower allostatic load. Social status also has an inverse relationship with allostatic load. In addition to environmental factors, genetic factors also likely influence allostatic load. For example, higher allostatic load is associated with shorter telomere length, and it has been suggested that individual allostatic load is 30% genetic and 70% environmental.

Higher allostatic load also is associated with multiple outcomes. Allostatic load is positively associated with measures of poor health and morbidity, such as poor physical performance and risk of physical decline, CVD, abdominal obesity, hypertension, diabetes, and arthritis. Individuals with higher allostatic load also have increased mortality risk. Higher allostatic load is related to poor mental health as well, including lower cognitive functioning, greater cognitive declines over time, decreased brain volume measurements, and psychiatric disorders.

**ALLOSTATIC LOAD RESEARCH IN ANIMALS**

Several researchers previously have suggested the inclusion of an allostatic load framework in studies of animal populations. Subsequently, various reports have included biomarkers of allostatic load, the concept of allostatic load, or both to frame research questions and discuss results. For example, fluctuations in numerous biomarkers in rodents (e.g., glucocorticoids, IL-6, tumor necrosis factor alpha [TNF-α], corticotro-
A case study: allostatic load in zoo-housed gorillas

Using zoo medical records and assays of banked serum, an allostatic load index was operationalized for western lowland gorillas (Gorilla gorilla gorilla, n = 27) housed at the Columbus Zoo and Aquarium (Powell, Ohio) between 1956 and 2014. This allostatic load index included seven biomarkers: albumin, cortisol, CRH, DHEA-S, glucose, IL-6, and TNF-α. Distributions of each biomarker were divided into quartiles (Table 1), and the number of biomarkers in the highest risk quartile represented each gorilla’s individual allostatic load. Because of significant differences between males and females in albumin and IL-6, sex-specific quartiles were determined for these biomarkers (Table 1). Both hypo- and hypersecretion of cortisol may reflect physiologic dysregulation,13,32,66 so a two-tailed quartile was used for this biomarker (bottom and top 12.5% of the sample distribution; Table 1).

Gorillas in this sample ranged from 6 to 52 yr of age (Table 2). Mean age for females was 25.2 (SD = 14.2) yr and for males was 20.0 (SD = 11.5) yr. At the time allostatic load was determined, 10 gorillas had died (Table 2). On average, sampled gorillas experienced 34.7 (SD = 31.7) stressful events (e.g., anesthetic events, zoo transfers, and aggressive encounters with wounding) over their lifespans (Table 2). Allostatic load ranged from 0 to 4 (Table 2). Allostatic load was significantly associated with age and number of stressful events experienced. Additionally, allostatic load differed significantly between male and female gorillas, with females having a twofold higher allostatic load than males. In terms of outcomes, allostatic load was significantly associated with indicators of poor health (e.g., triglycerides, creatinine) and mortality risk.16 Because disrupted early environments also are substantial stressors for many species, a follow-up study examined associations between rearing history and adult allostatic load.15 Males, regardless of rearing history, had low allostatic load. Females, however, had significant differences in allostatic load by rearing history. Wild-caught females had significantly higher mean allostatic load compared with mother-reared females, whereas nursery-reared females were between and not significantly different from either wild-caught or mother-reared females.

On the basis of higher allostatic load in females, it is hypothesized female gorillas may face substantially more stressors than silverbacks when in human care. Silverbacks are sometimes aggressive toward females,26 and in the wild, females subsequently limit proximity to the silverback to times of estrus or when they have an infant and require protection.18–62 The potential inability of females in human care to limit proximity to the silverback sufficiently may result in hypervigilance, increas-
ing their allostatic load compared with males. Together, these results suggest providing females greater control over their proximity to silverbacks may help reduce their allostatic load. This research provides evidence that allostatic load indices can be applied to animal populations in much the same way as human populations and may be useful for both captive and wild management strategies.

**CONCLUSIONS**

As a theory of stress response and physiologic dysregulation, allostasis and allostatic load provide a novel view on how stressors affect somatic health and function over the lifespan of an animal. Many animals adaptively hide illness and injury until their health is dire. Allostatic load indices are tools for identifying apparently healthy individuals who are at risk and in need of preventative care, with the goal of improving health and lengthening lifespan. Because allostatic load indices are designed to measure subclinical risk, they have the potential to enable earlier detection of potential health issues.

Future research on animal health and welfare may be enhanced by focusing on constructing allostatic load indices and analyzing health promoters and outcomes in ways similar to those used in human clinical research. Initially, validating associations between allostatic load and both predictive factors and outcomes should be the goal. Estimating allostatic load in animal species should be based on a mixture of multisystemic biomarkers reflecting both physiologic responses to acute stress (e.g., glucocorticoids, CRH) and indicators of long-term dysregulation from chronic stress (e.g., albumin, cholesterol). All biomarkers should be obtained at the same time, although they may be collected from different types of tissues. Research may commence immediately in many settings with the use of available data. For example, specific biomarkers typically are assayed during routine veterinary procedures in zoo collections, and others may be obtained from tissue samples banked during the same examination.

Researchers also will need to determine appropriate methods for constructing allostatic load indices and should be encouraged to explore different methods of construction, such as quartile versus decile cut-points, grouping males and females together versus establishing sex-specific cut-points, or accounting versus not accounting for medication use. One limitation to constructing allostatic load indices in animal research may be the lack of physiologic reference values for biomarkers of interest, thereby eliminating the possibility of using average or clinical cut-points. Fortunately, because allostatic load estimates subclinical risk among members of a particular population and traditionally is based on sample-specific distributions, this limitation should not hinder research progress. Additionally, such research efforts will be able to contribute biomarker data for inclusion in databases, such as Species 360 (formerly the International Species Information System), thereby helping establish standard reference values for use in future research efforts.

The predictive power of allostatic load may aid in informing human caretakers of which individuals are at greatest risk of health declines, providing greater opportunity for preemptive care and transforming animal welfare research. Using

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**Table 2.** Sex, age, number of stressful events experienced, age at death (if applicable), and estimated allostatic load at time of sampling among 27 western lowland gorillas previously or currently housed at the Columbus Zoo and Aquarium (1956–2014).

<table>
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<th>Age at sample (yr)</th>
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\[ \bar{X}, SD = 23, 13, 30.8, 13.2, 34.7, 31.7, 1.8, 1.3 \]

* Mean and standard deviation for age at death only calculated from deceased gorillas. Modified with permission from Edes et al.16
allostatic load allows comparisons between individuals and groups within the same population, as well as comparisons of overall health between and across populations, and consequently informs habitat and population protection measures. Because many zoos and field researchers already collect extensive biomarker data, allostatic load indices likely will be relatively inexpensive and easily implemented, providing an informative tool for improving zoo and sanctuary welfare, implementing preventive care, and measuring both intended and unintended effects of conservation strategies.

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LITERATURE CITED


20. Glei DA, Goldman N, Chuang Y-L, Weinstein M. Do chronic stressors lead to physiological dysreg-


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