⌛GSA65
GETTING BETTER WITH AGE

Frontiers in the Use of Biomarkers of Health in Research on Stress and Aging

Jennifer R. Piazza, David M. Almeida, Natalia O. Dmitrieva, and Laura C. Klein

Department of Human Development and Family Studies, Pennsylvania State University, University Park.

Assessment of biomarkers that reflect objective indicators of physiological processes has become increasingly popular in psychological research on stress and aging. The current article reviews biomarkers of the neuroendocrine and immune systems, including issues related to measurement and normative age-related changes. We also discuss how exposure to stressors can provoke changes in these biomarkers and propose that stressful experiences may accelerate age-related declines in these systems. We recommend that future research examining physical health and aging incorporate dynamic and multivariate methods for assessing links between stressors and biomarkers.

Key Words: Aging—Allostatic load—Daily diary—Hypothalamic–pituitary–adrenal axis—Immune system—Stress—Sympathetic–adrenal–medullary axis.

"The only thing we have in common is our obedience to certain fundamental biologic laws which govern all men [*sic*]."
Hans Selye, *The Stress of Life*, 1976, p. 454.

HANS Selye was correct in his observation that all people face similar biological changes across the life span, and nowhere is this more evident than in later adulthood. Hair grays, walking slows, faces wrinkle, and rates of illnesses increase. Yet, there is variability in these seemingly inevitable changes. Understanding why this variability exists and attempting to identify the factors that determine why some people age well and others do not has demanded innovation from aging researchers interested in psychophysiology. For years, self-reported measures of physical health dominated biopsychosocial data collection, and such measures continue to garner important information regarding the association between psychological and physical health across adulthood. Certain questions, however, cannot be answered with self-reported measures alone and, instead, require the collection of physiological data. To this end, biomarkers that reflect underlying physiological processes are being utilized in several research laboratories that focus on stress, health, and aging.

A biomarker is defined as a biological indicator—such as blood or saliva—that reflects underlying physiological processes, including both normative processes and pathogenic states (Baum & Grunberg, 1997). For example, elevated blood pressure is a biomarker of cardiovascular disease, elevated proinflammatory cytokines are biomarkers of inflammation, and elevated body temperature is a biomarker of infection. Biomarker assessments have become increasingly popular among behavioral and social aging researchers as a way to connect behavioral, environmental, and social factors to an individual's health and well-being (Gruenewald & Kemeny, 2007). In fact, the National Institutes of Health encourages research that assesses multiple behavioral and psychosocial pathways to health and well-being—

a task that requires an integration of biological and social science (Singer & Ryff, 2001). Biomarkers play a pivotal role in this journey, and the goal of this article is to provide researchers with an understanding of how biomarkers inform research on stress, health, and aging.

This article is divided into three broad sections. In the first section, we discuss how stressors of different domains may affect physical health. In the second section, we review three systems closely implicated in stress functioning (i.e., the sympathetic–adrenal–medullary [SAM] axis, the hypothalamic–pituitary–adrenal [HPA] axis, and the immune system) and discuss normative age-related changes that occur in each system. Page restrictions prohibit an exhaustive review of the literature, so we provide tables that describe the function and age-related changes of specific biomarkers and refer the reader to resources mentioned in text for more detailed information. In this section, we also address the impact psychological stress has on these systems and how it may accelerate age-related physical health declines. In the last section, we discuss the future of biomarkers and propose that researchers studying stress, health, and aging take a multivariate and dynamic approach.

STRESSOR DOMAINS: LIFE EVENTS, CHRONIC STRESSORS, AND DAILY HASSLES

There are two major approaches to examining the interrelationships between stress, health, and aging. The first approach focuses on major life events that are relatively rare in their occurrence and that require significant life adjustments, such as divorce or the death of a spouse (e.g., Dohrenwend, 2006; Holmes & Rahe, 1967; Wheaton, 1997). The second approach focuses on everyday events, or quotidian stressors (Almeida, 2005; Bolger, Davis, & Rafaeli, 2003; Pearlin, 1999), which are divided into two types: chronic stressors and daily hassles. Whereas chronic stressors

513

refer to ongoing persistent situations, such as caregiving for an ill spouse, daily stressors refer to minor events that arise from day-to-day living, such as meeting work deadlines (see review by Almeida, Piazza, Stawski, & Klein, in press). The mechanisms through which different types of stressors exert an effect on health vary, with life events associated with prolonged arousal and daily stressors associated with spikes in arousal (Almeida et al.). Not surprisingly, however, these constructs overlap. Take, for instance, the example of spousal caregiving. In this situation, a life event—that is, one's spouse being diagnosed with an illness—leads to the chronic stress of caregiving, which, in turn, results in increased exposure to daily stressors related to caregiving responsibilities. Indeed, many researchers suggest that it is the interaction and accumulation of stressors that lead to poor health outcomes over time (e.g., Almeida & Wong, 2009; Chiriboga, 1997; Pearlin, Menaghan, Lieberman, & Mullan, 1981).

In and of itself, the stress response does not lead to adverse health outcomes; rather, it protects an organism from harm by increasing alertness, mobilizing energy, and protecting against pathogens. Each time the stress response is activated, however, physiological adjustments must be made, and over time, these adjustments may lead to cumulative wear and tear (McEwen, 1998). Exposure to stressors may, therefore, accelerate the aging process, particularly when this exposure occurs in later adulthood, as physiological systems are already compromised. In the following section, we discuss this view in greater detail and also provide an overview of the biomarkers implicated in the stress response.

The SAM Axis, the HPA Axis, and the Immune System: A Brief Review

SAM Axis

Cannon (1914) was the first to describe hormones as biomarkers of stress when he detailed the physiological "fight-or-flight" response (Cannon, 1932; Cannon, Britton, Lewis, & Groeneveld, 1927). The fight-or-flight response begins with the activation of the sympathetic (SNS) branch of the autonomic nervous system (see Figure 1), which stimulates the release of the catecholamines, epinephrine (EPI) and norepinephrine (NE), from sympathetic neurons and the adrenal medulla (for review, see Klein & Corwin, 2007); this SNS-stimulated release of EPI and NE from the adrenal medulla is also known as the SAM axis. Effects of EPI and NE include decreased blood flow to the organs of the gastrointestinal tract, the skin, and the kidneys, which ensures maximum blood flow to the brain, heart, and skeletal muscles when a stressor is encountered.

Table 1 provides a description of several biomarkers of the SAM axis. Circulating or excreted levels of EPI and NE are the primary biomarkers of SAM axis activity (Baum & Grunberg, 1997) and can be measured reliably in urine, plasma, and cerebrospinal fluid samples. Circulating catecholamines are metabolized quickly (within 1–3 min) and indicate immediate changes in SAM axis activation. The invasive nature of blood sampling, as well as the short half-life and high frequency of pulse-like fluctuations in catecholamines, make it challenging to track circulating catecholamines outside of the laboratory. Analysis of urine provides a relatively noninvasive measure of catecholamine levels, and home collection of urine by participants has been used successfully in many field studies of chronic stress (e.g., Baum, Gatchel, & Schaeffer, 1983; Cohen, Doyle, & Baum, 2006; Lundberg & Frankenhaeuser, 1999). Because catecholamines are secreted slowly (over hours) while the bladder fills, however, urinary catecholamine assessments provide a relatively static index of SAM axis activity, which makes it difficult to examine the impact of stressor onset and cessation (Baum & Grunberg; Mason, 1968).

A rapidly growing literature suggests that the salivary enzyme α-amylase (sAA) can serve as a minimally invasive surrogate marker of SNS activity (for reviews, see Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007; Nater & Rohleder, 2009). The rationale is that catecholamine release in response to SNS activation stimulates salivary gland receptors that, in turn, alter activity of these glands (Nederfors & Dahlof, 1992). Although the main function of sAA is the enzymatic digestion of carbohydrates (Rohleder & Nater, 2009), sAA appears to be a viable surrogate marker of SAM axis activation, as it parallels stress-related increases in NE 5–10 min after NE release (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). With regard to diurnal patterns, sAA exhibits moderately low levels upon awakening, drops briefly at 30-min post-awakening and increases gradually throughout the day (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007).

Age Differences and Changes in SAM Axis Functioning

A number of normative age-related changes occur in the cardiovascular and sympathetic nervous systems, partly due to changes in SAM axis functioning (for reviews, see Crimmins, Vasunilashorn, Kim, & Alley, 2008; Uchino, Birmingham, & Berg, 2010). Systolic and diastolic blood pressure gradually increases across the adult life span until approximately age 70 years, at which time the increase appears to cease. Resting heart rate remains relatively stable across adulthood, whereas maximum heart rate gradually decreases (Uchino et al.). In response to acute psychosocial stressors, older age is associated with increased systolic blood pressure and modest increases in diastolic blood pressure (Uchino, Holt-Lunstad, Bloor, & Campo, 2005; Uchino et al.). In contrast, heart rate response to physical exercise and psychosocial stressors is attenuated in older age (for review, see Ferrari, Radaelli, & Centola, 2003).
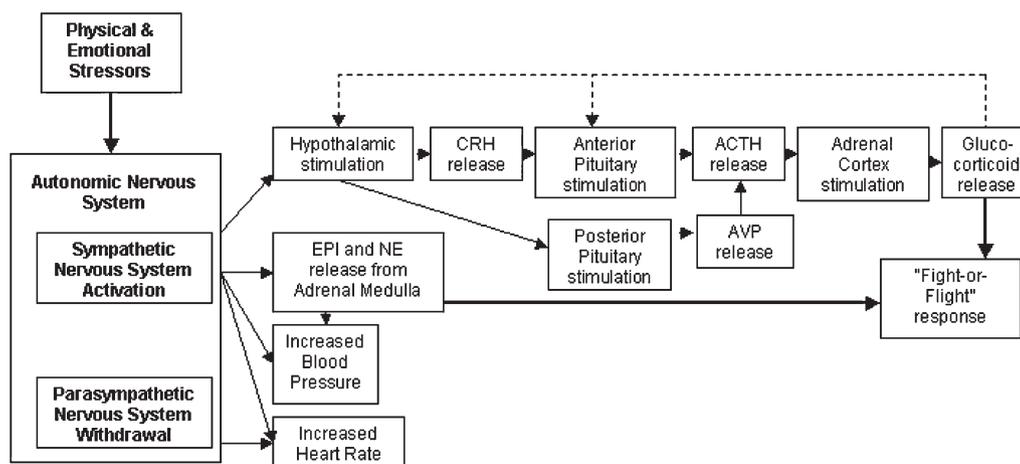
Figure 1. A simplified representation of the components of the autonomic nervous system and the hypothalamic–pituitary–adrenal axis stress system. CRH = corticotrophin-releasing hormone, ACTH = adrenocorticotropin hormone, EPI = epinephrine, NE = norepinephrine, AVP = arginine vasopressin. Solid lines represent direct or indirect stimulatory pathways. Dashed lines represent direct or indirect inhibitory pathways. (Adapted from Klein and Corwin, 2002.)

Fewer studies have examined age-related changes in baseline catecholamine levels and in catecholamine reactivity to stressors. Of those that have, age appears to be associated with higher average NE levels (e.g., Barnes, Raskind, Gumbrecht, & Halter, 1982; Blandini et al., 1992), an increase that may occur due to age-related increases in basal sympathetic NE activity and reduced metabolic clearance (i.e., neuronal reuptake) of NE among older adults (Seals & Esler, 2000). In contrast, basal EPI activity is reduced in older age (e.g., Esler et al., 1995), but lower

Table 1. Description and Age-Related Changes of Biomarkers of the SAM Axis

Biomarkers of SAM axis	Brief definition	Function	Age-related changes	Association with disease
SBP	Peak arterial pressure during heartbeat	Indicator of cardiovascular health; exerts maximum force on blood vessel walls during systole to insure delivery of oxygen to vital organs and skeletal muscle	Increase, followed by a possible plateau	High levels indicate hypertension and CHD
DBP	Lowest arterial pressure between heartbeats	Indicator of cardiovascular health; exerts minimum force on blood vessel walls during diastole to insure delivery of oxygen to vital organs and skeletal muscle	Increase, followed by a possible plateau	High levels indicate hypertension among younger adults; lower levels among older adults can lead to hypotension, which can result in falls
HR	Number of heartbeats per unit time (typically beats per minute or BPM)	Indicator of cardiovascular health; regulates blood flow and oxygen delivery to skeletal muscle	Decrease in maximum HR; stability of resting HR	High HR can indicate hypertension; reduced HR among older adults can lead to hypotension, which can result in falls
EPI	Catecholamine released by adrenal medulla following sympathetic nervous system activation	Produces sympathetic arousal in response to stress, including increased HR, glucose release from energy stores, and increased blood flow to skeletal muscle	Possible stability or decrease	High levels may be associated with poorer outcomes of CHD; elevations associated with anxiety
NE	Catecholamine released by adrenal medulla following sympathetic nervous system activation	Produces sympathetic arousal in response to stress, including increased HR, glucose release from energy stores, and increased blood flow to skeletal muscle	Possible increase	Higher levels associated with poorer outcomes of CHD; elevations associated with anxiety; dysregulation associated with depression
sAA	Enzyme produced by salivary gland	Digestion of carbohydrates and starch	Attenuation of diurnal increase; basal sAA activity does not appear to change	Deficient levels related to poor oral health

Notes: CHD = coronary heart disease; DBP = diastolic blood pressure; EPI = epinephrine; HR = heart rate; NE = norepinephrine; sAA = salivary alpha-amylase; SAM = sympathetic–adrenal–medullary; SBP = systolic blood pressure.

levels are not always evident because of reduced clearance of EPI in later adulthood (Seals & Esler). Older age is generally associated with higher NE reactivity to stressors (Barnes et al.; Palmer, Ziegler, & Lake, 1978) and possibly lower EPI reactivity to stressors (e.g., Esler et al.). Not all studies illustrate these patterns, however (e.g., Lindheim et al., 1992), and an examination of individual differences may help to elucidate these results. In addition, the diurnal increase and deceleration of the diurnal increase of sAA are attenuated from young adulthood to midlife (Nater et al., 2007), though recent studies suggest no age differences in basal sAA levels (for review, see Rohleder & Nater, 2009).

Psychological Stress and the SAM Axis

As previously described, the SAM axis is the body's primary physiological response to acute stressors (for review, see Klein & Corwin, 2007). Following the experience of a stressor, NE slows digestion and gastrointestinal motility, increases plasma glucose levels, and dilates pupils, whereas EPI increases heart rate and cardiac contractility, relaxes smooth muscle, and increases blood pressure and glucose release. The rise in catecholamine levels depends on stressor severity, doubling in their resting levels during daily activities, and rising three to five times their resting levels during moderately severe stressors (for review, see Frankenhaeuser, 1971). Other important determinants of SAM axis reactivity are various stressor characteristics, such as stressor controllability, pleasantness, uncertainty, and elicitation of fear or anger (e.g., Frankenhaeuser & Rissler, 1970; Patkai, 1971). As stressors increase in chronicity, the frequency and length of sympathetic activation also increases, possibly resulting in tissue damage. Several studies indicate that the effect of chronic stressors on the SAM axis resembles age-related changes, as evidenced by higher diastolic blood pressure (Jeckel et al., 2010), higher blood pressure reactivity (Chida & Hamer, 2008), higher sAA reactivity (Bosch et al., 1998), and blunted diurnal profiles of NE (Fujiwara et al., 2004; Hawk, Dougall, Ursano, & Baum, 2000), EPI (Hawk et al.), and sAA (Nater et al., 2007; Rohleder, Marin, Ma, & Miller, 2009) among people experiencing elevated levels of chronic or perceived stress.

HPA Axis

Whereas SAM axis activation is designed for an immediate response to threat, HPA axis activation is a longer-term hormonal response that is observed approximately 15–20 min following stressor onset (see review by Dickerson & Kemeny, 2004). Once a stressor is perceived by the cerebral cortex, it alerts the neurons of the paraventricular nucleus of the hypothalamus to release corticotrophin-releasing hormone (CRH) into the hypothalamic–pituitary portal blood flow system. CRH travels to the pituitary gland, where it stimulates the release of the adrenocorticotropin hormone (ACTH), as well as arginine vasopressin (AVP). Although

AVP acts centrally to support the fight-or-flight response, ACTH circulates to the cortex of the adrenal glands to stimulate glucocorticoid release, including corticosteroids (e.g., cortisol) (for reviews, see Klein & Corwin, 2007; Taylor et al., 2000). Corticosteroids themselves regulate continued HPA axis function through a negative feedback loop by dampening further CRH release from the hypothalamus and ACTH release from the anterior pituitary gland. Ultimately, cortisol mobilizes energy stores, serves as an anti-inflammatory hormone, and communicates with the immune system (Chrousos & Gold, 1992; for review, see Dickerson & Kemeny).

The primary biomarkers of the HPA axis include CRH, ACTH, cortisol, dehydroepiandrosterone-sulfate (DHEA-S) and its unsulfated form DHEA, and AVP (see Table 2). CRH is measured in cerebrospinal fluid, which means that this biomarker must be measured in controlled clinical settings. In contrast, ACTH, cortisol, and AVP can be assessed in blood, and unbound levels of cortisol can be measured in saliva. The ease of the salivary collection, in conjunction with cortisol's predictable diurnal pattern—peaking 30–45 min after awakening and gradually decreasing throughout the day (e.g., Van Cauter, Leproult, & Kupfer, 1996)—have made it possible for researchers to include this hormone as a biomarker across a number of laboratory (for reviews, see Dickerson and Kemeny, 2004; Hellhammer, Wüst, & Kudielka, 2009) and field studies (e.g., Almeida, Piazza & Stawski, 2009; Seltzer et al., 2009; Smyth et al., 1997).

Whereas cortisol is a catabolic hormone, DHEA-S is an anabolic hormone that is thought to be coreleased with cortisol (Granger & Kivlighan, 2003). DHEA-S and its unsulfated form, DHEA, are bioactive, work similarly in the body (Montanini et al., 1988), and can be assayed in cerebrospinal fluid, blood, urine, or saliva. Secreted primarily from the adrenal cortex in women and men, as well as from the testes in men, DHEA-S/DHEA is similar to cortisol in that it is released in response to ACTH stimulation as part of the HPA axis, making it a potential moderator of the HPA axis response to stress (Roberts, 1999). Similar to cortisol, DHEA-S levels are highest in the morning and decline throughout the day (Hucklebridge, Hussain, Evans, & Clow, 2005; Klein et al., 2008).

Age Differences and Changes in HPA Axis Functioning

Table 2 lists some of the age-associated changes that occur in the HPA axis. Increasing age is associated with higher mean cortisol levels, a disruption of the negative feedback loop, and a relatively flat diurnal pattern (for reviews, see Chahal & Drake, 2007; Epel, Burke, & Wolkowitz, 2007). With age, cortisol appears to exhibit an attenuated awakening response (e.g., Kudielka & Kirschbaum, 2003; Van Cauter et al., 1996; but see Almeida et al., 2009, Wust et al., 2000) and a less steep decline in the evening hours, resulting in a higher nadir (Deuschle et al., 1997; Van Cauter et al.).

Table 2. Description and Age-Related Changes of Biomarkers of the HPA Axis

Biomarkers of HPA axis	Brief definition	Function	Age-related changes	Association with disease
ACTH	Hormone released by anterior pituitary gland	Stimulates production of corticosteroids from adrenal cortex	Higher ACTH levels at nadir; reduced negative feedback	Elevations associated with disease states, including Addison's and Cushing's disease
CRH	Neuropeptide released by paraventricular nucleus of the hypothalamus	Initiates HPA axis response to stress; stimulates ACTH release from anterior pituitary	Increased activity in CRH but reduced CRH receptor binding in the pituitary	Elevations associated with depression and poor cognitive functioning
Cortisol	Corticosteroid released by zona fasciculata of adrenal cortex	Hormonal response to stress; mobilizes energy production, promotes glucogenesis, and conserves carbohydrates; reduces inflammation; feeds back to hypothalamus to reduce CRH release and dampen HPA axis activity	Higher cortisol levels at nadir; reduced negative feedback to hypothalamus	Persistently high levels associated with CHD, depression, anxiety, and poor cognitive functioning
AVP	Hormone released by the posterior pituitary gland	Acts centrally to support "fight-or-flight" response by stimulating ACTH release; also known as antidiuretic hormone; controls salt and water handling by kidney, thirst, and arterial blood pressure	Increased AVP release; reduced renal collecting duct sensitivity to AVP; loss of kidney AVP receptors; reduced AVP neuromodulation	Reduced urine concentrating abilities; poor blood pressure regulation; decreased cognitive functioning
DHEA-S/DHEA	Steroid produced by zona reticularis of adrenal cortex	Precursor of androgens; buffers the damaging effects of cortisol on the hippocampus and body	Declines	Lower levels related to CHD and poorer mental health

Notes: ACTH = adrenocorticotropin hormone; AVP = arginine vasopressin; CHD = coronary heart disease; CRH = corticotropin-releasing hormone; DHEA-S = dehydroepiandrosterone-sulfate; DHEA = dehydroepiandrosterone; HPA = hypothalamic–pituitary–adrenal.

Several studies also suggest that age is associated with greater HPA axis reactivity to stressors (e.g., Otte et al., 2005; Peskind et al., 1995), although this is not always the case (e.g., Kudielka, Schmidt-Reinwald, Hellhammer, Schurmeyer, & Kirschbaum, 2000; Nicolson, Storms, Ponds, & Sulon, 1996). More research is needed to ascertain age differences in DHEA-S/DHEA, but some studies suggest that levels steadily decline from early (i.e., 20–30 years old) to later adulthood (i.e., 70–80 years old) (Labrie, Belanger, Cusan, Gomez, & Candas, 1997).

Psychological Stress and the HPA Axis

HPA axis activation is adaptive in the short term, but repeated or prolonged activation can have detrimental effects on health, and lead to a dysregulation of the negative feedback loop. Specifically, prolonged exposure to stressors appears to uncouple cortisol from its ability to inhibit further CRH and ACTH secretion, which leads to an overproduction of cortisol (Dickerson & Kemeny, 2004). Elevations in cortisol, in turn, are associated with chronic health problems, psychological disorders, and problems with memory, learning, and attention (Sapolsky, 1996, 2000).

The changes that occur in the HPA axis as a result of exposure to chronic stressors appear to be similar to the changes that occur during the aging process. For example, the experience of trauma, persistent or chronic stressors, or stressors of high magnitude are associated with greater cortisol and ACTH reactivity (Rasmusson et al., 2004), a blunted diurnal rhythm of salivary cortisol (Adam & Gunnar, 2001; Bergman, Ahmad, & Stewart, 2008; Miller, Chen, & Zhou, 2007; Ranjit, Young, & Kaplan, 2005; Seltzer et al., 2009), lower evening DHEA levels (van Niekerk, Huppert, & Herbert, 2001), and lower overall DHEA-S levels (Jeckel et al., 2010), although there are exceptions to these findings (e.g., Miller et al., 2009; Rohleder et al., 2009; Young & Breslau, 2004).

The Immune System

Just as the SAM and HPA axes are essential to the stress response, so too is the immune system. The immune system protects the body from a variety of internal (e.g., defective cells) and external threats (e.g., bacteria) by destroying pathogens. It comprises two interrelated branches referred to as innate and adaptive immunity. The innate branch of the immune system provides the first line of defense against infection and includes cells and proteins that are nonspecific to particular antigens and include—but are not limited to—natural killer (NK) cells, phagocytes, and complement. Because of their nonspecificity, these cells and proteins are the first to mount a defense against pathogens. They also trigger a response by the adaptive branch of the immune system via cytokine communication. The adaptive branch creates antigen-specific immunity; therefore, it responds to

Table 3. Description and Age-Related Changes of Biomarkers of the Immune System

Immune system biomarkers	Brief definition	Function	Age-related changes	Association with disease
Epithelial barriers	Nonimmunological defense (e.g., skin, mucous, coughing)	Prevent pathogens from entering the body	Age-related functional decrease	Compromised barriers associated with an increased risk of pathogenic organism invasion
Complement	A series of plasma proteins that mediate the inflammatory response	Destroys pathogens by attacking their plasma membranes; promotes ingestion of foreign materials by phagocytes	Decreases with age	Lower levels may result in less efficient removal of non-self antigens, which may increase morbidity

Phagocytes
  Neutrophils (n)
  Granulocytes (g)
  Macrophages (m)
  Dendritic cells (dc)
	Immune cells that engulf pathogens	Ingest foreign particles (e.g., bacteria); typically first responders that initiate/promote continuation of the inflammatory response	Age-related impairment in the ability to destroy pathogens	Impairments may result in an increased susceptibility to illness and infection

C-reactive protein	Protein that increases during systemic inflammation; marker of inflammation	Enhances phagocytosis; assists in the binding of complement to foreign and/or damaged cells	Age-related increases	Higher levels associated with acute illness and increased risk of cardiovascular and other diseases
NK cells	Cytotoxic lymphocytes that destroy compromised host cells	Destroy virally infected cells; inhibit viral reproduction; destroy cancer cells	Many studies show age-related increase in number of NK cells, but a decrease in functionality	Decreased functionality associated with the development and/or progression of infection and disease
T lymphocytes	White blood cells derived from bone marrow that mature in thymus; responsible for cell-mediated immunity	Recognize nonself-antigens— Helper T cells: release cytokines that direct behavior of other immune cells; Regulatory T cells: mediate immune response suppression; Cytotoxic T cells: lysis virally infected cells; inhibit viral reproduction; some become memory cells to fight recurrent infection	More antigen-specific memory T cells with age, but fewer naive T cells; decreased functional capacity; atrophy of the thymus	Decreased number of naive cells and decreased functional capacity results in a reduced ability to mediate an effective response against novel antigens
B lymphocytes	White blood cells derived from bone marrow; responsible for humoral immunity	Recognize nonself-antigens; produce antibodies that neutralize antigens; label infected cells for destruction by phagocytes	Impaired functioning; fewer naive as compared with antigen-specific B cells; decreased antibody production	Impaired functioning results in a diminished response to vaccines and an inefficient response to antigens
Proinflammatory cytokines (e.g., IL-6, TNF-α)	Chemical messengers that influence systemic inflammation	Released by immune cells to promote inflammatory response; affect the differentiation of Helper T cells during an immune response	Most studies find age-related increases	Elevated levels associated with several age-related diseases (e.g., osteoporosis, atherosclerosis)

Note: IL-6 = interleukin 6; NK = natural killer; TNF-α = tumor necrosis factor.

threats more slowly. During initial antigen exposure, this branch responds by developing lymphocytes with a high degree of specificity and "memory" for the challenge, so that if the pathogen returns, a subset of lymphocytes can respond more rapidly. The adaptive branch consists of two major groups of cells: lymphocytes and antigen-presenting cells. The two major populations of lymphocytes are T lymphocytes, which are responsible for the antigen-specific killing of infected cells, and B lymphocytes, which are responsible for the mass production of antigen-specific antibodies. Because the innate and adaptive branches work in tandem, decrements in one branch can compromise the efficacy of the other (for reviews, see Bauer, Jeckel, & Luz, 2009; Graham, Christian, & Kiecolt-Glaser, 2006; Segerstrom &

Miller, 2004). Although many biomarkers of the immune system have been identified, Table 3 lists and describes those that have received the most attention in the stress and aging literature.

Age Differences and Changes in the Immune System

The immune system is highly efficient for the first 40 years of life, at which time certain aspects begin to show functional declines (i.e., immunosenescence; Aw, Silva, & Palmer, 2006). Had life expectancy remained unchanged from the turn of the 20th century—when people could expect to live an average of 50 years—understanding why and how the immune system changes with age would be

unnecessary. Yet, over the last century, life expectancy has nearly doubled (Centers for Disease Control and Prevention, National Center for Health Statistics, 2007), and the resulting health implications of a less than optimal immune system are apparent. Consequences of immunosenescence include higher rates of most illnesses, including cancer (Huang, Patel, & Manton, 2005), osteoporosis and arthritis (Aw et al.), increased susceptibility to infectious disease (Weiskopf, Weinberger, & Grubeck-Loebenstein, 2009), and less efficacious responses to vaccinations (Kovaiou, Herndler-Brandstetter, & Grubeck-Loebenstein, 2007). Despite the known health consequences of immunosenescence, the study of immune biomarkers in aging research has developed relatively recently (see review by Graham et al., 2006) and suggests that there are certain biomarkers implicated in immunosenescence.

In terms of the innate branch, research indicates that with age, cells involved in phagocytosis (e.g., macrophages) become less effective, levels of complement decrease, and although there are increases in the number of NK cells, there is also a decrease in their functionality (Kovaiou et al., 2007). The adaptive branch also undergoes age-related changes. The thymus gland shrinks with age, and as a result, fewer naive T cells are produced. Although the percentage of T cells does not decrease with age, the proportion of memory T cells (i.e., those cells that are only activated by a previously encountered antigen) and naive T cells (i.e., those cells that respond to novel pathogens) is altered, such that the number of memory T cells is greater than the number of naive T cells. A similar pattern occurs among the B lymphocytes, which also become less able to produce antibodies (for reviews, see Hawkley & Cacioppo, 2004; Kiecolt-Glaser & Glaser, 2001; Kovaiou et al.; Pfister & Savino, 2008). With fewer lymphocytes able to respond to newly introduced pathogens, an individual becomes less able to fend off new immunologic threats, resulting in increased morbidity and mortality (Weiskopf et al., 2009). In contrast to the declines that occur in some components of the immune system, those aspects that promote inflammation, such as interleukin-6 (IL-6), tumor necrosis factor-α, and C-reactive protein (CRP), typically increase with age (Graham et al., 2006), resulting in concomitant age-related diseases associated with inflammation, such as osteoporosis, osteoarthritis, and atherosclerosis (Huang et al., 2005).

Psychological Stress and the Immune System

Psychological stressors activate a number of changes in the immune system, and these alterations vary according to stressor duration, with short-term stressors provoking transient changes in the immune system, and progressively longer lasting stressors triggering greater changes (for review, see Segerstrom & Miller, 2004). In the face of an acute challenge, the immune system readies itself for possible exposure to pathogens, resulting in an upregulation of some aspects of innate immunity and a decrease in lymphocyte proliferation. These transient changes downregulate approximately 1 hr after the threat ceases and are evolutionarily adaptive, as they protect an organism from impending threat (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002). As the duration of stressors increases, however, such as during examination periods or when undergoing residential relocation, more robust changes in immune biomarkers occur, including lowered B-lymphocyte concentrations (McGregor, Antoni, Ceballos, & Blomberg, 2008), weaker vaccine responses (Glaser et al., 1992), slower wound healing (e.g., Marucha, Kiecolt-Glaser, & Favagehi, 1998), and decreased functioning of NK cell activity (Lutgendorf, Vitaliano, Tripp-Reimer, Harvey, & Lubaroff, 1999). As stressors become more chronic, the adaptiveness of the stress response begins to wane. Changes similar to those seen in aging start to occur, with decreases in adaptive immunity (for review, see Graham et al., 2006; Segerstrom & Miller), increases in inflammatory markers, such as proinflammatory cytokines and CRP (Koster et al., 2006), and decrements in several aspects of innate immunity (Gomez, Boehmer, & Kovacs, 2005).

Does Chronic Stress Accelerate Aging?

If chronic stress activates biomarker changes that are akin to those seen in aging, does it also have the potential to exacerbate normative age-related changes? Surprisingly, relatively few studies have examined this question, but of those that have, several suggest that stress does, indeed, exacerbate the aging process. For example, in their seminal study, Epel, Burke, and Woskowitz (2004) found that women reporting higher levels of perceived stress showed accelerated cellular aging compared to their lower-stressed peers. One intriguing question that was not explicitly examined in this study, however, is how age may influence this association. Studies that have examined the impact of age on the relationship between stress and health indicate that stress may actually be more harmful for older as compared to younger adults. For example, high job demands appear to be more detrimental to the health of older employees than to the health of younger employees (Schnorpfeil et al., 2003). Older adults may also take longer to physiologically recover from high-stress work weeks than their younger counterparts (Ritvanen, Louhevaara, Helin, Vaisanen, & Hanninen, 2006), a finding that coincides with the self-ratings of older adults who indicate needing more "recovery time" after work compared with younger adults (Kiss, De Meester, & Braeckman, 2008). Similarly, in a study of stressed individuals, age was linearly related to cortisol levels, indicating that among people exposed to stressors, older adults were affected to a greater extent than were their middle-aged counterparts (Jacobs, Mason, Kosten, Brown, & Ostfeld, 1984).

Studies also suggest that chronic stress may exacerbate normative aging processes of the immune system (see reviews

by Gruenewald & Kemeny, 2009; Hawkley & Cacioppo, 2004; Schneiderman, Ironson, & Siegel, 2005; but see Pedersen, Zechariae, & Bovbjerg, 2009). In one study, for example, the influenza vaccine was given to caregivers of spouses with dementia and noncaregiver controls. Noncaregivers were better able to mount a response to the vaccine than were caregivers, and among the caregivers, those younger than 70 years had a stronger response than did those older than 70 years (Kiecolt-Glaser et al., 1996). In contrast, nonelderly caregivers of relatives with multiple sclerosis did not appear to differ in their ability to mount a vaccine response than did noncaregivers (Vedhara et al., 2002). More postsurgery complications have also been noted among anxious older men when compared with nonanxious older men and both anxious and nonanxious younger men (Linn, Linn, & Jensen, 1983). Over a 6-year period, Kiecolt-Glaser and colleagues (2003) also found that spousal caregivers had a fourfold increase in levels of IL-6 compared with noncaregivers. Given that aging is positively associated with levels of IL-6, this additional increase may result in even greater health repercussions. Indeed, in a meta-analysis of over 300 studies examining stress and the immune system, Segerstrom and Miller (2004) concluded that "aging likely makes people more susceptible to negative immunological effects of stress" (p. 619).

Current research hints at the possibility of chronic stress exacerbating normative age-related changes in the neuroendocrine and immune systems. Not all studies show this pattern (e.g., Dijkstra, Charness, Yordon, & Fox, 2009; Strahler, Berndt, Kirschbaum, & Rohleder, 2010), however, so more work is needed before definitive conclusions can be drawn. Fortunately, there has been an increase in the number of large-scale longitudinal studies that collect both biological and psychological data, which will make it possible to assess the impact stressor type and duration have on aging.

FUTURE DIRECTIONS

Taking a Multivariate View: Allostasis and Allostatic Load

Our review thus far has highlighted how aging, stress, and the interaction between the two may affect biomarkers of the SAM axis, the HPA axis, and the immune system. Rather than operating independently, these systems interact, leading to dynamic, synergistic effects on the body. Examining how only one biomarker is related to one physiological system may provide an unacceptably narrow understanding of aging and stress. The concepts of allostasis and allostatic load (McEwen, 1998; Sterling & Eyer, 1988) were introduced in recognition of the need to integrate the effects of stress on the entire body and have moved researchers away from a univariate approach (i.e., use of one biomarker) of understanding the effects of stress on the body toward a multivariate approach (i.e., use of multiple biomarkers).

When faced with external challenges, such as stressful situations, the human body adapts by altering internal physiological processes. This process of adjustment and adaptation to meet external demands is referred to as allostasis (Sterling & Eyer, 1988). Allostasis is adaptive in the short term; continual accommodation of physiological systems in response to stressors, however, may result in allostatic load or a wearing down of bodily systems due to constant activity (McEwen, 1998). Operationalization of allostatic load varies from study to study, but one common way to measure it is through a summary score that represents the total number of biomarkers for which an individual scores in the uppermost or lowermost extreme (e.g., Crimmins, Kim, & Seeman, 2009; Seeman, Singer, Rowe, Horwitz, & McEwen, 1997). Studies using this method have shown that the more biomarkers for which a person scores in the highest risk quartiles, the greater is one's risk of cognitive and physiological declines, as well as mortality (e.g., Seeman et al.).

Statistical techniques, such as canonical correlation and recursive partitioning, have been used to more accurately account for the multidimensional and complex nature of allostatic load (e.g., Gruenewald, Seeman, Ryff, Karlamangla, & Singer, 2006; Karlamangla, Singer, McEwen, Rowe, & Seeman, 2002). Such methods allow researchers to identify the biomarkers that are most predictive of the outcome of interest. In general, however, much of the research on allostatic load has taken a fairly static view of aging, stressor exposure, and biomarkers. Because biological processes are labile and responsive to recent experiences and challenges, the understanding of aging, stressor exposure, biomarkers, and allostatic load may benefit from daily diary methods.

Taking a Dynamic View: Daily Stressors, Biomarkers, and Diary Designs

There are several virtues to daily diary studies (Bolger et al., 2003), one of the most notable being increased ecological validity, which oftentimes limits the generalizability of laboratory research. Given their relatively short-time frames, daily diary studies also address concerns regarding potential memory biases that may be an issue for retrospective studies with longer time frames. Perhaps most relevant to the study of stress, health, and aging, however, is the ability of daily diary studies to assess "within-person stressor reactivity."

"Stressor reactivity" refers to how people react either emotionally or physically to daily stressors, and is operationalized as the within-person association between stressors and a particular outcome--in this case biomarkers (Almeida, 2005; Cacioppo, 1998). In this sense, stressor reactivity does not refer to the average level of a given biomarker, but rather how biomarkers change as a function of stressor exposure. For example, prior research demonstrates that people who are less reactive to daily stressors are also

less susceptible to physical illness than are people who are more reactive to daily stressors (Cacioppo). A more dynamic way to approach the study of daily stressor reactivity, however, is to compare an individual's biomarker levels on stressor days versus stressor-free days. For example, rather than ask whether caregivers have higher salivary cortisol levels than do non-caregivers, researchers can examine whether caregivers' cortisol levels are higher on days when they experience stressors related to caregiving versus days when they do not experience caregiving-related stressors (e.g., Seltzer et al., 2009). This within-person approach makes it possible to rule out third variable explanations for the association between stressors and biomarkers, and permits a temporal examination of these two variables (for review, see Almeida, McGonagle, & King, 2009).

The next step in this daily approach is to embed intensive repeated measurements in traditional longitudinal designs to tease apart the pathways through which economic, social, and psychological factors affect daily stressor exposure and reactivity and how they may influence biomarkers (Almeida et al., 2009). Using data collected over an extended period of time would allow researchers to examine how preexisting circumstances may be associated with daily stress processes and biomarker changes. Moreover, examining the full life course would make it possible to explore how life cycle variation, in conjunction with economic and social factors, may alter these pathways (Almeida & Wong, 2009).

The use of daily diary data may also help researchers determine the physiological responses that are most adaptive to different types of stressors. Some researchers, for example, suggest that an adaptive response to stress consists of moderate activity within the SAM and HPA axes, with either moderate–moderate or high–low activation of the two systems, in contrast to the less adaptive high–high or low–low balance (Bauer, Quas, & Boyce, 2002). By collecting daily diary and biomarker data from the same individuals over time, it is possible to not only determine the responses that are most adaptive for people of different ages but also which biomarker combinations are most predictive of morbidity and mortality.

A Word of Caution

Clearly, the use of biomarkers in stress, health, and aging research provides a wealth of information. Yet, researchers must be well informed of the potential limitations that accompany this type of data collection. Biomarker assays tend to be costly, may require specialized training or a skilled technician, and—in isolation—are not always indicative of meaningful underlying processes (Mayeux, 2004). In addition, many biomarkers can only be collected in laboratory settings, thereby limiting the types of phenomena researchers may study. When biomarkers are collected in the field, however, questions arise regarding participant

compliance and sample integrity. Researchers, therefore, must decide whether reduced control is outweighed by the benefit of capturing stress processes as they occur in daily life. Regardless of where data are collected, proper collection procedures cannot be overemphasized. Arm movement, for example, can make blood pressure readings unreliable (Mourad et al., 2003); saliva collection method (e.g., cotton swab vs. passive drool) can affect DHEA-S/DHEA (Gallagher, Leitch, Massey, McAllister-Williams, & Young, 2006); and caffeine consumption can affect salivary cortisol levels (Dickerson & Kemeny, 2004). Finally, it is important to realize that not all research on stress, health, and aging requires biomarker data. In many situations, self-reported health information is not only acceptable but is ideally suited for the question at hand. Indeed, biomarkers are not a panacea to all research on stress, health, and aging. Instead, the use of biomarker data simply allows skilled researchers a chance to ask questions they may not otherwise be able to answer.

Summary and Conclusions

The study of stress and biological aging has a rich history. Over 50 years ago, Selye (1956) wrote in the final pages of *The Stress of Life*: "Stress is the sum of all of the wear and tear caused by any kind of vital reaction throughout the body at any one time. That is why it can act as a common denominator of all of the biologic changes which go on in the body; it is a kind of 'speedometer of life'" (p. 274). The frontier for research on stress and aging is to assess this speedometer within naturalistic settings across multiple time frames. Only by examining multiple daily stressors, in conjunction with individual differences and biological measures, can researchers determine the ways specific types of stressors, as well as their duration, are related to physiological processes across the life span.

Correspondence

Address correspondence to Jennifer R. Piazza, PhD, Department of Human Development and Family Studies, Pennsylvania State University, 118 Henderson Building, University Park, PA 16802. Email: jrp27@psu.edu.

References

Adam, E. K., & Gunnar, M. R. (2001). Relationship functioning and home and work demands predict individual differences in diurnal cortisol patterns in women. *Psychoneuroendocrinology*, 26, 189–208.

Almeida, D. M. (2005). Resilience and vulnerability to daily stressors assessed via diary methods. *Current Directions in Psychological Science*, 14, 64–68.

Almeida, D. M., McGonagle, K., & King, H. A. (2009). Assessing daily stress processes in social surveys by combining stressor exposure

and salivary cortisol. *Biodemography and Social Biology*, *55*, 219–237.

Almeida, D. M., Piazza, J. R., & Stawski, R. (2009). Inter-individual differences and intra-individual variability in the cortisol awakening response: An examination of age and gender. *Psychology and Aging*, *24*, 819–827.

Almeida, D. M., Piazza, J. R., Stawski, R. S., & Klein, L. C. (in press). The speedometer of life: Stress, health, and aging. In K. W. Schaie & R. Levey (Eds.), *Handbook of Aging*. New York: Elsevier.

Almeida, D. M., & Wong, J. D. (2009). Life transition and stress: A life course perspective on daily stress processes. In G. H. Elder & J. Z. Giele (Eds.), *The craft of life course research* (pp. 141–162). New York: Guilford Press.

Aw, D., Silva, A. B., & Palmer, D. B. (2006). Immunosenescence: Emerging challenges for an ageing population. *Immunology*, *120*, 435–446.

Barnes, R. F., Raskind, M., Gumbrecht, G., & Halter, J. B. (1982). The effects of age on the plasma-catecholamine response to mental stress in man. *Journal of Clinical Endocrinology and Metabolism*, *54*, 64–69.

Bauer, A. M., Quas, J. A., & Boyce, W. T. (2002). Associations between physiological reactivity and children's behavior: Advantages of a multisystem approach. *Journal of Developmental and Behavioral Pediatrics*, *23*, 102–113.

Bauer, M. E., Jeckel, C. M., & Luz, C. (2009). The role of stress factors during aging of the immune system. *Annals of the New York Academy of Sciences*, *1153*, 139–152.

Baum, A., Gatchel, R. J., & Schaeffer, M. A. (1983). Emotional, behavioral, and physiological effects of chronic stress at three mile island. *Journal of Consulting and Clinical Psychology*, *51*, 565–572.

Baum, A., & Grunberg, N. (1997). Measurement of stress hormones. In S. Cohen, R. C. Kessler & L. U. Gordon (Eds.), *Measuring stress: A guide for health and social scientists* (pp. 175–192). New York: Oxford University Press.

Bergman, B., Ahmad, F., & Stewart, D. E. (2008). Work family balance, stress, and salivary cortisol in men and women academic physicians. *International Journal of Behavioral Medicine*, *15*, 54–61.

Blandini, F., Martignoni, E., Deril, G. V. M., Biasio, L., Sances, G., Lucarelli, C., Rizzo, V., Costa, A., & Nappi, G. (1992). Free plasma catecholamine levels in healthy subjects: A basal and dynamic study. The influence of age. *Scandinavian Journal of Clinical and Laboratory Investigation*, *52*, 9–17.

Bolger, N., Davis, A., & Rafaeli, E. (2003). Diary methods: Capturing life as it is lived. *Annual Review of Psychology*, *54*, 579–616.

Bosch, J. A., Brand, H. S., Ligtenberg, A. J. M., Bermond, B., Hoogstraten, J., & Amerongen, A. V. N. (1998). The response of salivary protein levels and S-IgA to an academic examination are associated with daily stress. *Journal of Psychophysiology*, *12*, 384–391.

Brosschot, J. F., Benschop, R. J., Godaert, G. L. R., & Olff, M. (1994). Influence of life stress on immunological reactivity to mild psychological stress. *Psychosomatic Medicine*, *56*, 216–224.

Cacioppo, J. T. (1998). Somatic responses to psychological stress: The reactivity hypothesis. In M. Sabourin, F. Craik & M. Robert (Eds.), *Advances in psychological science* (Vol. 2, pp. 87–112). East Sussex, UK: Psychology Press.

Cannon, W. B. (1914). The emergency function of the adrenal medulla in pain and the major emotions. *American Journal of Physiology*, *33*, 356–372.

Cannon, W. B. (1932). *The wisdom of the body*. New York: Norton.

Cannon, W. B., Britton, S. W., Lewis, J. T., & Groeneveld, A. (1927). The influence of motion and emotion in medulloadrenal secretion. *American Journal of Physiology*, *79*, 433–465.

Centers for Disease Control and Prevention, National Center for Health Statistics. (2007). *Health, United States, 2007* Retrieved November 3, 2009, from http://www.cdc.gov/nchs/data/hus/hus07.pdf#executivesummary

Chahal, H. S., & Drake, W. M. (2007). The endocrine system and ageing. *Journal of Pathology*, *211*, 173–180.

Chida, Y., & Hamer, M. (2008). Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: A quantitative review of 30 years of investigations. *Psychological Bulletin*, *134*, 829–885.

Chiriboga, D. A. (1997). Crisis, challenge, and stability in the middle years. In M. E. Lachman & J. J. Boone (Eds.), *Multiple paths of midlife development. Studies on successful midlife development: The John D. and Catherine T. MacArthur Foundation series on mental health and development* (pp. 293–322). University of Chicago Press.

Chrousos, G. P., & Gold, P. W. (1992). The concepts of stress and stress system disorders: Overview of physical and behavioral homeostasis. *Journal of the American Medical Association*, *267*, 1244–1252.

Cohen, S., Doyle, W. J., & Baum, A. (2006). Socioeconomic status is associated with stress hormones. *Psychosomatic Medicine*, *68*, 414–420.

Crimmins, E., Vasunilashorn, S., Kim, J. K., & Alley, D. (2008). Biomarkers related to aging in human population. *Advances in Clinical Chemistry*, *46*, 161–216.

Crimmins, E. M., Kim, J. K., & Seeman, T. E. (2009). Poverty and biological risk: The earlier "aging" of the poor. *Journal of Gerontology: Biological Sciences and Medical Sciences*, *64A*, 286–292.

Deuschle, M., Gotthardt, U., Schweiger, U., Weber, B., Körner, A., Schmider, J., Standhard, H., Lammers, C. H., & Heuser, I. (1997). With aging in humans the activity of the hypothalamus-pituitary-adrenal system increases and its circadian rhythm flattens. *Life Sciences*, *61*, 2239–46.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin*, *130*, 355–391.

Dijkstra, K., Charness, N., Yordon, R., & Fox, M. (2009). Changes in physiological stress and self-reported mood in younger and older adults after exposure to a stressful task. *Aging Neuropsychology, and Cognition*, *16*, 338–356.

Dohrenwend, B. P. (2006). Inventorying stressful life events as risk factors for psychopathology: Toward resolution of the problem of intracategory variability. *Psychological Bulletin*, *132*, 477–495.

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*, *101*, 17312–17315.

Epel, E. S., Burke, H. M., & Wolkowitz, O. M. (2007). The psychoneuroendocrinology of aging: Anabolic and catabolic hormones. In C. M. Aldwin, C. L. Park & A. Spiro (Eds.), *Handbook of health psychology and aging* (pp. 119–141). New York: Guilford Press.

Esler, M., Kaye, D., Thompson, J., Jennings, G., Cox, H., Turner, A., Lambert, G., & Seals, D. (1995). Effects of aging on epinephrine secretion and regional release of epinephrine from the human heart. *Journal of Clinical Endocrinology and Metabolism*, *80*, 435–442.

Ferrari, A. U., Radaelli, A., & Centola, M. (2003). Aging and the cardiovascular system. *Journal of Applied Physiology*, *95*, 2591–2597.

Frankenhaeuser, M. (1971). Behavior and circulating catecholamines. *Brain Research*, *31*, 241–262.

Frankenhaeuser, M., & Rissler, A. (1970). Effects of punishment on catecholamine release and efficiency of performance. *Psychopharmacologia*, *17*, 378–390.

Fujiwara, K., Tsukishima, E., Kasai, S., Masuchi, A., Tsutsumi, A., Kawakami, N., Miyake, H., & Kishi, R. (2004). Urinary catecholamines and salivary cortisol on workdays and days off in relation to job strain among female health care providers. *Scandinavian Journal of Work, Environment and Health*, *30*, 129–138.

Gallagher, P., Leitch, M. M., Massey, A. E., McAllister-Williams, R. H., & Young, A.H. (2006). Assessing cortisol and dehydroepiandrosterone (DHEA) in saliva: Effects of collection method. *Journal of Psychopharmacology*, 20, 643–649.

Glaser, R., Kiecolt-Glaser, J. K., Bonneau, R. H., Malarkey, W., Kennedy, S., & Hughes, J. (1992). Stress-induced modulation of the immune

response to recombinant hepatitis B vaccine. *Psychosomatic Medicine*, *54*, 22–29.

Gomez, C. R., Boehmer, E. D., & Kovacs, E. J. (2005). The aging innate immune system. *Current Opinions in Immunology*, *17*, 457–462.

Graham, J. E., Christian, L. M., & Kiecolt-Glaser, J. K. (2006). Stress, age, and immune function: Toward a lifespan approach. *Journal of Behavioral Medicine*, *29*, 389–400.

Granger, D. A., & Kivlighan, K. T. (2003). Integrating biological, behavioral, and social levels of analysis in early child development: Progress, problems, and prospects. *Child Development*, *74*, 1058–1063.

Granger, D. A., Kivlighan, K. T., El-Sheikh, M., Gordis, E., & Stroud, L. R. (2007). Salivary alpha-amylase in biobehavioral research: Recent developments and applications. *Annals of the New York Academy of Sciences*, *1098*, 122–144.

Gruenewald, T. L., & Kemeny, M. E. (2007). Psychoneuroimmunological processes in aging and health. In C. M. Aldwin, C. L. Park & A. Spiro (Eds.), *Handbook of health psychology and aging* (pp. 97–118). New York: Guilford Press.

Gruenewald, T. L., Seeman, T. E., Ryff, C. D., Karlamangla, A. S., & Singer, B. H. (2006). Combinations of biomarkers predictive of later life mortality. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 14158–14163.

Hawk, L. W., Dougall, A. L., Ursano, R. J., & Baum, A. (2000). Urinary catecholamines and cortisol in recent-onset posttraumatic stress disorder after motor vehicle accidents. *Psychosomatic Medicine*, *62*, 423–434.

Hawkley, L. C., & Cacioppo, J. T. (2004). Stress and the aging immune system. *Brain, Behavior, and Immunity*, *18*, 114–119.

Hellhammer, D. H., Wüst, S., & Kudielka, B. M. (2009). Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology*, *34*, 163–171.

Holmes, T. H., & Rahe, R. H. (1967). The social readjustment rating scale. *Journal of Psychosomatic Research*, *11*, 213–218.

Huang, H., Patel, D. D., & Manton, K. G. (2005). The immune system in aging: Roles of cytokines, T cells and NK cells. *Frontiers in Bioscience*, *10*, 192–215.

Hucklebridge, F., Hussain, T., Evans, P., & Clow, A. (2005). The diurnal patterns of the adrenal steroids cortisol and dehydroepiandrosterone (DHEA) in relation to awakening. *Psychoneuroendocrinology*, *30*, 51–57.

Jacobs, S., Mason, J., Kosten, T., Brown, S., & Ostfeld, A. (1984). Urinary-free cortisol excretion in relation to age in acutely stressed persons with depressive symptoms. *Psychosomatic Medicine*, *46*, 213–221.

Jeckel, C. M. M., Lopes, R. P., Berleze, M. C., Luz, C., Feix, L., Argimon, I. I. D., Stein, L. M., & Bauer, M. E. (2010). Neuroendocrine and immunological correlates of chronic stress in 'strictly healthy' populations. *Neuroimmunomodulation*, *17*, 9–18.

Karlamangla, A. S., Singer, B. H., McEwen, B. S., Rowe, J. W., & Seeman, T. E. (2002). Allostatic load as a predictor of functional decline MacArthur studies of successful aging. *Journal of Clinical Epidemiology*, *55*, 696–710.

Kiecolt-Glaser, J. K., & Glaser, R. (2001). Stress and immunity: Age enhances the risks. *Current Directions in Psychological Science*, *10*, 18–21.

Kiecolt-Glaser, J. K., Glaser, R., Cacioppo, J. T., MacCallum, R. C., Snydersmith, M., Kim, C., & Malarkey, W. B. (1996). Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proceedings of the National Academy of Sciences*, *93*, 3043–3047.

Kiecolt-Glaser, J. K., McGuire, L., Robles, T. F., & Glaser, R. (2002). Psychoneuroimmunology: Psychological influences on immune function and health. *Journal of Consulting and Clinical Psychology. Special Issue: Behavioral Medicine and Clinical Health Psychology*, *70*, 537–547.

Kiecolt-Glaser, J. K., Preacher, K. J., MacCallum, R. C., Atkinson, C., Malarkey, W. B., & Glaser, R. (2003). Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proceedings*

of the National Academy of Sciences of the United States of America, *100*, 9090–9095.

Kiss, P., De Meester, M., & Braeckman, L. (2008). Differences between younger and older workers in the need for recovery after work. *International Archives of Occupational Environmental Health*, *81*, 311–320.

Klein, L. C., & Corwin, E. J. (2002). Seeing the unexpected: How sex differences in stress responses may provide a new perspective on the manifestation of psychiatric disorders. *Current Psychiatry Reports*, *4*, 441–448.

Klein, L. C., & Corwin, E. J. (2007). Homeostasis and the stress response. In E. J. Corwin (Ed.), *Handbook of Pathophysiology (3rd ed)* (pp. 159–172). Philadelphia, PA: Lippincott Williams & Wilkins.

Klein, L. C., Whetzel, C. A., Almeida, D. M., Bennett, J. M., Stawski, R. S., Banks, S. R., & Crouter, A. C. (2008). Salivary DHEA-S levels across the day: Evidence for a daily rhythm in a healthy adult population. *Psychosomatic Medicine*, *70*A-46.

Koster, A., Bosma, H., Penninx, B. W. J. H., Newman, A. B., Harris, T. B., van Eijk, J. T. M., Kempen, G. I. J. M., Simonsick, E. M., Johnson, K. C., Rooks, R. N., et al. (2006). Association of inflammatory markers with socioeconomic status. *Journal of Gerontology: Medical Sciences*, *61*, 284–290.

Kovaiou, R. D., Herndler-Brandstetter, D., & Grubeck-Loebenstein, B. (2007). Age-related changes in immunity: implications for vaccination in the elderly. *Expert Reviews in Molecular Medicine*, *9*, 1–17.

Kudielka, B. M., & Kirschbaum, C. (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology*, *28*, 35–47.

Kudielka, B. M., Schmidt-Reinwald, A. K., Hellhammer, D. H., Schurmeyer, T., & Kirschbaum, C. (2000). Psychosocial stress and HPA functioning: No evidence for a reduced resilience in healthy elderly men. *Stress*, *3*, 229–240.

Labrie, F., Belanger, A., Cusan, L., Gomez, J. L., & Candas, B. (1997). Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. *Journal of Clinical Endocrinology and Metabolism*, *82*, 2396–2402.

Lindheim, S. R., Legro, R. S., Bernstein, L., Stanczyk, F. Z., Vijod, M. A., Presser, S. C., & Lobo, R. A. (1992). Behavioral stress responses in premenopausal and post menopausal women and the effects of estrogen. *American Journal of Obstetrics and Gynecology*, *167*, 1831–1836.

Linn, B. S., Linn, M. W., & Jensen, J. (1983). Surgical stress in healthy elderly. *Journal of the American Geriatric Society*, *31*, 544–568.

Lundberg, U., & Frankenhaeuser, M. (1999). Stress and workload of men and women in high-ranking positions. *Journal of Occupational Health Psychology*, *4*, 142–151.

Lutgendorf, S. K., Vitaliano, P. P., Tripp-Reimer, T., Harvey, J. H., & Lubaroff, D. M. (1999). Sense of coherence moderates the relationship between life stress and natural killer cell activity in healthy older adults. *Psychology and Aging*, *14*, 552–563.

Marucha, P. T., Kiecolt-Glaser, J. K., & Favagehi, M. (1998). Mucosal wound healing is impaired by examination stress. *Psychosomatic Medicine*, *60*, 362–365.

Mason, J. W. (1968). A review of psychoendocrine research on pituitary-adrenal cortical system. *Psychosomatic Medicine*, *30*, 576–607.

Mayeux, R. (2004). Biomarkers: Potential uses and limitations. *NeuroRX*, *1*, 182–188.

McEwen, B. S. (1998). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York Academy of Sciences*, *840*, 33–44.

McGregor, B. A., Antoni, M. H., Ceballos, R., & Blomberg, B. B. (2008). Short communication: Very low CD19+ B-lymphocyte percentage is associated with high levels of academic stress among healthy graduate students. *Stress and Health: Journal of the International Society for the Investigation of Stress*, *24*, 413–418.

Miller, G. E., Chen, E., Fok, A. K., Walker, H., Lim, A., Nicholls, E. F., Cole, S., & Kobor, M. S. (2009). Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 14716–14721.

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin*, *133*, 25–45.

Montanini, V., Simoni, M., Chiossi, G., Baraghini, G. F., Velardo, A., Baraldi, E., & Marrama, P. (1988). Age-related changes in plasma dehydroepiandrosterone sulphate, cortisol, testosterone and free testosterone circadian rhythms in adult men. *Hormone Research*, *29*, 1–6.

Mourad, A., Carney, S., Gillies, A., Jones, B., Nanra, R., & Trevillian, P. (2003). Arm position and blood pressure: A risk factor for hypertension? *Journal of Human Hypertension*, *17*, 389–395.

Nater, U. M., & Rohleder, N. (2009). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology*, *34*, 486–496.

Nater, U. M., Rohleder, N., Schlotz, W., Ehlert, U., & Kirschbaum, C. (2007). Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology*, *32*, 392–401.

Nederfors, T., & Dahlof, C. (1992). Effects of the beta-adrenoceptor antagonists atenolol and propranolol on human whole saliva flow rate and composition. *Archives of Oral Biology*, *37*, 579–584.

Nicolson, N., Storms, C., Ponds, R., & Sulon, J. (1996). Salivary cortisol levels and stress reactivity in human aging. *Journals of Gerontology: Medical Sciences*, *52*, 68–75.

Otte, C., Hart, S., Neylan, T. C., Marmar, C. R., Yaffe, K., & Mohr, D. C. (2005). A mete-analysis of cortisol response to challenge in human aging: importance of gender. *Psychoneuroendocrinology*, *30*, 80–91.

Palmer, G. J., Ziegler, M. G., & Lake, C. R. (1978). Response of norepinephrine and blood pressure to stress increases with age. *Journals of Gerontology*, *33*, 482–487.

Patkai, P. (1971). Catecholamine excretion in pleasant and unpleasant situations. *Acta Psychologica*, *35*, 352–363.

Pearlin, L. I. (1999). The stress process revisited: Reflections on concepts and their interrelationships. In C. S. Aneshensel & J. C. Phelan (Eds.), *Handbook of the sociobiology of mental health* (pp. 395–415). New York: Kluwer Academic.

Pearlin, L. I., Menaghan, E. G., Lieberman, M. A., & Mullan, J. T. (1981). The stress process. *Journal of Health and Social Behavior*, *22*, 337–356.

Pedersen, A. F., Zechariae, R., & Bovbjerg, D. H. (2009). Psychological stress and antibody response to influenza vaccination: A meta-analysis. *Brain, Behavior, and Immunity*, *23*, 427–433.

Peskind, E. R., Raskind, M. A., Wingerson, D., Pascualy, M., Thal, L. J., Dobie, D. J., Veith, R. C., Dorsa, D. M., Murray, S., Sikkema, C., et al. (1995). Enhanced hypothalamic-pituitary-adrenocortical axis responses to physostigmine in normal aging. *Journals of Gerontology: Biological Sciences and Medical Sciences*, *50A*, M114–M120.

Pfister, G., & Savino, W. (2008). Can the immune system still be efficient in the elderly? An immunological and immunoendocrine therapeutic perspective. *Neuroimmunomodulation*, *15*, 351–364.

Ranjit, N., Young, E. A., & Kaplan, G. A. (2005). Material hardship alters the diurnal rhythm of salivary cortisol. *International Journal of Epidemiology*, *34*, 1138–1143.

Rasmusson, A. M., Vasek, J., Lipschitz, D. S., Vojvoda, D., Mustone, M. E., Shi, Q. H., Gudmundsen, G., Morgan, C. A., Wolfe, J., & Charney, D. S. (2004). An increased capacity for adrenal DHEA release is associated with decreased avoidance and negative mood symptoms in women with PTSD. *Neuropsychopharmacology*, *29*, 1546–1557.

Ritvanen, T., Louhevaara, V., Helin, P., Vaisanen, S., & Hanninen, O. (2006). Responses of the autonomic nervous system during periods of perceived high and low work stress in younger and older female teachers. *Applied Ergonomics*, *37*, 311–318.

Roberts, E. (1999). The importance of being dehydroepiandrosterone sulfate (in the blood of primates): A longer and healthier life? *Biochemical Pharmacology*, *57*, 329–346.

Rohleder, N., Marin, T. J., Ma, R., & Miller, G. E. (2009). Biologic cost of caring for a cancer patient: Dysregulation of pro- and anti-inflammatory signaling pathways. *Journal of Clinical Oncology*, *27*, 2909–2915.

Rohleder, N., & Nater, U. M. (2009). Determinants of salivary α-amylase in humans and methodological considerations. *Psychoneuroendocrinology*, *34*, 469–485.

Rohleder, N., Nater, U. M., Wolf, J. M., Ehlert, U., & Kirschbaum, C. (2004). Psychosocial stress-induced activation of salivary alpha-amylase: An indicator of sympathetic activity? *Annals of the New York Academy of Sciences*, *1032*, 258–263.

Sapolsky, R. M. (1996). Why stress is bad for your brain. *Science*, *273*, 749–750.

Sapolsky, R. M. (2000). Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Archives of General Psychiatry*, *57*, 925–935.

Schneiderman, N., Ironson, G., & Siegel, S. D. (2005). Stress and health: Psychological, behavioral and biological determinants. *Annual Review of Clinical Psychology*, *1*, 607–628.

Schnorpfeil, P., Noll, A., Schulze, R., Ehlert, U., Frey, K., & Fischer, J. E. (2003). Allostatic load and work conditions. *Social Science and Medicine*, *57*, 647–656.

Seals, D. R., & Esler, M. D. (2000). Human ageing and the sympathoadrenal system. *Journal of Physiology*, *528*, 407–417.

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*, 2259–2268.

Segerstrom, S. C., & Miller, G. E. (2004). Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychological Bulletin*, *130*, 601–630.

Seltzer, M. M., Almeida, D. M., Greenberg, J. S., Savla, J., Stawski, R. S., Hong, J., & Lounds, J. J. (2009). Daily stress and dysregulation of salivary cortisol in parents of children with disabilities: A report from the MIDUS Study. *Journal of Health and Social Behavior*, *50*, 1–15.

Selye, H. (1956). *The stress of life.*. New York: McGraw-Hill.

Singer, B., & Ryff, C. D. (2001). *New horizons in health: An integrative approach.*. Washington, DC: National Academy Press.

Smyth, J. M., Ockenfels, M. C., Gorin, A. A., Catley, D., Porter, L. S., Kirschbaum, C., Hellhammer, D. H., & Stone, A. A. (1997). Individual differences in the diurnal cycle of cortisol. *Psychoneuroendocrinology*, *22*, 89–105.

Strahler, J., Berndt, C., Kirschbaum, C., & Rohleder, N. (2010). Aging diurnal rhythms and chronic stress: Distinct alterations of diurnal rhythmicity of salivary alpha-amylase and cortisol. *Biological Psychology*, *84*, 248–256.

Sterling, P., & Eyer, J. (1988). Allostasis: A new paradigm to explain arousal pathology. In S. Fisher & J. Reason (Eds.), *Handbook of life stress, cognition and health* (pp. 629–649). Oxford, England: John Wiley & Sons.

Taylor, S. E., Klein, L. C., Lewis, B. P., Gruenewald, T. L., Gurung, R. A. R., & Updegraff, J. A. (2000). Female responses to stress: Tend-and-befriend, not fight-or-flight. *Psychological Review*, *107*, 411–429.

Uchino, B. N., Birmingham, W., & Berg, C. A. (2010). Are older adults less or more physiologically reactive? A meta-analysis of age-related differences in cardiovascular reactivity to laboratory tasks. *Journal of Gerontology: Psychological Sciences and Social Sciences*, *65B*, 154–162.

Uchino, B. N., Holt-Lunstad, J., Bloor, L. E., & Campo, R. A. (2005). Aging and cardiovascular reactivity to stress: Longitudinal evidence for changes in stress reactivity. *Psychology and Aging*, *20*, 134–143.

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*, 2468–2473.

van Niekerk, J. K., Huppert, F. A., & Herbert, J. (2001). Salivary cortisol and DHEA: association with measures of cognition and well-being

in normal older men, and effects of three months of DHEA supplementation. *Psychoneuroendocrinology*, *26*, 591–612.

Vedhara, K., McDermott, M. P., Evans, T. G., Treanor, J. J., Plummer, S., Tallon, D., Cruttenden, K. A., & Schifittoe, G. (2002). Chronic stress in nonelderly caregivers: Psychological, endocrine, and immune implications. *Journal of Psychosomatic Research*, *53*, 1153–1161.

Weiskopf, D., Weinberger, B., & Grubeck-Loebenstein, B. (2009). The aging of the immune system. *Transplant International*, *22*, 1041–1050.

Wheaton, B. (1997). The nature of chronic stress. In B. H. Gottlieb (Ed.), *Coping with chronic stress.* (pp. 43–73). New York, NY, US: Plenum Press.

Wust, S., Wolf, J., Hellhammer, D. H., Federenko, I., Schommer, N., & Kirschbaum, C. (2000). The cortisol awakening response—normal values and confounds. *Noise and Health*, *7*, 77–85.

Young, E. A., & Breslau, N. (2004). Cortisol and catecholamines in posttraumatic stress disorder: An epidemiologic community study. *Archives of General Psychiatry*, *61*, 394–401.