Sociodemographic Factors and Residential Location Influence Allostatic Load and Frailty in Poland

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Arts in the Graduate School of The Ohio State University

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2017

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Abstract

In 2005, The World Health Organization listed work-related stress as a leading challenge to health for the 21st century. Mammals maintain homeostasis via an evolved neurophysiological response to life’s stressors - allostasis. Over time, chronic exposures and responses to stressors lead to physiological dysregulation. Compounded over a lifespan, these produce a somatic burden, an allostatic load. Compensatory allostatic responses and related somatic alterations across multiple systems cannot be measured directly. However, individual allostatic load may be assessed by combining arrays of neurophysiological biomarkers. To better understand how personal factors may contribute to physiological dysregulation, we examined associations of age, sex, area of residence, and frailty with a 10-biomarker estimate of allostatic load in 211 residents of Poland (ages 55-91 years) residing in either Nekla, a small village, or Poznan, an urban metropolis. We hypothesized men, older participants, residents of Poznan, and those showing higher frailty would all exhibit greater allostatic load. Overall, men and women did not differ significantly in their allostatic load (p=0.146). Nor was allostatic load higher among residents of Nekla (p=0.063). However, men from Nekla did show higher allostatic load than those from Poznan (p=0.039), women did not (p=0.510). Allostatic load also was not associated significantly with either age or frailty. In this sample, lifestyle appears to influence stress-related physiological dysfunction only in men.
Acknowledgments

I wish to express my sincere gratitude to my advisor, Dr. Douglas E. Crews, for his endless patience, guidance, and enthusiastic encouragement. I have truly valued his willingness to give his time so generously. Thank you to Dr. Cohen and Dr. Piperata for their valuable advice. I am particularly grateful for my family and friends and their generous support. Finally, I would like to thank Elizabeth Freeman and Emily Wolfe for their unending kindness.
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Introduction

Allostasis is an integrated theory of how evolutionary forces have shaped adaptive stress responses (Sterling and Eyer 1988; McEwen and Stellar 1993; Korte et al 2005; Brune 2008). As a process allostasis is a neurophysiological adaptation to stressors conserved across all vertebrate species (Nelson 2011). Via allostasis organisms regulate their internal milieu in response to harmful stimuli (stressors) thereby mitigating related damage. Growth of neurological structures for initiating allostatic stress responses occurs in utero and infancy as the hypothalamic-pituitary-adrenal axis (HPA) and sympathetic nervous system (SNS) grow and mature into an integrated system. Thereafter, the soma responds to stressors through dynamic alterations in physiology, allostasis (Sterling and Eyer 1988; McEwen and Stellar 1993; Schulkin 2004). Left unchecked, stressors during growth and development may imprint tissue-specific responses or limit development during critical periods, possibly exacerbating physiological dysregulation throughout life (Barker 1998; Kuzawa and Quinn 2009).

Rapidity, length, and strength of allostatic responses to stimuli are shaped by differences in DNA, environments, sociocultural settings, and lifestyles (Seeman et al. 1997; McEwen 1998a, McEwen 1998b; Schulkin 2004; Beckie 2012; Maus et al. 2014). Contemporary environmental, demographic, socioeconomic, and cultural stressors likely
deviate from threats experienced by our hominin ancestors; however we share similar DNA, cognitive and physiological substrates, and response patterns (Crews 2003; Bulley et al. 2016). Adverse fetal, infant and childhood experiences, disruptive interpersonal relations, and insecure economic status are major stressors experienced in modern contexts (McEwen 1998a; McEwen 1998b; Schulkin 2004). Chronic activation of allostasis, long-term exposures to stressors, and persistent over- or under-activity all lead to allostatic load, i.e. systemic damage due to wear-and-tear on the soma and senescent losses of cells (Sterling and Eyer 1988; McEwen and Stellar 1993; McEwen 1998a; McEwen 1998b; Singer and Ryff 1999; Edes and Crews 2017). Over the lifespan, short- and long-term physiological disruptions and both appropriate and inappropriate activation of allostasis leave individuals vulnerable to physiological dysregulation and allostatic load (Juster et al 2010; Schulkin 2004; Leahy and Crews 2012; Edes and Crews 2017). Theoretically, allostatic load is the sum of dysregulation of physiological function resulting from chronic stressor exposures, activation of allostasis, and systemic exposures to mediating hormones (e.g. cortisol, epinephrine, and norepinephrine), along with secondary influences on cardiovascular, immune, and metabolic function.

One goal of human biological research is determining how sociocultural factors influence phenotypic variation (Segerstrom and Miller 2004). To accomplish life history tasks in a constantly changing environment, individuals must respond adaptively when their somatic stability or survival are threatened. Allostasis is the adaptive neuroendocrine-physiological system vertebrates evolved to respond to current stressors (Nelson 2011) and to previously encountered ones by initiating an anticipatory response
(Sterling and Eyer 1988). Today this neurophysiological response system is observed across mammals including non-human primates (Nelson 2011) and is modulated by social factors (Marks and Nesse 1994; Korte et al 2005; Brune 2008; Leahy and Crews 2012; Edes et al 2016; Edes and Crews 2017). We propose differences in lifestyles as determined by residential location along with sex and age will modulate allostatic load and frailty among older people (ages 69+).

To test this hypothesis we estimated allostatic load using a 10-biomarker index and examined associations with sex, age, life-time area of residence, and frailty in Nekla, a village, and Poznan, a major urban metropolis, in Poland. We also hypothesized a significant association of allostatic load with frailty. Frailty is a clinical phenotype characterized by sarcopenia (loss of muscle cells) leading to losses of strength, endurance, and resilience, as well as unintended weight loss (Fried et al. 2001; Fried et al. 2004; Walston 2005; Walston et al. 2006). Frailty has been assessed with as few as five biomarkers to over 40 (Studenski et al. 2004). The five-biomarker index includes reduced walking speed, physical activity, strength, endurance, and weight loss. These indices are associated with current and future morbidity, future hospitalization, and higher mortality risks (Fried et al. 2001; Fried et al. 2004; Crews 2005; Walston 2005; Walston et al. 2006). We hypothesize allostatic load and frailty will be significantly higher at older age (69+ years) in this sample. Moreover, we hypothesize allostatic load and frailty will be significantly higher amongst residents of Poznan than those in Nekla. Further, we hypothesize allostatic load and frailty will be significantly higher among men. Finally, we
hypothesize allostatic load will be a significant independent predictor of frailty controlling for sex, age, and residence.

**Life History Theory**

Allostasis is advantageous, particularly over the short term. However, chronic activation of this response system may reduce long-term somatic function promoting negative health outcomes (Sterling and Eyer 1988; McEwen and Stellar 1993; McEwen 1999; Edes and Crews 2017). Allostasis allows organisms to evade harmful outcomes of stressful stimuli thereby maximizing the probability of current survival (McEwen and Seeman 1999; Crews 2003; Bracha 2004; Miller et al 2011). One postulate of life history theory is that multiple energetic trade-offs and short-term adjustments throughout life occur as the soma seeks to maximize its survival and increase its probability of reproduction given current environmental and cultural constraints (Lande 1982; Williams and Nesse 1991; Singer and Ryff 1999; Miller et al 2011).

Life history theory also predicts critical periods occur during growth when developing organisms are particularly sensitive to environmental stimuli and responses to current stressors may initiate alterations that will partly determine adult health and later life well-being (see Barker 1994; Bogin 1996; Cameron and Demerath 2002). Phenotypic responses to disruptions during such sensitive periods influence organ size, cell numbers, biological function, and physiological pathways, including stress responses and the HPA. Moreover, such disturbances in development may contribute to premature cell senescence, sarcopenia, osteopenia, osteoporosis, chronic disease risk, disability and death, thereby increasing allostatic load and frailty throughout life (Katz and Cowley
Different cultural and ecological settings may lead to differential responses to stressors and, subsequently, variability in adult allostatic load.

**Adaptive Stress Responses**

Humans assess potential threats cognitively. Specifically a neurophysiological network, involving the amygdala, hippocampus, thalamus, and sensory cortex aids in recalling stressful encounters and shaping perceptions of current stressors (LeDoux 2003; McEwen 2012; Danese and McEwen 2012). Human social complexity, along with needs for cognitive skills to perceive others’ intentions, organize their own thoughts, and survive to achieve reproductive success, may have provided selective pressure leading to increase neocortical tissue (see Dunbar 2009), perhaps concurrently, enhancing stress responses (Miloyan et al. 2016). For example, catecholamines (i.e. norepinephrine and epinephrine) secreted by the adrenal medulla enhance emotional memory by the amygdala. Thereby, threatening stimuli are associated with allostatic responses, and anticipatory responses to future stressors are enhanced. Sensory neurons of the prefrontal cortex detect and evaluate environmental change, anticipate threats, and initiate allostasis via the HPA axis (McEwen 1999, 2007, 2012; Schulkin 2004; Rosen and Schulkin 2004; Danese and McEwen 2012). Initiation of allostasis is influenced by multiple factors, from inherited DNA and early life and childhood experiences and social setting to personal experiences, behavior, lifestyle, and current social milieu (McEwen 2007).
Response to Stressful Stimuli

Regardless of their origins, stressors activate allostasis, thereby mediating internal systems, stimulating rapid, and often anticipatory, responses (see Sterling and Eyer 1988; McEwen and Stellar 1993; Edes and Crews 2017; Peters 2017). Activated first are sensory neurons that signal the neural network of a change in inputs (see Peters et al. 2017) activating the hypothalamus. The hypothalamus then activates the SNS and pituitary to release neuroendocrine hormones that lead to soma-wide changes (McEwen 1999, 2007). Initiated within the central nervous system (CNS), allostasis includes sensory, neural, hormonal, and immunological networks. Following sensory perception, neurological processing activates the autonomic nervous (ANS) and neuroendocrine-immune systems, thereby coordinating allostasis (Charmandari et al 2005). The ANS, comprised of the SNS and parasympathetic nervous systems (PSNS), modulates basic internal processes and functions (e.g., heart rate and respiration) (Seaward 2006; Edes and Crews 2017). The SNS initiates release of neurotransmitters activating the HPA to release modulating hormones, initiating allostasis as the soma responds to potential threats. The PSNS acts to restore somatic function (e.g., digestion) and energy expenditures to their currently optimal levels (see Seaward 2006; Peters et al. 2017). Jointly, the HPA and the sympathetic-adrenal-medullary axis (SAM), initiate allostasis via a hormonal cascade that regulates downstream processes, including cardiovascular, immune, and metabolic function (Rosen and Schulkin 2004; Edes and Crews 2017).

By releasing corticotropin releasing hormone (CRH; a peptide hormone) directly into the anterior pituitary, the hypothalamus stimulates release of adrenocorticotropic
hormone (ACTH) into circulation (Charmandari et al. 2005; Everly and Lating 2012). ACTH then binds to receptors on the adrenal cortex inducing release of glucocorticoids (e.g. cortisol and cortisone) and mineralocorticoids (e.g., aldosterone and deoxycorticosterone) (Charmandari et al 2005; Seaward 2006; Everly and Lating 2013). Glucocorticoids promote immediate release of energy, thereby preparing the organism for physical exertion in response to perceived threats to well-being or survival. These alterations promote gluconeogenesis in the liver, providing glucose for ready energy, as cortisol promotes metabolism of fatty acids via lipolysis and leads to increased arterial blood pressure and serum glucose (Charmandari et al 2005; Everly and Lating 2013; Seaward 2006). Mineralocorticoids further modulate blood pressure by enhancing potassium elimination, promoting sodium retention, and increasing blood volume (Meaney et al 1991; Seaward 2006; Everly and Lating 2013; Edes and Crews 2017).

In addition to activating pituitary release of ACTH, the hypothalamus, via a direct neural pathway, signals the adrenal medulla to secrete catecholamines (e.g., norepinephrine and epinephrine) (Evely and Lating 2013; Edes and Crews 2017). Jointly, norepinephrine and epinephrine prepare the soma to counter potential threats by promoting decreased bloodflow to non-essential systems (e.g., digestive), increase heart rate and blood pressure, and mobilization of fatty acids for catabolism to glucose (Sterling 2004; Seaward 2006; Everly and Lating 2013; Edes and Crews 2017). Over time, continued responses to stressors, ill effects of stressors, and cellular senescence produce physiological dysregulation, an allostatic load. Here we determine allostatic load for a sample of 211 residents of Poland aged 55 to 99 years.
Primary Mediators of Allostasis

Primary mediators of stress response include hormones of the HPA axis: cortisol, norepinephrine, epinephrine, and dehydroepiandrosterone sulfate (DHEAs); a cortisol antagonist (Seeman et al 1997; McEwen 1998a, 1998b; 2012). Both glucocorticoids and catecholamines can damage somatic and cognitive tissues and organ function, further dysregulating physiology (Seward 2006). During a stress event, circulating epinephrine may increase 300-fold from the resting state (Seward 2006). Conversely, low DHEAs may impair regulation of stress-responsive hormones (Schulkin 2004; Edes and Crews 2017). Theoretically, circulating titers of cortisol, epinephrine, and norepinephrine increase, while DHEAs decreases during stress responses and then they return to non-stressed levels. Due to stressor exposures, allostasis, and failure of allostasis or failure to limit allostasis, damage accumulates as allostatic load (Seeman et al. 1997; Singer et al. 2004; McEwen 1998a, 1998b, 2007).

Primary mediators of allostasis target diverse physiological systems. Among the secondary effects of allostasis are mobilization of glucose and cholesterol to supply immediate energy for activity. During allostasis, heart rate, blood pressure, and coronary output increase and bloodflow is diverted to neural and muscle tissues from the immune and digestive systems (Chrousos 1995; Seeman et al. 1997; Glaser et al.1999; McEwen 2012). Tertiary outcomes of allostasis, specifically allostatic load, are hypertension, cardiovascular and metabolic diseases, obesity, and altered patterns of circulating
immune cytokines. Prolonged and repeated exposure to harmful stimuli may result in chronic activation of allostasis. Over time, stressors and allostasis damage systems, leading to suboptimal functional states and increased allostatic load (McEwen and Stellar 1993; Singer and Ryff 1999; Schulkin 2004; Leahy and Crews 2012).

Ideally, primary and secondary mediators of allostasis return to pre-stressor states following activation and resolution of stressors. However, chronic activation may manifest as permanent, detrimental alterations in secondary mediators of allostasis which may lead to tertiary outcomes: cardiovascular, metabolic, and inflammatory diseases, and increased morbidity and mortality (McEwen and Stellar 1993; McEwen 1998a, 1998b; Seward 2006). Elevated glucocorticoids and catecholamines promote metabolism of white blood cells, leading to imbalanced and suppressed immune function, elevated inflammation, and impaired healing (Cohen et al. 1991; Chrousos 1995; Glaser et al. 1999; Bierhaus et al. 2003; Kiecolt-Glaser et al. 2003). Elevated stress response also is associated with metabolic dysfunction: insulin resistance, hyperglycemia, hypercholesterolemia, hypertension, and visceral obesity (Kirschbaum et al. 1993; Sterling 2004; Rosmond 2005; Hotamisligil 2006; Chandola et al. 2006).

Waist-hip ratio assess adipose distribution and reflects long-term energy storage; higher ratios indicates dysregulated metabolism (McEwen 1998a, 1998b, 2012; Singer et al. 2004; Gianaros 2009). Similarly, percent HbA1c assesses average glucose concentration over the past 60 days, indicating metabolic dysregulation (Singer et al. 2004; Seward 2006). In addition, amino acids released by habitual activation of the HPA, may remodel the hippocampus, amygdala, and prefrontal cortex neurons, thereby
impairing cognitive function (Seward 2006). Furthermore, shortened dendrites following stressors may impede emotional regulation, resulting in behavioral issues, depression, and impaired attention span (McEwen 2007; McEwen and Gianaros 2011; Danese and McEwen 2012).

**Physiological Dysregulation**

Understanding how cultural variability shapes phenotypes and the physiological pathways leading to allostatic load aids in understanding patterns of human variation across populations. Current medical practice relies upon evidence-based research to develop treatment modalities, while public health and health intervention programs depend upon predictive research to aid in promoting well-being of populations. These and other approaches rely upon an evidence-based understanding of how sociocultural and socio-demographic differences may impede or enhance stressors and human physiological responses to them (McEwen and Stellar 1993, McEwen 1998a, McEwen 1998b, Singer and Ryff 1999; Schulkin 2004). For example, the World Health Organization listed work-related stress as a leading challenge of the 21st century (Mauss et al 2014). Among the multiple influences on adult and later-life well-being and health are childhood and adult lifeways, childhood experiences, ancestry, diet, socioeconomic status, and social support. Here, we explore samples from Nekla and Poznan, a village and a city located in Greater Poland, to determine influences of sex, age, area of residency, and life-styles on allostatic load and frailty.

Many key physiological parameters, for example body temperature and blood oxygenation, are maintained within narrow ranges around homeostatic setpoints
(McEwen 1998a, McEwen 1998b, Singer and Ryff 1999; Schulkin 2004). Others such as blood pressure and heart rate are maintained via allostasis. Allostasis is stability through change (Sterling and Eyer 1988). Allostatic responses evolved to maximize survival through dynamic adaptive responses across systems that are not limited to specific ranges or set points (McEwen 1998a, 1998b, 2007, 2012). Unlike homeostasis, allostatic responses vary with circumstances and do not have a specific baseline to which they return. Allostatic physiological systems adjust to and accommodate variability as they respond to current and anticipated or perceived stressors (Koob and Le Moal 1997). Failure of allostatic response generally follows three patterns; 1) failure to habituate to a stressor, 2) overexpression of allostasis, 3) inadequate responses to stressors (McEwen 1998a; McEwen 1998b; Seplaki et al 2005).

**Assessing Allostatic Load**

Pathways mediating stress responses are complex and nonlinear. Because growth and development occur in complex settings and phenotypic variation increases with age, determining environmental, lifestyle, and biological influences on adult outcomes is complicated (Lu and Halfon 2003; Wingfield 2004; Crews and Bogin 2010; Glover 2011). Allostasis is a latent, internal, non-measurable, and nonlinear process of sensory, neurological and physiological responses to stressful stimuli; as such, allostatic load cannot be directly measured (Sterling and Eyer 1988; Sterling 2004; Leahy and Crews 2012; McEwen 1998a, 1998b, 2015; Edes and Crews 2017). Allostatic alterations across multiple systems, and their compensatory responses for one another cannot be measured either directly or simultaneously (Sterling and Eyer 1988; Sterling 2004; Seeman et al. 2009).
Currently allostatic load is assessed by combining arrays of physiological biomarkers across systems that mediate or are modulated during allostasis, the neuroendocrine, metabolic, cardiovascular, and immune (Seeman et al. 1997; McEwen 1998a, 1998b, 2012; Sterling 2004; Leahy and Crews 2012).

**Biomarkers of Allostatic Load**

The original assessment of allostatic load was a composite of four primary and six secondary biomarkers (Seeman et al. 1997). Their choice was based on evidence-based research indicating these hormones and physiological biomarkers respond to stressors and exact physiological costs on the soma (See Table 1; Seeman et al. 1997; McEwen 1998a, 1998b, 2007; Singer et al. 2004; McEwen 2015). These composites are useful for estimating the latent physiological state of allostatic load and show significant associations with lifestyles, stressor exposures, and morbid outcomes across populations (Seeman 1999; McEwen 1998a, 1998b, 2007, 2015; Karlamangla 2002; Seward 2006; Crews et al. 2015; Kusano et al. 2016).

**Table 1. 10 Biomarkers of Allostatic Load (after Seeman et al. 1997)**

<table>
<thead>
<tr>
<th><strong>Primary Mediators</strong></th>
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<tr>
<td>Cortisol [nmol/g creatinine]</td>
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<tr>
<td>Norepinephrine [mg/g creatinine]</td>
<td></td>
</tr>
<tr>
<td>Epinephrine [mg/g creatinine]</td>
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<tr>
<td>Dehydroepiandrosterone sulfate DHEA-S [mg/dl]</td>
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<table>
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<tr>
<th><strong>Secondary Mediators</strong></th>
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<tr>
<td>Systolic blood pressure (SBP) [mmHg]</td>
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</tr>
<tr>
<td>Diastolic blood pressure (SBP) [mmHg]</td>
<td></td>
</tr>
<tr>
<td>High density lipoprotein/Total-Cholesterol ratio [mg/dl]</td>
<td></td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol (LDL) [mg/dl]</td>
<td></td>
</tr>
<tr>
<td>Waist Hip Ratio</td>
<td></td>
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<tr>
<td>Glycated hemoglobin HbA1c [%]</td>
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Early life sociocultural conditions, including social support, lifestyles, and environmental settings continue to modulate responses to current stressors among adults and influence their allostatic load (Seeman 1999; McEwen 1998a, 1998b, 2007, 2015; Seward 2006; Singer et al. 2004; Kusano et al. 2016). In 2013, through collaborations with the Nekla Cultural Center, Nekla Medical Center, and the Poznan Center for Geriatrics and Gerontology, we obtained blood samples, overnight urine collections, anthropometric measurements, and self-reported data from 104 residents of Nekla and 107 from Poznan, Poland. Here we examine these data to determine influences of sex, age, and residential location on allostatic load and frailty. In addition, we examined associations between allostatic load and frailty.
Methods

Background and Demographics

Greater Poland is the fifth largest state in Poland. It includes an area of 29,826 km² and a population total of 3,477,755 people. Greater Poland includes 111 towns, with 55% of the population residing in urban cities compared to the national average of 60% (Konecka-Szydłowska 2016). Nekla includes 3,712 residents in a total area of 19.8 km², while Poznan has 541,561 people in a total area of 261.9 km² (Poland by Numbers, accessed on 20 February 2017). Greater Poland experienced increased integration into the global economy following political reforms during the late 20th century, producing a rapidly accelerating local economic landscape. In addition, between 1990 and 2000, Poland’s Gross Domestic Product (GDP) per capita almost doubled, by about 22% to 40% (Domanński 2003).

During the late 20th and early 21st centuries, international investments through transnational corporations and privatization transformed many rural landscapes and turned cities into economic centers throughout Greater Poland (Paszkowski 1996; Łowicki 2008; Konecka-Szydłowska 2016). Nekla is one of the smallest towns in Greater Poland. It encourages foreign and domestic investment to narrow its economic gap compared to larger urban centers (Poland by Numbers, accessed 20 February 2017; Nekla Community Website, accessed 20 February 2017). Nekla is located 36 km east of Poznan with convenient access to international rail routes (e.g. Berlin-Moscow) and domestic highways (i.e. A-2 motorway) providing easy access and opportunities for development (Łowicki 2008; Nekla Community website, accessed 20 February 2017). Recently a $139
million wind farm was commissioned by a Polish affiliate of E.ON Climate and Renewables for the Poznan area (A USA based company; see Power Technology 2017).

Even with recent advancements, Nekla’s infrastructure remains transitional. For example, 30% lack access to modern sewer systems and gas supply lines (Nekla Community Website, accessed 20 February 2017). Nekla’s total community expenditures were 25.5 million Polish Zloty PLN in 2014. The largest village expense was on education (12.5 million PLN) followed by social assistance (2.6 million PLN), and healthcare (108,900 PLN) (Poland by Numbers, accessed 20 February 2017). Attainment of higher education differs between Nekla (13.2%) and Poznan (31.7%), with a higher proportion of college educated women than men in both areas (Census 2011).

Poznan, the economic and cultural hub of Greater Poland, is a popular tourist destination being home to museums, parks, and historic monuments (Local-Life 2017). Currently, Poznan has the highest direct foreign investment per capita and is second only to Warsaw in assessed prosperity (Domanński 2003; Konecka-Szydłowska 2016). Poznan boasts a robust economy, with a mean gross monthly salary of 4,549 PLN and a 2.4% registered unemployment rate compared to a 406 PLN salary and 11.9% unemployment rate in Greater Poland on average (Poland by numbers, accessed on 20 February 2017). Poznan’s total annual budget in 2014 was 2.58 billion PLN. The largest portion of which was spent on education (810 million), followed by social assistance (272.8 million), and health (24.5 million). Pronounced differences in education, income, infrastructure, development, and lifeways make Nekla and Poznan useful settings to explore how differences in lifestyle may influence allostatic load among Poland’s citizenry.
Sampling

During 2013, 104 residents of Nekla aged 55 years and over and 107 similarly aged residents of Poznan participated in anthropometric, biochemical, physiological, and self-reported assessments of their health. Included were 53 women and 51 men from Nekla and 71 women and 36 men from Poznan. Participants were recruited via local announcements, personal contacts, and newspaper advertisements. Those who responded were informed of the details and significance of the project upon first contact. This included a health assessment, phlebotomy, anthropometry, a 12-hour overnight urine collection, and a self-reported questionnaire (the Short Form (36) Health Survey; SF 36). The SF 36 survey assesses physical and mental health based upon a series of questions on physical functioning, general health, bodily pain, vitality, activity, and social functioning. The SF 36 is useful for identifying shifts in quality of life, including physical ability, over time (Stewart et al. 1988).

Measurement Protocols for Physical Exams

Anthropometric and biochemical measurements were obtained following strict study protocols and were incorporated into physical exams according to guidelines in the Helsinki Declarations and approved by the Research Ethics Committee (no 425/13) of the Poznan University of Life Sciences. Waist circumference was measured using a fiberglass tape placed at the narrowest portion of the torso above the iliac crests. Hip circumference was measured at the point of greatest protuberance of the most posterior aspect of the pubic symphysis and buttocks. Systolic and diastolic blood pressures were measured according to Systolic Hypertension in the Elderly Population protocols using a
Litman® stethoscope and Baumanometer®, with the cuff placed an inch above the antecubital fossa. Calibrated measurements were repeated three times: the mean of the last two are utilized for all data analysis. Systolic blood pressure measures the pressure blood exerts against arterial walls during heart contraction (systole), while diastolic blood pressure measures pressure on your arteries between contractions (diastole), both are biomarkers of cardiovascular activity (Seward 2006).

Total cholesterol, low-density lipoprotein cholesterol (LDLc), high density lipoprotein (HDLc), glycated hemoglobin (HbA1c), were assayed from serum samples obtained during physical examination of participants. Participants' 12-hour overnight urine samples were retrieved at their physical examinations and assayed for DHEAs, cortisol, norepinephrine, and epinephrine at the Diagnostic Laboratory “Diagnostyka” of Poznan University following International Standards. These data were used to assess allostatic load and its correlates in this sample of 211 residents of Poland.

**Frailty Index**

Biomarkers of frailty include reduced strength and endurance, slow walking speed, poor balance, reduced physical activity, and unexpected weight loss (Fried et al. 2001; Fried et al. 2004; Rockwood et al. 2005; Walston 2005; Walston et al. 2006). To estimate frailty we used eight biomarkers. These included maximum hand grip strength, a reliable proxy to evaluate muscle strength (Rantanen et al. 1999; Velghe et al. 2016), using a dynamometer to assess their maximum grip strength and walking speed, a reliable assessment of current functional abilities (Fried et al. 2001; Fried et al. 2004), by measuring seconds a participant took to walk 50 feet on a flat surface.
We also obtained self-reports of participants’ physical activity levels and capabilities using the SF-36, an evidence-based, multipurpose, and self-administered health survey designed to assess the general health of participants (Ware and Sherbourne 1992; Ware 2000). Therein, participants were requested to evaluate eight aspects of their current health using a five-point Likert scale. Questions included here asked about self-perceived physical limitations, physical health issues, bodily pain, general physical well-being, and somatic dysfunction as an impression of their current overall general health (Ware and Sherbourne 1992; Ware 2000). Applied widely in biomedical research, clinical practice, and health policy initiatives, the SF-36 is easy to administer and useful for evaluating current health (McHorney et al. 1993). For physical activities the Likert scale ranged from “not limited at all” to “limited a lot” for vigorous activity, lifting or carrying groceries, climbing several flights of stairs, bending and kneeling, walking more than 1 km, and bathing or dressing oneself.

To estimate frailty, we examined sample distributions of available quantitative biomarkers to determine the most limited (frail) quartile which was scored 1 and all others 0. Slow walking speed and poor grip strength indicate frailty. Self-reports of limitations on vigorous activity, lifting groceries, climbing several flights of stairs, bending, walking more than 1 km, and bathing/dressing oneself, were assigned highest-risk and a score of 1 when participants reported they were “limited a lot”. We estimated frailty by summing the scores for walking speed, grip strength, and self-reported limitations from each participant, resulting in a possible score ranging from 0-8.
Assessing Allostatic Load

A variety of methods have been used to assess allostatic load (see reviews McEwen and Seeman 1999; McEwen 2007, 2015; Juster et al 2010; Beckie 2012; Leahy and Crews 2012; Edes and Crews 2017). The most common employs Seeman et al.’s (1997) original model (See Table 1). First we examined distributions of all 10 biomarkers to determine quartile cutpoints. Next, we scored each participant's biomarkers as indicating either high or low risk depending on evidence-based research showing their association with earlier morbidity and mortality. For all biomarkers, except DHEAs and HDLc, highest risk occurs in the highest quartile of their distributions. For these eight biomarkers, when an individual’s value was above the critical cut point it was scored 1, otherwise zero. DHEAs and HDLc were scored 1 when falling in the lowest quartile of their respective distributions and zero otherwise (see Seeman et al. 1997; McEwen and Seeman 1999; McEwen 2007, 2015). Each participant's allostatic load was then determined by summing their biomarker scores (see Seeman et al. 1997).

In the MacArthur Studies of Successful Aging, this combination of 10 biomarkers consistently predicted cognitive and physical declines over seven year follow up (Rowe and Kahn 1997). Allostasis is dynamic. By using quartiles and a ‘count-based’ index rather than other possible methodologies, our allostatic load estimate captures subclinical variability and risks that underlie physiological dysfunction (see Edes and Crews 2017). Similar estimates of allostatic load are associated significantly with environmental and lifestyle conditions associated with physiological dysregulation and morbidity (Seplaki et al 2005).
Statistical Methods

Bivariate differences in allostatic load between men and women and by residence were examined using t-tests (Table 3). Due to a statistically significant difference between the sexes in preliminary analyses, we recalculated allostatic load using sex-specific biomarker distributions. These were used as estimators of allostatic load in all further analyses. In addition to bivariate associations, we examined joint associations of age, sex, area of residence, and frailty with allostatic load using linear regression. Sex and area of residence were measured by two dummy variables (men=0 and women=1; Nekla=0 and Poznan=1). All statistical analyses were performed in SPSS 24.
Results

Table 2. Means, standard deviations, ranges, and lower bound of the fourth quartile for age, hormonal and physiological biomarkers, allostatic load, and frailty among 211 residents of Greater Poland.

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Range</th>
<th>4th Quartile Cut Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>69.63</td>
<td>7.18</td>
<td>55.00-91.00</td>
<td></td>
</tr>
<tr>
<td>Cortisol [nmol/g creatinine]</td>
<td>84.94</td>
<td>59.11</td>
<td>11.40-321.00</td>
<td>112.0</td>
</tr>
<tr>
<td>Norepinephrine [mg/g creatinine]</td>
<td>35.54</td>
<td>15.18</td>
<td>9.60-103.80</td>
<td>44.20</td>
</tr>
<tr>
<td>Epinephrine [mg/g creatinine]</td>
<td>6.02</td>
<td>6.26</td>
<td>0-35.70</td>
<td>9.30</td>
</tr>
<tr>
<td>DHEAs [mg/dl]</td>
<td>125.41</td>
<td>81.75</td>
<td>15.80-499.30</td>
<td>61.30</td>
</tr>
<tr>
<td>Systolic blood pressure [mmHg]</td>
<td>138.77</td>
<td>18.62</td>
<td>97.00-192.00</td>
<td>151.00</td>
</tr>
<tr>
<td>Diastolic blood pressure [mmHg]</td>
<td>78.41</td>
<td>12.88</td>
<td>47.00-123.00</td>
<td>86.00</td>
</tr>
<tr>
<td>High density lipoprotein/Total-Cholesterol ratio</td>
<td>29.33</td>
<td>23.22</td>
<td>11.11-347.62</td>
<td>21.15</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol [mg/dl]</td>
<td>125.65</td>
<td>39.63</td>
<td>8.20-227.00</td>
<td>153.00</td>
</tr>
<tr>
<td>Waist Hip Ratio</td>
<td>0.889</td>
<td>0.079</td>
<td>0.660-1.080</td>
<td>0.940</td>
</tr>
<tr>
<td>Glycated hemoglobin HbA1c [%]</td>
<td>6.22</td>
<td>0.75</td>
<td>4.80-10.60</td>
<td>6.40</td>
</tr>
<tr>
<td>Allostatic Load</td>
<td>2.50</td>
<td>1.62</td>
<td>0.00-8.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Frailty</td>
<td>2.42</td>
<td>1.70</td>
<td>0.00-7.00</td>
<td>4</td>
</tr>
</tbody>
</table>
Variation in Biomarkers

In the full sample, age averaged 69.63 years (range=55-91; sd=1.62; Table 2). Average age of 87 male participants was 69.8 years (range=55-91; SD=7.95), among the 124 women it averaged 69.5 years (range=55-87; SD=6.62; Table 3). Men from Nekla comprised 24% of the full sample (N=51), women 25% (N=53), men from Poznan comprised 17% (N=36), and women 33% (N=71). Within this sample LDLc averages 125.6 mg/dl falling within the normal range compared to clinical standards (100-129 mg/dl; NIH 2013), while its 4th quartile cut-point 153 mg/dl, is within the borderline high range (130-159 mg/dl; NIH 2013). Systolic blood pressure averages 138.7 mmHg and diastolic blood pressure 78.4 mmHg, at and near the lower range for prehypertension (SBP 120-139/DBP 80-89 mmHg; NIH 2013). The fourth quartile cut point for systolic blood pressure is 151 mmHg, that for diastolic blood pressure is 86 mmHg, both falling within stage 1 hypertension (SBP 140-159/DBP 90-99 mmHg; NIH 2013; Table 2). The high density lipoprotein cholesterol/total-cholesterol ratio averages 29.33 for full sample, above the borderline high clinical cut point (25 mg/dl; Mayo Clinic accessed on 20 September 2017).

Within the full sample, cortisol averages 83.94 nmol/g creatinine (Table 2). Cortisol averages for both men (91.30 nmol/g creatinine) and women (78.78 nmol/g creatinine), both fall within the normal range for clinical standards (1-119 nmol/g creatinine; 0.7-85 nmol/g creatinine respectively; Mayo Clinic accessed on 20 September 2017; Table 3). The 4th quartile cut-point for creatinine is 112.00 nmol/g, within the normal clinical distribution (Mayo Clinic accessed on 20 September 2017). Average
catecholamine levels for norepinephrine (35.54 mg/g creatinine) and epinephrine (6.02 mg/g creatinine) both fall within the normal clinical range (2-24 mg/g; 15-100 mg/g respectively; American College of Physicians accessed on 20 September 2017). The 4th quartile cut-points for norepinephrine and epinephrine also fall within normal clinical standards (44.2 mg/g creatinine; 9.3 mg/g creatinine; Mayo Clinic accessed on 20 September 2017; Table 3). DHEAs averages 154.85 mg/dl for men and 104.76 mg/dl for women both fall within normal clinical ranges (130-550 mg/dl; 60-330 mg/dl; Table 3; Mayo Clinic accessed on 20 September 2017). Percent HbA1C for full sample is 6.22%, within the prediabetic clinical range (5.7-6.4%; Table 3; Mayo Clinic accessed on 20 September 2017). Waist-hip-ratio for men averages 0.95 and for women 0.85, both falling within clinical standards for obesity (>0.90; >0.85 respectively; Table 3; World Health Organization accessed on 20 September 2017).

Table 3. Means, standard deviations, ranges, and p-values from Table 1 for age, hormonal and physiological biomarkers, allostatic load, and frailty comparing 87 men and 124 women residing in Greater Poland.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Men N=87</th>
<th>Women N=124</th>
<th>Range</th>
<th>Range</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>69.80 ± 7.95</td>
<td>69.53 ± 6.62</td>
<td>55-91</td>
<td>55-87</td>
<td>0.828</td>
</tr>
<tr>
<td>Cortisol</td>
<td>91.30 ± 67.29</td>
<td>78.78 ± 52.28</td>
<td>11.40-310.00</td>
<td>16.13-320.97</td>
<td>0.148</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>32.28 ± 12.83</td>
<td>37.83 ± 16.30</td>
<td>9.63-69.57</td>
<td>14.53-103.75</td>
<td>0.006</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>5.63 ± 5.12</td>
<td>6.29 ± 6.95</td>
<td>0-21.82</td>
<td>0-35.66</td>
<td>0.433</td>
</tr>
<tr>
<td>DHEAs</td>
<td>154.85 ± 95.98</td>
<td>104.76 ± 62.67</td>
<td>22.90-499.30</td>
<td>15.80-327.20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP</td>
<td>141.70 ± 18.47</td>
<td>136.26 ± 18.48</td>
<td>106.50-192.00</td>
<td>96.50-178.00</td>
<td>0.038</td>
</tr>
<tr>
<td>DBP</td>
<td>79.47 ± 12.27</td>
<td>77.27 ± 13.28</td>
<td>53.00-109.50</td>
<td>47.00-123.00</td>
<td>0.210</td>
</tr>
<tr>
<td>HDL/T-Chol</td>
<td>26.70 ± 7.60</td>
<td>31.17 ± 29.53</td>
<td>11.10-48.70</td>
<td>13.87-347.62</td>
<td>0.110</td>
</tr>
<tr>
<td>LDLc</td>
<td>117.40 ± 38.19</td>
<td>131.43 ± 39.74</td>
<td>37.00-222.00</td>
<td>8.20-227.00</td>
<td>0.011</td>
</tr>
<tr>
<td>WHR</td>
<td>0.947 ± 0.063</td>
<td>0.849 ± 0.063</td>
<td>0.777-1.078</td>
<td>0.656-1.078</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1c</td>
<td>6.31 ± 0.80</td>
<td>6.15 ± 0.71</td>
<td>4.80-9.20</td>
<td>5.30-10.60</td>
<td>0.127</td>
</tr>
<tr>
<td>Allostatic Load</td>
<td>2.70 ± 1.75</td>
<td>2.36 ± 1.50</td>
<td>0-8</td>
<td>0-7</td>
<td>0.146</td>
</tr>
<tr>
<td>Frailty</td>
<td>2.05 ± 1.46</td>
<td>2.68 ± 1.82</td>
<td>0-6</td>
<td>0-7</td>
<td>0.006</td>
</tr>
</tbody>
</table>
Allostatic Load

Allostatic load averaged 2.50 (sd=1.62; range= 0-8) in the full sample (Table 2). Allostatic load was higher among men, 2.70 (sd=1.74; range= 0-8) than women 2.36 (SD=1.5; range= 0-7) but not significantly so (p=0.146; Table 3). However, this may be because men and women vary in their biomarker profiles (Table 3). Therefore, we examined sex differences in biomarkers. Systolic blood pressure (p=0.038), DHEAs (p<0.001), and waist-hip-ratio (p<0.001) were significantly higher in men, while norepinephrine (p=0.006) and LDLc (p=0.011) were higher in women (Table 3). Given significant differences between men and women, we re-estimated allostatic load using sex-specific biomarker cut-points and examine this estimate in all additional analyses.

### Table 4. Means and standard deviations for age, hormonal and physiological biomarkers, allostatic load, and frailty comparing residents of Nekla and Poznan by t-tests for 87 men

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nekla (N=51)</th>
<th>Poznan (N=36)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>69.12</td>
<td>70.67</td>
<td>0.360</td>
</tr>
<tr>
<td>Cortisol</td>
<td>101.94</td>
<td>70.67</td>
<td>0.069</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>32.82</td>
<td>31.51</td>
<td>0.636</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>5.00</td>
<td>6.54</td>
<td>0.179</td>
</tr>
<tr>
<td>DHEAs</td>
<td>163.96</td>
<td>141.94</td>
<td>0.304</td>
</tr>
<tr>
<td>SBP</td>
<td>148.33</td>
<td>132.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP</td>
<td>83.55</td>
<td>74.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL/T-Chol</td>
<td>25.52</td>
<td>28.38</td>
<td>0.097</td>
</tr>
<tr>
<td>LDLc</td>
<td>126.28</td>
<td>104.83</td>
<td>0.006</td>
</tr>
<tr>
<td>WHR</td>
<td>0.94</td>
<td>0.95</td>
<td>0.498</td>
</tr>
<tr>
<td>Hba1c</td>
<td>6.30</td>
<td>6.33</td>
<td>0.865</td>
</tr>
<tr>
<td>Allostatic Load</td>
<td>2.94</td>
<td>2.17</td>
<td>0.039</td>
</tr>
<tr>
<td>Frailty</td>
<td>2.22</td>
<td>1.81</td>
<td>0.205</td>
</tr>
</tbody>
</table>

Following adjustments for sex differences in biomarkers, allostatic load among men from Nekla averaged 2.94 (sd=1.73), higher than observed among Poznan men, 2.17 (sd=1.67; p=0.039; Table 4). Among Nekla women average allostatic load was 2.57
(SD=1.39), also above that of Poznan women 2.38, but not significantly so (SD=1.74; p=0.510; Table 5).

**Table 5.** Means and standard deviations for age, hormonal and physiological biomarkers, allostatic load, and frailty comparing residents of Nekla and Poznan by t-tests for 124 women

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Nekla (N=53)</th>
<th>Poznan (N=71)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>68.23</td>
<td>70.51</td>
<td>0.60</td>
</tr>
<tr>
<td>Cortisol</td>
<td>87.56</td>
<td>72.23</td>
<td>0.107</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>35.45</td>
<td>39.61</td>
<td>0.160</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>3.77</td>
<td>8.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DHEAs</td>
<td>99.00</td>
<td>109.05</td>
<td>0.381</td>
</tr>
<tr>
<td>SBP</td>
<td>143.68</td>
<td>131.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP</td>
<td>81.91</td>
<td>74.20</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL/T-Chol</td>
<td>28.79</td>
<td>32.94</td>
<td>0.377</td>
</tr>
<tr>
<td>LDLc</td>
<td>129.40</td>
<td>132.94</td>
<td>0.630</td>
</tr>
<tr>
<td>WHR</td>
<td>0.84</td>
<td>0.86</td>
<td>0.095</td>
</tr>
<tr>
<td>HbA1c</td>
<td>6.28</td>
<td>6.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Allostatic Load</td>
<td>2.57</td>
<td>2.38</td>
<td>0.510</td>
</tr>
<tr>
<td>Frailty</td>
<td>3.00</td>
<td>2.44</td>
<td>0.086</td>
</tr>
</tbody>
</table>

We also compared biomarker averages between area of residence by sex using t-tests. Mean systolic and diastolic blood pressure, and LDLc were significantly higher in men residing in Nekla than those in Poznan (p<0.0001; p<0.0001; p=0.006 respectively; Table 4). Among women systolic and diastolic blood pressure were significantly higher among women residing in Nekla than those in Poznan (p<0.0001; p=0.001 respectively; Table 5), while epinephrine was higher amongst women from Poznan (p<0.0001; Table 5). In addition, among women, WHR (p=0.095) in Poznan, and HbA1c (p=0.08) and frailty (p=0.086) in Nekla were borderline significantly higher than their counterparts (Table 5).
Table 6. In multivariate analyses, only area of residence is associated significantly with allostatic load among 211 residents of Greater Poland aged 55-94 years, but neither sex nor age is associated significantly

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Sample (N=211)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.014</td>
<td>1.647</td>
<td>0.696</td>
</tr>
<tr>
<td>Area of Residence</td>
<td>-0.132</td>
<td>1.647</td>
<td>0.063</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.027</td>
<td>1.647</td>
<td>0.696</td>
</tr>
<tr>
<td>Men (N=87)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.037</td>
<td>1.709</td>
<td>0.729</td>
</tr>
<tr>
<td>Area of Residence</td>
<td>-0.221</td>
<td>1.709</td>
<td>0.045</td>
</tr>
<tr>
<td>Women (N=124)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.057</td>
<td>1.603</td>
<td>0.538</td>
</tr>
<tr>
<td>Area of Residence</td>
<td>-0.068</td>
<td>1.603</td>
<td>0.464</td>
</tr>
<tr>
<td>Nekla (N=104)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.024</td>
<td>1.572</td>
<td>0.538</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.12</td>
<td>1.572</td>
<td>0.464</td>
</tr>
<tr>
<td>Poznan (N=107)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.025</td>
<td>1.712</td>
<td>0.988</td>
</tr>
<tr>
<td>Sex</td>
<td>0.059</td>
<td>1.712</td>
<td>0.545</td>
</tr>
</tbody>
</table>

β: Standardized regression coefficient, SE: Standard Error of the Estimate

Age, Sex, Area of Residence, and Allostatic Load

In multivariate analyses, only area of residence (p=0.063) was associated significantly with allostatic load when including sex and age in the full model (Table 6). When subdivided by sex, Nekla men showed significantly higher allostatic load (p=0.045; Table 6).

Frailty

Within the total sample, frailty averaged 2.42 (range=0-7; SD=1.70; Table 2). Frailty was significantly higher among women 2.68 (range=0-7; SD=1.82; Table 3), than among men 2.05 (range=0-6; SD=1.46; p=0.006; Table 3). Amongst both men and women, frailty was higher in Nekla but not significantly so (p=0.086; p=0.205; respectively, Table 4).
**Allostatic Load and Frailty**

Associations of allostatic load, age, sex, and area of residence with frailty were examined. When stratified by sex, allostatic load was not associated significantly with frailty among men or women ($p=0.801$; $p=0.591$ respectively, Table 7). Nor did allostatic load associate significantly with frailty when examined within either Nekla or Poznan individually ($p=0.834$; $p=0.773$ respectively, Table 7). In the full sample allostatic load also did not associate significantly with frailty either ($p=0.755$).

**Table 7.** Multivariate associations of age, area of residence, and allostatic load with frailty while controlling for age and sex among 211 residents of Greater Poland aged 55-94 years

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men (N=87)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allostatic Load</td>
<td>-0.028</td>
<td>1.449</td>
<td>0.801</td>
</tr>
<tr>
<td>Age</td>
<td>0.179</td>
<td>1.449</td>
<td>0.099</td>
</tr>
<tr>
<td>Area of Residence</td>
<td>-0.162</td>
<td>1.449</td>
<td>0.144</td>
</tr>
</tbody>
</table>

| Women (N=124)      |       |      |         |
| Allostatic Load    | 0.047 | 1.734| 0.591   |
| Age                | 0.291 | 1.734| 0.001   |
| Area of Residence  | -0.201| 1.734| 0.023   |

| Nekla (N=104)      |       |      |         |
| Allostatic Load    | 0.02  | 1.586| 0.834   |
| Age                | 0.216 | 1.586| 0.025   |
| Sex                | 0.254 | 1.586| 0.009   |

| Poznan (N=107)     |       |      |         |
| Allostatic Load    | 0.027 | 1.674| 0.773   |
| Age                | 0.263 | 1.674| 0.006   |
| Sex                | 0.174 | 1.674| 0.067   |

| Full Sample (N=211)|       |      |         |
| Allostatic Load    | 0.021 | 1.622| 0.755   |
| Age                | 0.237 | 1.622| <0.0001 |
| Sex                | 0.215 | 1.622| 0.001   |
| Area of Residence  | -0.177| 1.622| 0.009   |

β: Standardized Regression Coefficient, SE: Standard Error of the Estimate
However, among men, although neither age nor area of residence was significantly associated with frailty, age was borderline significant (p=0.099; Table 7). Conversely, both age (p=0.001) and residence in Poznan were significantly associated with frailty amongst women (p=0.023; Table 7), positively and negatively respectively. Amongst residents of Nekla, age (p=0.025) and sex (p=0.009) were both positively associated with frailty (Table 7). However, amongst residents of Poznan, only age was associated positively with frailty (p=0.006), while sex showed a borderline significance (p=0.067; Table 7). In the full sample age and sex both were associated positively with frailty (p<0.0001; p=0.001, Table 7), while area of residence was negatively associated (p=0.009; Table 7).
Discussion

Individual genetic endowment, socioeconomic status, lifestyle, and the culture milieu in which an individual is embedded, are expected to contribute independently to variation in allostatic load and frailty (McEwen and Seeman 1999; Crews 2005). Theoretically, chronic exposures to life’s stressors lead to physiological dysregulation, an effect that is compounded over the lifespan, contributing to allostatic load (Seeman et al. 1997; McEwen 1998a, 1998b, 2007, 2015). Capturing this somatic burden is complicated because allostasis is a dynamic physiological response which cannot be measured directly (Seeman et al. 1997; McEwen 1998a, 1998b, 2012; Sterling 2004; Leahy and Crews 2012). This challenge is mitigated by measuring composites of biomarkers identified by evidence-based research to reflect allostasis and stressor-related somatic dysregulation across individuals (Seeman et al. 1997; McEwen 1998a, 1998b, 2007). Such estimates of allostatic load provide an integrated multisystem assessment of chronic activation of stress responses and associated adverse physiological changes/outcomes.

Frailty is a well-defined clinical phenotype, assessed as a screening tool in medical settings. Assessments of frailty based upon losses of somatic capabilities following muscle loss (sarcopenia), specifically by age-associated somatic declines in strength, endurance, and physical activity that contribute to increased vulnerability, disability, and death of individuals (Fried et al. 2001; Fried et al. 2004; Crews 2005; Walston 2005). Frailty indices provide useful measures of this clinical phenotype as it is associated with adverse somatic outcomes including decreased resiliency to stressors and increased morbidity and mortality (Fried et al. 2001; Fried et al. 2004; Crews 2005;
An open question is how allostatic load may influence frailty in older people across cultural settings.

We examined influences of sex, age, and life-time area of residence on allostatic load and frailty among 211 residents of Nekla and Poznan, Poland to test three hypotheses. Our first hypothesis was that men would show higher allostatic load than women. They did, but not always significantly so. We also hypothesized a lower allostatic load would characterize residents of Nekla compared to those in Poznan. However, allostatic load was higher among residents of Nekla than Poznan, specifically, among men, but not different among women (Table 4). Finally, we hypothesized allostatic load would be associated significantly with age and frailty, but it was not in this older sample.

Allostatic load was higher among men than women in both Nekla and Poznan. Such differences may reflect variation in male and female biology or they may reflect differences in how men and women interact with their environment and cultural settings (Juster et al 2016; Stephens et al 2016). Also, aspects of lifestyle, for example occupational activities, socioeconomic status, or educational differences may influence stress-related physiology differentially among men and women (Walston 2006). Men from Nekla also show higher allostatic load than do Poznan men, perhaps related to differences in daily activities or less access to healthcare in more rural settings (see for example Adeyi et al. 1997; Bakken et al. 1999; Lipowicz et al. 2016).

Nekla and Poznan provide a dichotomy of Poland’s current socio-economic variation. Differences in economic pursuits, diets, and access to healthcare may expose
Nekla men to more stressors than men in Poznan or women in Nekla. Recent structural
transitions affecting financial security and socio-cultural stability in Poland (Lipowicz et
al. 2016), across the nation also may have affected men more than women. In addition,
changing social norms may have altered perceptions of social security and support,
reducing socioeconomic stability and diminishing economic opportunities for men in
more rural areas like Nekla (Wróblewska 2002; Bolanowski et al 2010).

Previous research on Polish men (1988-1993) observed higher allostatic load
among those experiencing more economic stress and reduced access to jobs and
healthcare (Lipowicz 2007; Lipowicz et al. 2016). Significantly higher allostatic load
among men than women may reflect similar differential exposures. As Lipowicz et al.
(2015) observed, higher allostatic load among rural living men than those in urban
settings may reflect a general tendency among inhabitants of rural Poland toward greater
stressor exposures. In this older sample, age was not a significant predictor of allostatic
load. Similar results have been reported for other older samples including in Japan and
Samoa (Crews et al. 2014; Kusano et al. 2016). In the US, older samples also show such
an association (Crimmins et al. 2003; Gruenewald et al. 2012). One suggestion is that
social support and lifestyle may buffer physiological dysregulation among this Polish
sample as is observed among older Kuwaitis (Al-Kandari and Crews 2011).

We also hypothesized allostatic load and frailty would be associated significantly
in both Nekla and Poznan. No significant associations between allostatic load and frailty
were observed. However, sex, age, and area of residence all were associated positively
with frailty. In the full sample frailty was associated positively with age and was higher
among women from both Nekla and Poznan than in men. Although allostatic load was higher in men, frailty was not, and was in fact significantly higher in women (Table 3). This observation is similar to the United States and in the 11 European countries studied where frailty was associated significantly with age among women, but not men (Fried et al. 2001; Etman 2012; Szanton et al. 2009).
Conclusion

Many factors, e.g.: individual genetic endowment, socioeconomic status, and environmental conditions, may influence individual variation in allostasis and allostatic load. Compounded over time, allostasis results in somatic ‘wear and tear’, an allostatic load. Because not all allostatic responses and somatic alterations can be measured directly, allostatic load is a latent physiological state. However, using arrays of neurophysiological biomarkers we can estimate this somatic burden. Here we examined influences of sex, age, and area of residence on a 10-biomarker index of allostatic load. Overall, men and women from Poland do not differ significantly in their average allostatic load. Nor is allostatic load higher among residents of Nekla, a rural setting, than Poznan, an urban setting. However, men from Nekla do show higher allostatic load than those from Poznan, while women do not. Allostatic load is not associated significantly with either age or frailty. However, in this older sample, lifestyle as assessed by area of residence influences stress-related allostatic load among men and frailty among women. Age, sex, and area of residence all significantly modulate frailty and women from Nekla show greater frailty than men or women from Poznan.

Estimates of allostatic load allow biological anthropologists to explore demographic, environmental, and cultural influences on health disparities and adverse health outcomes across populations. Such research aids development of public health programs tailored to both individual and community needs. That area of residence influences allostatic load in men, but not women, while it influences frailty in women but not men, emphasizes the need for a life history perspective when examining whether and
how social environments may mediate individual health, stressor responses, and frailty. Future research on health in rural settings likely will need to incorporate social and economic factors when assessing cumulative effects of environmental systems on human somatic dysregulation.


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http://dx.doi.org/10.1098/rsbl.2015.0396


