Allostatic Load in Relation to Periodontal Disease, Tooth Loss, and Mortality:

Findings from the 1914 Glostrup Aging Study

Christian R. Salazar

Submitted in partial fulfillment of the

requirements for the degree

of Doctor of Philosophy

under the Executive Committee of the

Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY

2013

ABSTRACT

Allostatic Load in Relation to Periodontal Disease, Tooth Loss, and Mortality:

Findings from the 1914 Glostrup Aging Study

Christian R. Salazar

As the proportion of adults aged 65 years and older continues to grow across the globe and edentulism rates decline, there is widespread concern about a rise in the prevalence of periodontal disease, characterized by chronic inflammation of tooth-supporting tissues induced by persistent infection. Compared to their younger counterparts, older adults experience a higher burden of periodontal disease, which can result in tooth loss, poor nutritional intake, higher prevalence of other chronic diseases, and a decrease in overall quality of life. While cross-sectional studies have underscored the role of chronic stress on periodontal disease progression in older adults, longitudinal evidence is currently lacking. This dissertation draws on prospective data from a birth cohort of older Danish adults (1914 Glostrup Aging Study) with 25 years of follow-up. Using physiological markers than span the metabolic, inflammatory, and cardiovascular systems, I developed a composite measure of allostatic load (AL) at age 80, defined as the cumulative biological damage that results from a whole-body adaptation to chronic stress. First, I identified social and behavioral predictors of high scores on AL. In men, those with no vocational training, unskilled occupation, low income, and a sedentary lifestyle were more likely to have high AL, consistent with a "weathering" pattern of biological systems resulting from chronic adversity over the life course. To test the hypothesis that high AL is longitudinally associated with periodontal disease, I evaluated bidirectional longitudinal associations using multiple measures of AL and periodontal disease. Results showed a positive

nonlinear association of AL at age 70 with periodontal disease at age 85, but no association between periodontal disease at age 70 with AL at age 80. This finding confirms previous cross-sectional data, and supports the role of chronic stress on infection-induced inflammation. To test the hypothesis that high scores on AL is associated with mortality risk, I examined this association longitudinally from ages 70 – 95. Compared to low AL, high AL was positively associated with all-cause mortality, and even stronger when cardiovascular disease mortality was considered. AL-mortality associations were higher among those who were dentate as compared to edentate, suggesting that dentate status modifies the relationship. Findings from this dissertation contribute to our understanding of the consequences of stress on periodontal disease in relation to aging and offer potential avenues for intervention.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

v

ACKNOWLEDGEMENTS

First and foremost, I give very special thanks to my mentor, sponsor, and friend, Dr. Pam Factor-Litvak, for her guidance over the years. Years ago, she introduced me to this area of research, and I have learned so much from her along the way. I'm truly grateful for her mentorship. I'd like to thank Dr. Ralph Katz, who has been a key figure in my professional development. He placed great confidence in me by recruiting me as a pre-doctoral fellow; I was the first ever non-dentist in his T32 oral epidemiology training program. I thank Dr. Doug Morse, who is quite simply an amazing mentor and friend. He introduced me to colleagues in Copenhagen, and facilitated the process of gaining access to the Glostrup study. I thank Dr. Gene Fisch for his statistical feedback and guidance during the initial stages of the analyses. I give special thanks to Dr. Kirsten Avlund at the University of Copenhagen, for her endless support of my dissertation at every step of the way. I give thanks to Dr. Poul-Holm Pedersen, who made me feel very welcome when I visited Copenhagen. I thank Mrs. Emilie Godfrey and Liliane Zaretsky, for going above and beyond to help me complete my dissertation. To members of my PhD cohort, I thank them dearly for providing much needed support in this journey.

A very special thanks goes out to my parents and family members for their endless support, as always. Last but certainly not least, I dedicate this dissertation to my lovely wife, Michelle. Without her love and patience through the years, I could not have the endurance to complete this work. She is the rock of my life. To my unborn son, he has provided me with so many immeasurable gifts along the last stages of this journey. He is my inspiration.

"If you can't explain it simply, you don't understand it well enough."

Albert Einstein

CHAPTER 1:

THE ROLE OF STRESS IN PERIODONTAL DISEASE PROGRESSION IN OLDER

ADULTS: A SYSTEMATIC REVIEW

1.1. ABSTRACT

Background: Older adults exhibit a higher prevalence of periodontal disease than younger adults. While several risk factors for periodontal disease have been identified, variations in periodontal disease extent and severity in older adults cannot be fully explained by these factors alone. Stress has been implicated as a potential factor that may influence periodontal disease progression.

Objective: We conducted a systematic review of the relationship between stress, factors that lead to stress and periodontal disease progression.

Methods: Two databases, (PUBMED and MEDLINE) were searched for original epidemiological studies from 1970 – 2012, and animal studies from 2000 – 2012. Plausible mechanistic pathways are described.

Results: Eleven epidemiological and 5 animal studies were identified. Three psychosocial factors (social strain, financial strain, and being widowed) and number of negative life events were found to be independent predictors of periodontal disease. The evidence is less consistent when measures of depression and anxiety were evaluated as exposures. Two cross-sectional studies report positive associations between allostatic load and periodontal disease. Selected animal studies corroborate the epidemiological evidence and highlight the role of glucocorticoids and catecholamines.

Conclusions: The weight of the evidence suggests a positive relationship between stress, psychosocial factors and periodontal disease progression. Allostatic load serves as an appropriate and promising theoretical model for future studies.

Key words: Periodontal disease, Stress, Psychosocial factors, Allostatic load, Physiological dysregulation, Older adults

1.2. Introduction

Declines in fertility and increases in life expectancy have led to drastic shifts in the age distribution of the world's population, where the proportion of older individuals (aged 65 years or older) has risen steadily from 5.0% of the total population in 1950 to 7.5% in 2009, and is expected to more than double to 16% (1.5 billion persons) by 2050[1,2]. Although more developed nations have relatively higher proportions of older adults, the growth is considerably more rapid in less developed regions[3]. The fastest growing segment of the older population is the portion aged 80 years or over, where the growth rate is 4.0% per year.

Paralleling this global upward trend is a steady increase in the number of older adults retaining their natural teeth[4]. In the US, the proportion of adults over 65 years who are edentulous (missing all natural teeth) declined from 46% in the early 1970's to 27% by 2004 [5,6]. A similar pattern of decline has been observed in other industrialized nations in Europe (Finland, Sweden, UK, Denmark), Australia and Japan [7]. As rates of edentulism decline, the number of natural teeth potentially at risk for oral diseases rises; hence, there is widespread concern that older adults may have an increasingly greater prevalence of periodontal diseases[8].

"Periodontal disease" is an umbrella term that includes gingivitis, an inflammation of the gingiva, and "periodontitis", used to define a set of inflammatory conditions affecting the alveolar bone in the jaw and supporting soft tissues that help anchor teeth in place [9]. The pathogenesis of periodontitis is complex but it is generally agreed that the initiating etiologic event involves infection with a group of predominantly gram-negative anaerobic bacteria that colonize the subgingival area[10]. Progression of disease is dependent upon a complex inter-relationship between microbial activity and the host's inflammatory response to microbial challenge, which progressively leads to connective tissue degradation and alveolar bone loss.

Differences in the clinical presentation of periodontitis reflect its complex multifactorial etiology, and these varying presentations have recently led to classification of the disease as either "chronic" or "aggressive" [11]. Compared to chronic periodontitis, aggressive periodontitis is characterized by its relatively early onset, rapid progression, and familial aggregation.

1.2.1. Measures of periodontal disease

Case definitions for periodontal disease are determined by an array of measures involving clinical signs and symptoms assessed predominantly with a periodontal probe (**table 1.1**). Periodontal probing depth (PD) is defined as the distance from the free gingival margin to the bottom of the pocket in millimeters (mm)[12]. Presence of bleeding on probing (BOP) serves as an objective indicator for gingival inflammation and is associated with active gingitivitis and periodontitis [13]. Clinical attachment level (CAL) is the distance from the cemento-enamel junction to the bottom of the pocket and is associated with gingival recession. In some studies, radiographically assessed alveolar bone loss serves as an additional measure [14]. Differences in clinical measures utilized and variations in the clinical thresholds for defining disease (e.g., attachment levels and/or depth of pockets) add to the complexity and non-comparability across epidemiologic studies of older adults. Studies that measure disease from randomly selected quadrants in the mouth as opposed to full mouth underestimate the true prevalence and severity of disease [15-18]. Moreover, estimates of disease experience in older adults may be additionally attenuated if a considerable proportion of individuals with aggressive periodontitis had substantial tooth loss earlier in life and, for these individuals, disease severity is determined from retained teeth considered "healthy survivors" [19].

1.2.2. Epidemiology of periodontal disease in older adults

While numerous cross-sectional and longitudinal studies have documented the prevalence and severity of periodontal disease in adults, data regarding disease progression in older adults (70+ years) have been scarce, largely due to a low proportion of available subjects who have retained their natural dentition at older age and the difficulties associated with following older adults prospectively. Several cross-sectional studies of periodontitis in older adults have described moderate levels of disease in a majority of individuals/teeth, with severe levels confined to a small but substantial minority. The New England Elders Dental Study, which assessed the periodontal status of 554 adults aged 70+ years in the Northeast region of the US, reported a prevalence of 66% with moderate periodontal pocketing (defined as at least one tooth with no more than a 4-6 mm pocket) [20,21]. Similar rates of disease prevalence have been observed in other parts of the US [22,23], and in other regions of the world such as China, India, Italy, and the Netherlands [24-27]. Severe periodontitis (defined as >6 mm pocket depth) are evident in approximately one fifth of adults aged 70+ years, and are estimated to be higher among the oldest old (85+ years) [21]. Similar conclusions can be drawn from prevalence studies of alveolar bone loss where a minority of older adults is reported to have advanced bone loss [28,29]. Since the prevalence and severity of periodontal disease increases with increasing age[30-32], age was initially considered a potential risk factor. However, the general consensus today challenges that notion and argues that older adults experience a cumulative effect of prolonged exposure to true risk factors of periodontal disease rather than a heightened susceptibility [19,33].

The inflammatory response and extent of tissue destruction associated with periodontitis are influenced in part by genetic and environmental risk factors [34]. While some evidence suggests that aggressive periodontitis has a stronger genetic component than does chronic

disease, risk factors are largely similar for both forms of the disease [35]. Established risk factors for periodontal disease onset and progression include tobacco consumption [36-40], poorly controlled diabetes mellitus [41,42], and poor oral hygiene [43,44]. Certain systemic diseases and conditions such as obesity, osteoporosis and rheumatoid arthritis are associated with a higher prevalence of periodontitis [41,45,46]. Studies have shown that sex/gender (males vs. females) [47,48], race/ethnicity (Blacks vs. Whites) [49] and socioecionomic status (low vs. high) [50,51] are similarly associated with higher levels of periodontal disease, although many of these factors are not independent of each other and are likely risk markers/indicators rather than true risk factors [52]. However, variations in periodontal disease severity in older adults cannot be fully explained by these factors alone [53]. Gaps remain in our understanding of the factors that contribute to the increased prevalence and progression of periodontal disease. It has been posited that factors leading to, or related to, stress may account for at least some of the remaining variability [54].

The current models of stress and stress system disorders retain the notion that both psychological and physiological components of stress are capable of invoking peripheral physiological responses in an organism in order to maintain homeostasis and promote survival[55]. To better understand the potential role of stress on periodontal disease, we undertook a systematic review of epidemiological and laboratory studies addressing this relationship.

1.3. Methods

We collected peer-reviewed reports from the publicly available database PubMed, which comprises citations from MEDLINE and other scientific data sources. Search terms included those that (a) involved the outcome of interest: "periodontal disease", "periodontal inflammation", and "periodontitis"; and (b) stress factors: "stress", "anxiety", and "life events".

Our initial search yielded 1,894 articles using combinations of the above-mentioned keywords. Original research articles were included if: (a) at least one objective measure of periodontal disease presented in table 1.1 was clinically assessed; (b) the study population included a minimum of 100 participants; and (c) the work was published in print or online between January 1, 1970 and November 30, 2012. We excluded articles that did not control for established risk factors or markers for periodontal disease in their models, namely smoking, sex/gender and age. Since further restriction of research articles to older adult populations (70+ years) would have yielded no articles after applying our inclusion and exclusion criteria, we chose not to restrict. In total, 11 original research articles were abstracted.

To systematically review the literature and evaluate this relationship with laboratory evidence using animal models, we used the same keywords for the outcome, as described previously. Search terms for stress factors included: "physical stress", "emotional stress", and "psychological stress". Additional terms were included to focus the search to animal studies: animals, animal models, rats, and mice. We included original research articles in which (a) experimental treatments were administered to live animals; (b) animal tissues, cell systems or organ preparations were examined in the laboratory; and (c) the overarching objective was to understand potential stress mechanism(s) involved in periodontal disease. To obtain the latest information, we included original research articles published in print or online from January 1, 2000 to November 30, 2012. Our initial search yielded 27 articles, but was reduced to 5 after applying our inclusion criteria.

The data that described the design, exposure, outcome and main results of each study were systematically abstracted. Given that measurement of stress can vary between the studies included in this review, we did not combine the study results using any method of quantitative

meta-analysis. Rather, we describe our findings in narrative form, with the results classified according to frequently cited stressors or group of stressors. In our discussion of potential mechanistic pathways, we describe specific theoretical models and review literature pertaining to the use of these models.

1.4. Results

1.4.1. Epidemiological studies

Nine cross-sectional and three case-control studies are presented in **table 1.2**. The geographical distribution of the study populations is wide-ranging and covers a broad spectrum of developed and developing nations. Exposures of stress were ascertained through a variety of validated instruments that assessed aspects of psychological well-being. These exposures could be arranged into three broad categories: a) psychosocial factors, b) depression and anxiety, and c) negative life events.

Three frequently cited psychosocial factors were social strain, financial strain and marital status. In a cross-sectional study of adults residing in Erie County, New York, Moss et al. found a strong dose-dependent positive association between "role" strain, defined as a global measure of social strain that comes from fulfilling multiple role demands, and severe periodontal disease (Odds Ratio [OR]=2.84, 95% Confidence Interval [CI]: 1.08 – 7.46)[56]. In a similar population, Genco et al reported that high levels of financial strain was positively associated with CAL (adjusted OR = 1.70, 1.09 – 2.65) and alveolar bone loss (adjusted OR = 1.91, 1.15 – 3.17) after controlling for age, gender, and smoking[57]. In a cross-sectional study of middle-aged Swedish adults, being widowed was a statistically significant predictor periodontal disease severity, but the association was removed after controlling for age[58]. However, findings from NHANES (2001

– 2004) data of older adults (60+ years) showed that being widowed was positively associated with extent of periodontal disease as compared to married individuals, after controlling for age, gender, race/ethnicity, education, and smoking (adjusted OR=1.27, 1.03 –1.58)[59].

We found that a majority of selected studies (seven out of 11) evaluated the relationship between objective measures of depression and anxiety with periodontal disease[56,58,60-64]. Depression and anxiety were measured with CES-D and other validated instruments, and over half (4 studies) reported no association. In a cross-sectional study of middle-aged Brazilian adults, Hilgert et al. found no association between stress symptoms, as measured by Lipp's Stress Symptoms for Adult Inventory, and periodontitis[61]. However, in the same sample, they found a positive association between salivary levels of cortisol and CAL $\geq$ 4 mm (OR=6.9, 1.7 – 27.1), and PD $\geq$ 4 mm (OR=10.7, 1.9 – 54.1), after adjusting for age, gender and smoking. In a large cross-sectional study of Chinese adults, Ng et al. found that individuals who exhibited high anxiety and depressive traits had higher extent of CAL $\geq$ 4 mm in multivariable models (OR=1.51, 1.09 – 2.72 and OR=1.62, 1.15 – 2.35, respectively) [65]. In another large cross sectional study of Taiwanese adults, Chiou et al reported positive associations between levels of psychosocial stress and CAL $\geq$ 6 mm[60].

A total of five of 11 of our selected studies investigated the relationship between stressful life events and emotional stressors with periodontal disease[57,58,64-66]. Of these, two reported no association. In one case-control study of British adults, Croucher et al found a positive association between number of major life events, as measured by the Holmes-Rahe Social Readjustment Scale, and presence of at least one tooth site with PD $\geq$ 5.5 mm, independent of smoking, education, and number of missing teeth[66]. Hugoson et al similarly found a positive association between number of traumatic events and periodontitis, and this relationship was

stronger among individuals with poor coping skills as compared to those with good coping skills[58]. Genco et al similarly reported an improvement in periodontal health associated with better coping skills.

1.4.2. Laboratory studies using animal models

Five selected experimental studies involving rodent models are presented in **table 1.3**. These studies utilize a variety of stress-inducing stimuli such as exposures to isolation, maternal separation, increased population density, enclosure with flexible wire mesh to immobilize the animal (also known as restraint stress), and cat shock, a technique in which a cat is introduced to the environment with the goal of eliciting fear and anxiety. Physical stressors included in these studies involved exposure to loud noise and cold temperatures. To evaluate the effects of these exposures on periodontal disease progression, periodontitis was induced using ligatures tied around molar teeth of rats, which promote the growth and proliferation of oral microorganisms. Changes in oral microflora following use of ligatures increase the likelihood of periodontitis[67]. Periodontal pocket formation, extent of loss of attachment, and alveolar bone loss were ascertained after the animal had been sacrificed and underlying tissues were subjected to radiographic and histopathological analysis.

Using experimental trials on mice, Shapiro et al. found that exposure to either physical or emotional stressors increased the host's inflammatory response to *Porphyromonas gingivalis*, a known pathogen that is etiologically linked to periodontitis, relative to controls[68]. Exposed mice also exhibited higher levels of nitric oxide compared to unexposed mice, an important mediator of the inflammatory response. Using Wistar rats, Takada et al. showed that exposure to restraint stress increased circulating levels of cortisol and adrenaline[69]. Rats exposed to restraint stress

also exhibited a higher degree of alveolar bone loss than those unexposed. In another study,

Huang et al. found that rats exposed to cold temperatures and cat shock stress exhibited higher

levels of periodontal attachment loss than controls[70]. Exposed rats also showed higher levels of

periodontal tissue hypoxia; decreased oxygenation to periodontal tissues is thought to promote a

favorable microenvironment for proliferation of bacteria etiologically linked to periodontal

disease. Using Sprague Dawley rats, Rivera et al. similarly showed that rats subjected to

prolonged restraint stress had significantly higher levels of gingival and alveolar bone

inflammation relative to controls[71]. In another study, Benatti et al. evaluated whether there is a

potential effect modification by nicotine of the relationship between restraint stress and

periodontal disease progression. The investigators found that rats exposed to nicotine and stress

exhibited significantly greater alveolar bone loss than those exposed to restraint stress or nicotine

alone[72].

1.5. Discussion

Our systematic review of the epidemiological evidence suggests that psychosocial

factors, depression, anxiety, and negative life events may contribute to periodontal disease

progression, independent of other strong risk factors. Laboratory studies involving animals

corroborate with the results obtained from epidemiological studies, suggesting that a biological

mechanism is plausible. Importantly, findings from this systematic review are consistent with

other systematic reviews that have investigated this relationship[73-76].

The associations between psychosocial factors and periodontal disease showed

remarkable consistency across epidemiological studies. Similar consistency was observed with

associations between negative life events and periodontal disease[65,66]. Studies that evaluated the

relationship using depression and anxiety as exposures showed the least consistency[62-64]. This may be due in part to a lack of uniformity in the method for defining and quantifying exposure to depression and anxiety. Definitions of depression and anxiety have ranged from subjective assessments of stressful situations, some of which were collected with validated questionnaires[56,58,63,64] and others not[62], to physiological measurements at specific points in time such as salivary cortisol[61]. Single measures of salivary cortisol are limited by the wide variability in levels across a 24-hour period; serial measures are needed for a more stable construct. In summary, stress can best be understood as part of a complex and dynamic system of positive and negative transactions between individuals and their environment, occurring universally in varying degrees, and exhibiting different effects upon individuals over their life course[77].

Use of animal studies in our systematic review was essential for understanding the potential mechanistic pathways involved. Findings from our selected animal studies supported our initial hypothesis that both physical and emotional stressors can influence the progression of periodontal disease. Moreover, these results underscore the importance of stress mediators such as glucocorticoids and catecholamines in the pathway(s) between stress and periodontal disease.

1.5.1. Potential Mechanisms

Although a number of pathways have been proposed to explain the stress-periodontal disease association[74,75], there has not been complete agreement on which mechanism(s) are likely involved. In general, mechanisms have been grouped into 2 broad categories: (a) 'health-impairing behaviors' associated with stress, such as increases in tobacco and alcohol consumption, poor oral hygiene, and poor nutritional intake[35] ; and (b) 'pathophysiological

factors' that lead to increases in stress hormones which can indirectly influence inflammatory and immunological profiles and increase the susceptibility to periodontal disease[76,78].

Health-impairing behaviors

A number of health-impairing behaviors associated with stress are likely to influence periodontal heath. Evidence suggests a positive bidirectional association between depression and smoking, particularly among young adults[79]. As an established risk factor for periodontal disease, smoking may serve as a mediating factor between depression and periodontal disease progression. While associations between alcohol consumption and depression have been well documented, the relationship between alcohol consumption and periodontal disease is unclear[80]. Smoking may confound a purported association between alcohol consumption and periodontal disease. Similarly, studies that investigate the relationship between oral hygiene and depression show inconsistent results, possibly due to confounding by other lifestyle factors[81].

Pathophysiological factors

A hallmark of the body's response to physical and perceived stress is the activation of the hypothalamic-pituitary-adrenal axis, the sympathetic (SNS) and parasympathetic (PNS) branches of the autonomic nervous system by the brain[82,83]. In a highly coordinated fashion, secretion of corticotropin-releasing hormone by the hypothalamus and adreno-corticotropic hormone by the pituitary gland lead to the release of glucocorticoids by the adrenal cortex[84]. Glucocorticoids exert a myriad of effects throughout the body that range from promoting immunosuppression to microbial infection (indirectly), altering levels of cytokine and growth factors, and modifying blood glucose levels[85]. There is a general suppressive effect of glucocorticoids on inflammatory cytokine production, such as interleukins (IL)-1, IL-2, IL-6, and tumor necrosis factors (TNFs),

thereby altering inflammatory activity[86]. Similarly, activation of the SNS results in the release of catecholamines (epinephrine and norepinephrine) from the adrenal medulla. Catecholamines regulate cardiovascular functioning, are involved in the mobilization and redistribution of immune cells throughout the body, and can synergize with or oppose the action of glucocorticoids[86,87]. Nonlinear interactions and complex biological feedback between these primary mediators (e.g., glucocorticoids, catecholamines, and inflammatory cytokines) constitute the dynamic and highly regulated response elicited during stress[88].

1.5.2. Allostatis and allostatic load as a theoretical framework

While hormonal mediators of the stress response have protective effects on the body in the short term, over-production can lead to pathophysiology and damage over the long term[89]. Chronic exposure to stress hormones from excessive cycles of response creates changing patterns of energy demand. To meet this challenge, a whole-body adaptation known as allostasis[90,91] occurs where physiological systems operate at new levels in order to maintain stability or homeostasis. With allostasis, biological set points of physiological system parameters change to a new equilibrium, thereby increasing efficiency and allowing for the organism's continued survival during repeated challenge[92]. Over time, however, cumulative damage to tissues and major organ systems result from this adaptation. This biological 'wear and tear' is referred to as allostatic load[93].

Four types of allostatic physiological conditions can lead to allostatic load (**figure 1.2**). The first involves multiple hits from novel stressors, which, over time, result in over-exposure to stress hormones and eventual biological damage. The next three conditions involve a failure of the organism to adequately manage the hormonal stress response. In the first instance, there is a

lack of habituation to repeated stressors of the same kind. In the second, the body experiences a delayed response due to an inability to properly shut down the stress system. Lastly, an inadequate physiological response in one regulatory system, such as the HPA axis, leads to compensatory over-activity of other allostatic systems, such as those that increase circulating pro-inflammatory cytokines as an example, which under normal circumstances are kept in balance by glucocorticoids and catecholamines. These allostatic load subtypes illustrate the damaging effects that hormonal mediators of stress can have on the body and contribute to gradients of health.

Allostatic load differs from traditional approaches to measurement of disease risk. The emphasis on biological system interconnectedness departs from conventional methods that focus on the role of one aspect of the system. Moreover, allostatic load considers moderate levels of physiological dysregulation when accounting for biological risk over the life course, consistent with the cumulative risk model proposed by Kuh and Ben-Shlomo[94,95]. Over time, individuals accumulate allostatic load at different rates, and older adults generally have higher levels than younger individuals. Differences in allostatic load scores in a given population reflect the myriad prior exposures associated with stress that ultimately contribute to disease. For periodontitis, exposure to stress can promote health-impairing behaviors, such as intensification of smoking and alcohol consumption, negligence of oral hygienic practices, and negative changes in dietary habits, thereby accelerating the course of disease.

Allostatic load has been operationalized using a variety of approaches[100]. Initial efforts involved a straightforward summative approach where markers of regulatory biological systems were used to calculate an aggregate score with equal weighting of each contributing factor (**table 1.4**). More complex scoring methodologies have been recently developed to expand the range

and scope of physiological measurements although no "gold standard" approach has yet been
accepted. The goal is to capture a multisystem array of information that includes the multiple
regulatory systems involved in adaptive allostatic processes.

Predictors of allostatic load in older age

Although emergent patterns of increasing dysregulation in physiological systems define
the aging process[96,97], it is unclear which factors contribute to differences in rates of
accumulation. A growing body of literature has emerged to suggest that social and environmental
elicitors of negative emotions, pathogens, and physical challenges lead to activations of
physiological systems designed to maintain balance[98]. Findings from the MacArthur and SEBAS
cohorts showed that high scores on allostatic load were associated with lower levels of social
support, negative social relationships, and poorer self-rated general health[99-101]. In addition,
accumulating evidence supports the notion that lower socioeconomic status is associated with
higher levels of allostatic load[102-106]. Taken together, these data show that some individuals seem
resilient to diseases of aging and present with a profile of positive health and well-being that may
protect stress regulatory systems from dysregulation. More research is needed to identify social
and behavioral factors that predict allostatic load profiles at older ages.

Associations between allostatic load and periodontal disease

Two recent cross-sectional studies have investigated the relationship between allostatic
load and periodontal disease (**table 1.5**). Findings from NHANES III provided some of the first
evidence to support an association between allostatic load and periodontitis in adults 18 years
and over [107]. Allostatic load was assessed with 7 physiological markers that are commonly

associated with periodontitis: central obesity (waist circumference), high blood pressure (systolic and diastolic), hypertriglyceridemia, low HDL, high plasma glucose, high CRP and fibrinogen. Results showed that high levels of allostatic load were significantly associated with a higher extent of gingival inflammation, periodontal attachment loss, and periodontal pockets. In addition, a significant association was also found between allostatic load and ischemic heart disease in the same population, suggesting a potential common stress pathway for both conditions. Recent findings from the NHANES survey (1999-2004)[108] similarly found a significant positive association between allostatic load and periodontal disease using 10 markers: BMI, diastolic and systolic blood pressure, CRP, albumin, glycosylated hemoglobin, total cholesterol, triglycerides, homocysteine, and creatinine clearance. However, longitudinal studies are warranted to further investigate the temporal association of this relationship.

1.6. Conclusions

The older adult population is growing faster than any other age group worldwide. The prevalence, and severity of periodontal disease among older adults are expected to rise globally as individuals experience longer life spans and retention of their natural teeth. Our systematic review showed that the role of stress in the progression of periodontal disease is firmly supported by current epidemiological and laboratory evidence. While the exact mechanism is a matter of debate, the association has a plausible pathophysiological basis. One theoretical model that captures cumulative biological damage resulting from chronic exposure to stress over the life course is allostatic load. Despite epidemiological studies that suggest that high allostatic load predicts a multitude of diseases at older ages, the evidence for associations with periodontal disease is cross-sectional. Longitudinal studies are necessary to prospectively evaluate the

directionality of this relationship. Much more needs to be known about which specific biomarkers, and/or combination of biomarkers, are most useful in predicting periodontal disease. These findings could contribute immensely to our understanding of the role of stress and inflammation in relation to aging and offer avenues for intervention.

1.7. Dissertation Aims

The current dissertation draws on data from the 1914 Glostrup Aging Study, a birth cohort from Glostrup, Denmark, with the overarching goal of examining the relationship between allostatic load and periodontal disease in older adults. In the parent study, comprehensive information was collected with regard to biological, and social conditions at five year intervals from ages 70 (1984) through 85 (1999). Clinical periodontal assessments were performed at ages 70 and 85. Physical examinations were conducted and blood samples drawn at ages 70 and 80, which make it possible to analyze for a variety of biological markers of allostatic load. Information on mortality was collected for all participants in the study until the end of follow-up in 2009.

Aim 1 (chapter 2): The hypothesis is that low socio-economic position and maladaptive health behaviors are associated with high scores of allostatic load. To test this hypothesis, I first develop an index of allostatic load with available physiological markers at ages 70 and 80. Then I evaluate the associations between social/behavioral characteristics of participants at age 75 and allostatic load at age 80.

Aim 2 (chapter 3): The hypothesis is that there are positive bidirectional associations between allostatic load and periodontal disease. To test this, I evaluate a series of cross-sectional and longitudinal associations: (i) cross-sectional associations between allostatic load and periodontal inflammation at age 70; (ii) prospective association between allostatic load at age 70 and periodontal disease at age 85; and (iii) prospective association between periodontal disease at age 70 and allostatic load at age 80.

Aim 3 (chapter 4): The hypothesis is that allostatic load is associated with mortality risk, and periodontal disease/edentulism mediates this relationship. To test this, I first evaluate the relationship between periodontal disease/edentulism and mortality risk (all-cause and cardiovascular disease mortality). Then I evaluate the relationship between periodontal allostatic load and mortality risk. Lastly, I explore whether periodontal disease/edentulism mediates and/or modifies the relationship between allostatic load and mortality risk.

1.8. References

1.   United Nations. Dept. of Economic and Social Affairs. Population Division. World

        population ageing 2009. New York, N.Y.: United Nations, Dept. of Economic and Social

        Affairs, Population Division; 2010.

2.   Lutz W, Sanderson W, Scherbov S. The coming acceleration of global population ageing.

        Nature 2008;451:716-9.

3.   Bongaarts J. Human population growth and the demographic transition. Philos Trans R Soc

        Lond B Biol Sci 2009;364:2985-90.

4.   Ettinger RL. The unique oral health needs of an aging population. Dent Clin North Am

        1997;41:633-49.

5.   United States. Dept. of Health and Human Services., National Institute of Dental and

        Craniofacial Research (U.S.). Oral health in America : a report of the Surgeon General.

        Rockville, Md.: U.S. Public Health Service, Dept. of Health and Human Services; 2000.

6.   Dye BA, Tan S, Smith V, et al. Trends in oral health status: United States, 1988-1994 and

        1999-2004. Vital Health Stat 11 2007:1-92.

7.   Suominen-Taipale AL, Alanen P, Helenius H, Nordblad A, Uutela A. Edentulism among

        Finnish adults of working age, 1978-1997. Community Dent Oral Epidemiol 1999;27:353-

        65.

8.   Joshi A, Douglass CW, Feldman H, Mitchell P, Jette A. Consequences of success: do more

        teeth translate into more disease and utilization? J Public Health Dent 1996;56:190-7.

9.   Armitage GC. Development of a classification system for periodontal diseases and

        conditions. Annals of periodontology / the American Academy of Periodontology

        1999;4:1-6.

10. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005;366:1809-20.

11. Consensus report. Periodontal diseases: epidemiology and diagnosis. Annals of periodontology / the American Academy of Periodontology 1996;1:216-22.

12. Robertson PB, Buchanan SA, Armitage GC, Newbrun E, Taggart EJ, Hoover CI. Evaluation of clinical and microbiological measures to predict treatment response in severe periodontitis. Journal of periodontal research 1987;22:230-2.

13. Greenstein G, Caton J, Polson AM. Histologic characteristics associated with bleeding after probing and visual signs of inflammation. Journal of periodontology 1981;52:420-5.

14. Armitage GC. The complete periodontal examination. Periodontology 2000 2004;34:22-33.

15. Kingman A, Albandar JM. Methodological aspects of epidemiological studies of periodontal diseases. Periodontology 2000 2002;29:11-30.

16. Hunt RJ, Fann SJ. Effect of examining half the teeth in a partial periodontal recording of older adults. J Dent Res 1991;70:1380-5.

17. Kingman A, Morrison E, Loe H, Smith J. Systematic errors in estimating prevalence and severity of periodontal disease. Journal of periodontology 1988;59:707-13.

18. Hunt RJ. The efficiency of half-mouth examinations in estimating the prevalence of periodontal disease. J Dent Res 1987;66:1044-8.

19. Locker D, Slade GD, Murray H. Epidemiology of periodontal disease among older adults: a review. Periodontology 2000 1998;16:16-33.

20. Douglass CW, Jette AM, Fox CH, et al. Oral health status of the elderly in New England. J Gerontol 1993;48:M39-46.

21. Fox CH, Jette AM, McGuire SM, Feldman HA, Douglass CW. Periodontal disease among New England elders. Journal of periodontology 1994;65:676-84.

22. Beck JD, Koch GG, Rozier RG, Tudor GE. Prevalence and risk indicators for periodontal attachment loss in a population of older community-dwelling blacks and whites. Journal of periodontology 1990;61:521-8.

23. Gilbert GH, Heft MW. Periodontal status of older Floridians attending senior activity centers. Journal of clinical periodontology 1992;19:249-55.

24. Baelum V, Luan WM, Chen X, Fejerskov O. A 10-year study of the progression of destructive periodontal disease in adult and elderly Chinese. Journal of periodontology 1997;68:1033-42.

25. Karsten RH, Truin GJ, Burgersdijk RC, Kalsbeek H, van 't Hof MA, Mulder J. Periodontal treatment need of the Dutch 15-74-year-old population. Community dentistry and oral epidemiology 1992;20:310-1.

26. Maity AK, Banerjee K, Pal TK. Low levels of destructive periodontal disease in a rural population in west Bengal, India. Community dentistry and oral epidemiology 1994;22:60-1.

27. Strohmenger L, Cerati M, Brambilla E, Malerba A, Vogel G. Periodontal epidemiology in Italy by CPITN. Int Dent J 1991;41:313-5.

28. Jenkins WM, Kinane DF. The 'high risk' group in periodontitis. Br Dent J 1989;167:168-71.

29. Salonen LW, Frithiof L, Wouters FR, Hellden LB. Marginal alveolar bone height in an adult Swedish population. A radiographic cross-sectional epidemiologic study. Journal of clinical periodontology 1991;18:223-32.

30. Burt BA. Periodontitis and aging: reviewing recent evidence. J Am Dent Assoc 1994;125:273-9.

31. Johnson BD, Mulligan K, Kiyak HA, Marder M. Aging or disease? Periodontal changes and treatment considerations in the older dental patient. Gerodontology 1989;8:109-18.

32. Van der Velden U. Effect of age on the periodontium. Journal of clinical periodontology 1984;11:281-94.

33. Papapanou PN, Lindhe J, Sterrett JD, Eneroth L. Considerations on the contribution of ageing to loss of periodontal tissue support. J Clin Periodontol 1991;18:611-5.

34. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontology 2000 1997;14:216-48.

35. Stabholz A, Soskolne WA, Shapira L. Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis. Periodontology 2000 2010;53:138-53.

36. Ogawa H, Yoshihara A, Hirotomi T, Ando Y, Miyazaki H. Risk factors for periodontal disease progression among elderly people. Journal of clinical periodontology 2002;29:592-7.

37. Gustafsson A, Asman B, Bergstrom K. Cigarette smoking as an aggravating factor in inflammatory tissue-destructive diseases. Increase in tumor necrosis Factor-alpha priming of peripheral neutrophils measured as generation of oxygen radicals. Int J Clin Lab Res 2000;30:187-90.

38. Gelskey SC. Cigarette smoking and periodontitis: methodology to assess the strength of evidence in support of a causal association. Community dentistry and oral epidemiology 1999;27:16-24.

39. Haber J. Cigarette smoking: a major risk factor for periodontitis. Compendium 1994;15:1002, 4-8 passim; quiz 14.

40. Haber J, Wattles J, Crowley M, Mandell R, Joshipura K, Kent RL. Evidence for cigarette smoking as a major risk factor for periodontitis. Journal of periodontology 1993;64:16-23.

41. Kinane DF. Periodontitis modified by systemic factors. Annals of periodontology / the American Academy of Periodontology 1999;4:54-64.

42. Loe H. Periodontal disease. The sixth complication of diabetes mellitus. Diabetes Care 1993;16:329-34.

43. Loe H, Theilade E, Jensen SB. Experimental Gingivitis in Man. The Journal of periodontology 1965;36:177-87.

44. Silness J, Loe H. Periodontal Disease in Pregnancy. Ii. Correlation between Oral Hygiene and Periodontal Condtion. Acta Odontol Scand 1964;22:121-35.

45. Kuo LC, Polson AM, Kang T. Associations between periodontal diseases and systemic diseases: a review of the inter-relationships and interactions with diabetes, respiratory diseases, cardiovascular diseases and osteoporosis. Public Health 2008;122:417-33.

46. Heitz-Mayfield LJ. Disease progression: identification of high-risk groups and individuals for periodontitis. Journal of clinical periodontology 2005;32 Suppl 6:196-209.

47. Albandar JM. Global risk factors and risk indicators for periodontal diseases. Periodontology 2000 2002;29:177-206.

48. Burt BA, Eklund SA. Dentistry, Dental Practice, and the Community. Philadelphia, PA: W.B. Saunders Company; 1999.

49. Borrell LN, Taylor GW, Borgnakke WS, et al. Factors influencing the effect of race on established periodontitis prevalence. Journal of public health dentistry 2003;63:20-9.

50. Locker D, Leake JL. Risk indicators and risk markers for periodontal disease experience in older adults living independently in Ontario, Canada. J Dent Res 1993;72:9-17.

51. Oliver RC, Brown LJ, Loe H. Variations in the prevalence and extent of periodontitis. J Am Dent Assoc 1991;122:43-8.

52. Borrell LN, Papapanou PN. Analytical epidemiology of periodontitis. Journal of clinical periodontology 2005;32 Suppl 6:132-58.

53. Marcenes WS, Sheiham A. The relationship between work stress and oral health status. Social science & medicine 1992;35:1511-20.

54. da Silva AM, Newman HN, Oakley DA. Psychosocial factors in inflammatory periodontal diseases. A review. Journal of clinical periodontology 1995;22:516-26.

55. Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. JAMA : the journal of the American Medical Association 1992;267:1244-52.

56. Moss ME, Beck JD, Kaplan BH, et al. Exploratory case-control analysis of psychosocial factors and adult periodontitis. Journal of periodontology 1996;67:1060-9.

57. Genco RJ, Ho AW, Grossi SG, Dunford RG, Tedesco LA. Relationship of stress, distress and inadequate coping behaviors to periodontal disease. Journal of periodontology 1999;70:711-23.

58. Hugoson A, Ljungquist B, Breivik T. The relationship of some negative events and psychological factors to periodontal disease in an adult Swedish population 50 to 80 years of age. Journal of clinical periodontology 2002;29:247-53.

59. Sabbah W, Tsakos G, Chandola T, et al. The relationship between social network, social support and periodontal disease among older Americans. Journal of clinical periodontology 2011;38:547-52.

60. Chiou LJ, Yang YH, Hung HC, et al. The association of psychosocial factors and smoking with periodontal health in a community population. Journal of periodontal research 2010;45:16-22.

61. Hilgert JB, Hugo FN, Bandeira DR, Bozzetti MC. Stress, cortisol, and periodontitis in a population aged 50 years and over. J Dent Res 2006;85:324-8.

62. Solis AC, Lotufo RF, Pannuti CM, Brunheiro EC, Marques AH, Lotufo-Neto F. Association of periodontal disease to anxiety and depression symptoms, and psychosocial stress factors. Journal of clinical periodontology 2004;31:633-8.

63. Persson GR, Persson RE, MacEntee CI, Wyatt CC, Hollender LG, Kiyak HA. Periodontitis and perceived risk for periodontitis in elders with evidence of depression. Journal of clinical periodontology 2003;30:691-6.

64. Castro GD, Oppermann RV, Haas AN, Winter R, Alchieri JC. Association between psychosocial factors and periodontitis: a case-control study. Journal of clinical periodontology 2006;33:109-14.

65. Ng SK, Keung Leung W. A community study on the relationship between stress, coping, affective dispositions and periodontal attachment loss. Community dentistry and oral epidemiology 2006;34:252-66.

66. Croucher R, Marcenes WS, Torres MC, Hughes F, Sheiham A. The relationship between life-events and periodontitis. A case-control study. Journal of clinical periodontology 1997;24:39-43.

67. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. Journal of periodontology 1992;63:322-31.

68. Shapira L, Frolov I, Halabi A, Ben-Nathan D. Experimental stress suppresses recruitment of macrophages but enhanced their P. gingivalis LPS-stimulated secretion of nitric oxide. Journal of periodontology 2000;71:476-81.

69. Takada T, Yoshinari N, Sugiishi S, Kawase H, Yamane T, Noguchi T. Effect of restraint stress on the progression of experimental periodontitis in rats. Journal of periodontology 2004;75:306-15.

70. Huang S, Lu F, Zhang Z, Yang X, Chen Y. The role of psychologic stress-induced hypoxia-inducible factor-1alpha in rat experimental periodontitis. Journal of periodontology 2011;82:934-41.

71. Rivera C, Monsalve F, Suazo I, Becerra J. Stress increases periodontal inflammation. Exp Ther Med 2012;4:883-8.

72. Benatti BB, Nogueira-Filho GR, Diniz MC, Sallum EA, Sallum AW, Nociti FH, Jr. Stress may enhance nicotine effects on periodontal tissues. An in vivo study in rats. Journal of periodontal research 2003;38:351-3.

73. Peruzzo DC, Benatti BB, Ambrosano GM, et al. A systematic review of stress and psychological factors as possible risk factors for periodontal disease. J Periodontol 2007;78:1491-504.

74. Rosania AE, Low KG, McCormick CM, Rosania DA. Stress, depression, cortisol, and periodontal disease. Journal of periodontology 2009;80:260-6.

75. Genco RJ, Ho AW, Kopman J, Grossi SG, Dunford RG, Tedesco LA. Models to evaluate the role of stress in periodontal disease. Annals of periodontology / the American Academy of Periodontology 1998;3:288-302.

76. Boyapati L, Wang HL. The role of stress in periodontal disease and wound healing. Periodontology 2000 2007;44:195-210.

77. Selye H. Stress in health and disease. Boston: Butterworths; 1976.

78. Wiebe DJ, McCallum DM. Health practices and hardiness as mediators in the stress-illness relationship. Health Psychol 1986;5:425-38.

79. Chaiton MO, Cohen JE, O'Loughlin J, Rehm J. A systematic review of longitudinal studies on the association between depression and smoking in adolescents. BMC Public Health 2009;9:356.

80. Amaral Cda S, Vettore MV, Leao A. The relationship of alcohol dependence and alcohol consumption with periodontitis: a systematic review. J Dent 2009;37:643-51.

81. Sakki TK, Knuuttila ML, Vimpari SS, Hartikainen MS. Association of lifestyle with periodontal health. Community dentistry and oral epidemiology 1995;23:155-8.

82. Wurtman RJ. Stress and the adrenocortical control of epinephrine synthesis. Metabolism 2002;51:11-4.

83. Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev 1984;5:25-44.

84. Rosol TJ, Yarrington JT, Latendresse J, Capen CC. Adrenal gland: structure, function, and mechanisms of toxicity. Toxicol Pathol 2001;29:41-8.

85. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 2000;21:55-89.

86. Spencer RL, Kalman BA, Dhabhar FS. Coping with the environment: Neural and endocrine mechanisms. New York: Oxford University Press; 2000.

87. Dowdell K, Whitacre C. Coping with the environment: Neural and endocrine mechanisms. New York: Oxford University Press; 2000.

88. McEwen BS. Interacting mediators of allostasis and allostatic load: towards an understanding of resilience in aging. Metabolism 2003;52:10-6.

89. McEwen BS, Seeman T. Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. Ann N Y Acad Sci 1999;896:30-47.

90. Sterling P, Eyer J. Handbook of life stress, cognition, and health. New York: John Wiley and Sons; 1988.

91. Schulkin J. Allostasis, homeostasis and the costs of physiological adaptation. New York: Cambridge University Press; 2004.

92. Sterling P. Allostasis: A model of predictive regulation. Physiol Behav 2011.

93. McEwen BS. Protective and damaging effects of stress mediators. N Engl J Med 1998;338:171-9.

94. Kuh D, Ben-Shlomo Y, Lynch J, Hallqvist J, Power C. Life course epidemiology. Journal of epidemiology and community health 2003;57:778-83.

95. Ben-Shlomo Y, Kuh D. A life course approach to chronic disease epidemiology: conceptual models, empirical challenges and interdisciplinary perspectives. Int J Epidemiol 2002;31:285-93.

96. Perry HM, 3rd. The endocrinology of aging. Clin Chem 1999;45:1369-76.

97. Lipsitz LA, Goldberger AL. Loss of 'complexity' and aging. Potential applications of fractals and chaos theory to senescence. JAMA 1992;267:1806-9.

98. McEwen BS, Stellar E. Stress and the individual. Mechanisms leading to disease. Archives of internal medicine 1993;153:2093-101.

99. Seeman T, Glei D, Goldman N, Weinstein M, Singer B, Lin YH. Social relationships and allostatic load in Taiwanese elderly and near elderly. Social science & medicine 2004;59:2245-57.

100. Seplaki CL, Goldman N, Glei D, Weinstein M. A comparative analysis of measurement approaches for physiological dysregulation in an older population. Exp Gerontol 2005;40:438-49.

101. Seplaki CL, Goldman N, Weinstein M, Lin YH. Measurement of cumulative physiological dysregulation in an older population. Demography 2006;43:165-83.

102. McEwen BS, Gianaros PJ. Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. Ann N Y Acad Sci 2010;1186:190-222.

103. Seeman T, Epel E, Gruenewald T, Karlamangla A, McEwen BS. Socio-economic differentials in peripheral biology: cumulative allostatic load. Ann N Y Acad Sci 2010;1186:223-39.

104. Seeman TE, Singer BH, Ryff CD, Dienberg Love G, Levy-Storms L. Social relationships, gender, and allostatic load across two age cohorts. Psychosomatic medicine 2002;64:395-406.

105. Seeman TE, Crimmins E, Huang MH, et al. Cumulative biological risk and socio-economic differences in mortality: MacArthur studies of successful aging. Social science & medicine 2004;58:1985-97.

106. Geronimus AT, Hicken M, Keene D, Bound J. "Weathering" and age patterns of allostatic load scores among blacks and whites in the United States. Am J Public Health 2006;96:826-33.

107. Sabbah W, Watt RG, Sheiham A, Tsakos G. Effects of allostatic load on the social gradient in ischaemic heart disease and periodontal disease: evidence from the Third National Health and Nutrition Examination Survey. J Epidemiol Community Health 2008;62:415-20.

108. Borrell LN, Crawford ND. Social Disparities in Periodontitis among United States Adults: The effect of allostatic load. J Epidemiol Community Health 2009.

Table 1.1. Clinical measures of periodontal disease utilized in epidemiological studies

Clinical measure	How assessed	Interpretation of measure
Periodontal pocket depth (PD)	Distance in millimeters from the bottom of the pocket to the top of the gingiva or gingival margin	Amount of active disease present
Clinical attachment loss (CAL)	Distance in millimeters from the bottom of the pocket to the cemento-enamel junction (CEJ)	Amount of gingival recession present and is indicative of past periodontal experience
Bleeding on probing (BOP)	Presence of blood when pocket is gently probed	Indicator of active periodontal inflammation
Radiographs	X-rays of maxillary bone	Extent of alveolar bone loss

Table 1.2. Epidemiological studies examining associations between stress factor(s) and periodontal disease

First author and year (Reference No.)	Type of study	Periodontal disease measure	Population size (age range)	Geographical location	Stress factor(s) assessed	Principal Findings
Moss et al 1996 (56)	Case-control	CAL and PD	71 cases, 77 controls (25 – 74)	USA	Self reported strain, depressive symptoms based on a 53-item validated instrument	Role strain was positively associated with case status: CAL ≥ 6 mm and PD ≥ 5 mm (OR=2.84, 1.08 – 7.46).
Genco et al 1999 (57)	Cross-sectional	CAL and radiographs	1,426 (25 – 74)	USA	Life events and psychosocial stress as measured by 5 validated instruments	Financial strain was positively associated with CAL ≥ 4 mm (OR = 1.70, 1.09 – 2.65) and alveolar bone loss (OR=1.68, 1.20 – 2.37)
Hugoson et al 2002 (58)	Cross-sectional	PD	298 (50 – 80)	Sweden	Wellness as measured by 4 items on a CES-D scale, loneliness as measured by a 4 items on a validated instrument, marital status	Being widowed compared to married was positively associated with PD ≥ 4 mm (crude OR=2.69, 1.28 – 5.64), but the association was attenuated after controlling for age.

Sabbah et al 2011 (59)	Cross-sectional	CAL, PD,	1,632 (60+)	USA	Psychosocial factors	Being widowed was positively associated with CAL $\geq$ 3 mm (adjusted OR=1.27, 1.03 −1.58)
Chiou et al 2010 (60)	Cross-sectional	CAL and PD	11,723 ($\geq$ 18)	Taiwan	Psychosocial stress as measured by a 12-item validated instrument	Psychosocial stress was positively associated with CAL $\geq$ 6 mm (OR=1.69, 1.01 − 2.77)
Hilgert et al 2006 (61)	Cross-sectional	CAL, PD, and BOP	235 (50 – 86)	Brazil	Stress symptoms as measured by a validated instrument, cortisol measured in saliva	No association between stress and periodontal disease, but high levels of cortisol was positively associated with CAL $\geq$ 4 mm (OR=6.9, 1.7 – 27.1), and PD $\geq$ 4 mm (OR=10.7, 1.9 – 54.1)
Solis et al 2004 (62)	Cross-sectional	CAL and PD	153 (19 – 67)	Brazil	Self reported anxiety, depression, and psychiatric symptoms	No associations
Persson et al 2003 (63)	Cross-sectional	PD and radiographs	701 (60 – 75)	USA	Depression as measured by a 15-item validated instrument	No association

Castro et al 2006 (64)	Case-control	CAL	165 (35 – 60)	Brazil	Life events, anxiety, and depression as measured by 4 validated instruments	No associations
Ng et al 2006 (65)	Cross-sectional	CAL, PD, and BOP	1000 (25 – 64)	China	Life events as measured by a 12-item validated instrument, symptoms of stress, depression and anxiety as measured by 2 validated instruments	Factors that were positively associated with CAL $\geq$ 4 mm were: depression (OR=1.62, 1.15 – 2.35) anxiety (OR=1.51, 1.09 – 2.72), job strain (OR=1.47, 1.21 – 2.01), and financial strain (OR=1.38, 1.13 – 1.71)
Croucher et al 1997 (66)	Case-control	PD	100; 50 cases, 50 controls (18 – 60)	UK	Number of traumatic life events as measured by a 43-item validated instrument	Number of traumatic life events was positively associated with at least one tooth with PD $\geq$ 5 mm

Abbreviations: PD= Periodontal pocket depth, CAL= Clinical attachment loss, BOP = Bleeding on Probing

Table 1.3. Animal studies examining associations between stressors and periodontal disease[a]

First author & year (Reference No.)	Animal type	Stress factor(s) assessed	Study objective(s)	Principal Findings
Shapira et al. 2000 (68)	24 Sabra mice	Cold temperatures and isolation	To examine whether physical and emotional stressors modify the inflammatory response to *Porphyromonas gingivalis*, a bacterium etiologically linked to periodontitis	Compared to controls, exposure to either physical or emotional stressors increased inflammatory response to *P. gingivalis* through upregulation of nitric oxide by macrophages
Takada et al. 2004 (69)	100 Wistar rats	Restraint stress	To evaluate whether restraint stress increases stress mediators and modifies extent of alveolar bone loss	Rats exposed to restraint stress had a higher level of cortisol and adrenaline, and higher degree of alveolar bone loss than rats not exposed
Huang et al. 2011 (70)	66 Wistar rats	Restraint stress, cold temperatures, cat shock stress	To examine whether psychological stressors exacerbates periodontal disease by increasing tissue hypoxia	Rats exposed to psychological stressors exhibited higher levels of attachment loss and tissue hypoxia compared to unexposed rats
Rivera et al. 2012 (71)	32 Sprague Dawley rats	Restraint stress	To examine the effect of chronic exposure to restraint stress on severity of periodontal disease	Exposure to restraint stress increased levels of plasma corticosterone, gingival and alveolar bone inflammation compared to controls
Benatti et al. 2003 (72)	20 Wistar rats;	Restraint stress	To evaluate whether there is a potential effect modification by nicotine of the relationship between stress and periodontal disease progression	Rats exposed to nicotine and stress exhibited significantly greater alveolar bone loss than those exposed to stress or nicotine alone

[a] All animals were induced with experimental periodontitis using ligatures on molar teeth

Table 1.4. Physiological markers utilized in allostatic load derivation

Physiological system	Marker assessed
Hypothalamic-Pituitary-Adrenal Axis	Cortisol, dehydroepiandrosterone sulfate
Autonomic Nervous System	Norepinephrine, epinephrine
Inflammation	IL-6, TNF-α, C-reactive protein
Cardiovascular	Diastolic and systolic blood pressure, heart rate
Metabolic	Glucose, glycosylated hemoglobin, insulin, lipids (total cholesterol, high density lipoprotein, low density lipoprotein, relative weight (BMI and WHR)

Abbreviations: BMI= Body mass index, WHR= weight/height ratio

Table 1.5. Cross-sectional studies from NHANES of associations between periodontal disease and allostatic load

First author and year (Reference No.)	Periodontal disease measure	Population included (age range)	Allostatic load markers	Principal Findings
Sabbah et al 2008 (107)	CAL and PD	NHANES 1988 – 1994 4,295 (17+)	Central obesity (waist circumference), systolic and diastolic blood pressure, triglycerides, high density lipoprotein (HDL)-cholesterol, plasma glucose, CRP and fibrinogen	Allostatic load was positively associated with CAL and PD. Similar associations were found between allostatic load and ischaemic heart disease
Borrell and Crawford 2010 (108)	CAL and PD	NHANES 1999 – 2004 4710 (18 – 60)	BMI, diastolic and systolic blood pressure, CRP, albumin, glycosylated hemoglobin, total cholesterol, triglycerides, homocysteine, and creatinine clearance	Allostatic load was positively associated with periodontitis cases[a] after adjustment for age, sex, race/ethnicity, marital and nativity status, smoking, health insurance, income, education, and time since last dental visit (PR=1.55, 1.05 – 2.29)

Abbreviations: PR= Prevalence ratio

[a] Periodontitis was defined as having at least two sites with CAL $\geq$ 4 mm and at least one site with PD $\geq$ 4 mm

CHAPTER 2:

SOCIAL AND BEHAVIORAL DETERMINANTS OF ALLOSTATIC LOAD IN A COHORT

OF OLDER DANISH ADULTS

2.1. ABSTRACT

Background: Allostatic load is a cumulative measure of physiological dysregulation across multiple systems over the life course. It is not entirely clear which factors lead to higher rates of accumulation in some individuals compared to others.

Objective: Using data from the 1914 Glostrup Aging Study, we examined predictors of allostatic load in a cohort of 330 fully functioning 80-year olds residing in Glostrup, Denmark based upon social and behavioral factors measured at age 75 with a structured questionnaire.

Methods: We used a count-based formulation to create a summary allostatic load measure incorporating 10 physiological markers from blood sampled at age 80. Analysis of variance was performed to compare mean allostatic load scores across each social/ behavioral factor. We constructed proportional odds models to examine associations between social/behavioral characteristics and allostatic load.

Results: In men, high allostatic load was associated with no vocational training versus (vs.) some training (odds ratio=2.08, 95% confidence interval: 1.04 – 4.14), unskilled vs. managerial occupation (OR=2.63, 1.16 – 5.97), low vs. high income (OR=1.76, 0.89 – 3.50), renting vs. owning a home (OR=1.92, 0.98 – 3.78), and low vs. high physical activity (OR=2.58, 1.23 – 5.41). After adjustment for other social/behavioral characteristics, physical activity was an independent predictor of allostatic load (OR=2.71, 1.21 – 6.05). Similar but non-significant associations were observed between selected social/behavioral characteristics and allostatic load in women.

Conclusions: These data support the hypothesis that aspects of socioeconomic position and maladaptive behaviors in early old age predict unequal accumulation of physiological

dysregulation, consistent with a "weathering" pattern of biological systems resulting from chronic adversity over the life course.

Key words: Physiological dysregulation; Allostatic load; Older adults; Socioeconomic position; Physical activity

2.2. Introduction

The aging process is characterized by emergent patterns of increasing structural and functional decline in physiological systems, even in the absence of apparent disease[1]. Exposure to stressors over the life course is thought to accelerate aging by promoting physiological dysregulation and influencing disease trajectories[2]. In the short-term, such exposures activate a highly-coordinated hormonal response mediated by the hypothalamic-pituitary-adrenal axis and autonomic nervous system[3]. Over the long run, however, over-activation of the stress response leads to compensatory changes in secondary physiological systems (e.g., metabolic and inflammatory) in a whole-body adaptation known as allostasis[4,5]. While allostasis is necessary for maintenance of internal stability or homeostasis during repeated challenge[6], the process results in cumulative damage to tissues and major organ systems, referred to as allostatic load[7]. The model of allostatic load serves as a multisystem construct of physiological dysregulation that has been useful in predicting morbidity and mortality at older age[8,9].

Evidence suggests that levels of allostatic load increase linearly over time from early age to mid-life, and then plateaus at age 60[10], consistent with the cumulative risk model proposed by Kuh and Ben-Shlomo[11,12]. Older adults consequently have a higher burden of allostatic load than younger individuals. However, there is great variability in the rate of allostatic load accumulation between individuals. These differences are hypothesized to be a function of an individual's overall lifetime exposure and resiliency to social and environmental stressors, as well as sex differences due to hormonal or genetic influences[13].

While it is not entirely clear which factors accelerate rates of allostatic load accumulation in some individuals compared to others, there is emerging evidence to suggest that socioeconomic conditions play a role[14,15]. Various cross-sectional and cohort studies across

different populations have documented gradients of socioeconomic position in allostatic load,

using biological markers that capture physiological dysregulation across metabolic,

inflammatory, and neuroendocrine systems[16-23]. Measures of socioeconomic adversity have been

shown to be strong predictors of high inflammatory burden throughout the life course from

childhood through adulthood[24-26]. Studies that examine predictors of multisystem physiological

dysregulation at older age are sparse, and additional research is necessary to fully elucidate

biological mechanisms through which socioeconomic adversity could lead to a decline in health.

Potential pathways through which socioeconomic conditions can influence allostatic load

have been suggested to involve a host of psychosocial, environmental, and behavioral exposures.

Maladaptive behaviors such as increased smoking, alcohol consumption, and sedentary lifestyles

are known to be associated with socioeconomic disadvantage[27]. Very few studies have

comprehensively examined behavioral determinants of allostatic load, and none have explored

these associations in older adults. From a public health perspective, identification of behavioral

risk factors of physiological dysregulation at older age is important since these are potentially

modifiable characteristics amenable to intervention.

The aim of the present study is to examine associations between social and behavioral

factors, including aspects of socioeconomic experience over the life course, and allostatic load in

a birth cohort of Danish older adults from the Glostrup Aging Study. We developed an index of

allostatic load at age 80, which includes lipid profile markers measured in blood (high density

lipoprotein [HDL] and ratio of total cholesterol/HDL), body mass index (BMI), non-fasting

blood glucose, cardiovascular measures (systolic and diastolic blood pressure, heart rate), and

serum levels of albumin. We also incorporate inflammatory cytokines IL-6 and TNF-α into our

index of allostatic load because they are significant predictors of overall death in the present

cohort[28]. The availability of multiple measures of social position allows us to not only explore the influence of chronic adversity on physiological dysregulation at older age but also over the life course. Moreover, by examining behavioral characteristics at early old age, we are able to elucidate pathways through which measures of social position could influence allostatic load accumulation at old age.

2.3. Methods

2.3.1. Study Population

The present study uses data from the 1914 Glostrup Aging Study, a birth cohort initiated at age 50 in Glostrup, Denmark; a full description of the parent study is presented elsewhere (**see Methods appendix**). The study population at age 75 (baseline for the current study; **figure 2.1**) was composed of 748 participants from 2 sub-samples: The first was a cohort of 576 survivors of the 70-year old assessment of the parent study, and the second was a random sample of 172 men and women born in 1914 and living within the 11 municipalities around Glostrup who formed part of a larger ongoing cohort study evaluating the functional capacity and overall health of older Nordic urban individuals (NORA study). Overall, participants and non-participants did not differ significantly with regard to demographic characteristics and prevalence of selected diseases such as cancer, stroke, diabetes mellitus, bronchitis, and ischemic heart diseases[29]. During home visits, participants completed structured questionnaires ascertaining information about social and lifestyle factors. At follow-up (age 80), 30% of participants (n=189) had died. Of the survivors (n=559), 362 (65%) participated in the medical evaluation that included blood work and clinical assessments. Complete data on physiological markers used for assessment of AL was available for 330 participants.

2.3.2. Social and behavioral characteristics at baseline (age 75)

Measures of social and behavioral factors were assessed with structured questionnaires administered at age 75; each variable is summarized in **Table 2.1**. The original questionnaire developed for the parent study at age 50 followed WHO criteria[30] for assessing risk factors of cardiovascular disease. Questionnaire items that evaluated occupation, physical activity, and lifestyle factors were tailored to the Danish population. Efforts were made to maintain item consistency across each of the 5-year follow up assessments. A trained examiner administered and then reviewed the questionnaire for completeness.

Measures of social position included variables that captured information about vocational training, occupation, and material wealth. Vocational training is the number of years above primary or elementary education, which is typically 7 years. In analyses, this variable was trichotomized into '*no training*', '*less than three years*' and '*greater than or equal to three years of training*'. The three-year cut-point corresponds to the transition between secondary and higher education. The variable "occupation" was defined as the longest held occupation before retirement and had 4 categories: "*unskilled*", "*skilled*", "*managerial*", and "*other*" (mostly self-employed). Low income was defined as compensation from old-age pension alone while high income was defined as old-age pension supplemented with income from other sources. Housing status was defined as the housing arrangement at the time of the interview ("*renter*", "*owner*", or "*other*" [mostly institutionalized]). In analyses, we chose not to report comparisons with the 'other' category because institutionalized individuals may have a distinct profile different from renters and owners that would otherwise complicate interpretation. Taken together, we felt that income and housing status capture information about current economic experience and accumulated wealth over the life course.

Behavioral characteristics include variables that assess smoking habits, alcohol consumption, and physical activity in early old age. Smoking was assessed through a battery of items evaluating type of consumption (cigarettes, cigars, pipe smoking), intensity (amount per day), and duration (in years). In analyses, smoking was categorized into three groups: current smoker, ex-smoker, and never smoker. Current smokers were those who reported smoking any product daily or occasionally. Ex-smokers were those who reported having smoked in the past either daily or occasionally and who were not currently smoking. Never smokers are those who were not currently smoking and had not smoked in the past. Smoking information was also available longitudinally from ages 70 – 80. This allowed us to investigate changes in smoking behavior in relation to allostatic load in exploratory analyses.

Alcohol consumption was captured with a questionnaire item that inquired about the number of beverages and intensity (daily intake). In analyses, physical activity was measured as a six-category variable that assessed information regarding the amount of exercise performed per week, and ranged from "no physical activity" to "rigorous exercise (>4 hours per week)".

2.3.3. Physiological markers at age 80

Physiological markers derived from blood were collected at the Copenhagen County Hospital (CCH) in Glostrup. Similarly, physiological markers from clinical measurements were recorded at CCH. Non-fasting venous blood was collected with the participant in a supine position and employing the least stasis[31,32]. Whole blood samples were analyzed on the same day of collection. Assays for evaluation of lipid levels were performed at CCH. Standard techniques were used to determine levels of total cholesterol, high-density lipoprotein [HDL], and triglycerides. Serum TNF-α and IL-6 were measured in serum and detected by commercially

available enzyme-linked immunosorbent assay (ELISA) kits (catalogue numbers HSTA50 and HS600, R&D systems, Minneapolis MN)[28]. Detection thresholds for assays were 0.1-0.2 pg/ml. The coefficient for intra-assay and inter-assay variability was 15.7% and 25.0% for TNF-α and 8.7% and 15.1% for IL-6, respectively.

Hospital staff assessed height and weight during the medical evaluation visit. Measurements were made with participants wearing light clothing and no shoes. Height was calculated to the nearest 0.5 cm and weight to the nearest 100 g. Body mass index (BMI) was computed as weight in kg/height in m^2. Resting heart rate, systolic and diastolic blood pressures were measured in a supine position.

2.3.4. Missing data patterns and loss to follow-up

At baseline (age 75), participants (n=748) were largely women (55%), renters (60%), with high income (69%; **table 2.2**). Approximately 45% of all data regarding behavioral characteristics (i.e., smoking, alcohol consumption, and physical activity) were missing. While participants with and without missing behavioral data did not differ with respect to income and last occupation, participants with missing data were more likely to have no vocational training (p-values ranged from 0.02 – 0.04) and were more likely to be institutionalized (p-values ranged from 0.02 – 0.03; **supplemental table 2.1).** A majority of participants with complete behavioral data were current smokers (43%), consumed alcohol daily – 3 times per week (42%), and exercised less than 1 hour per week (46%).

A complete description of loss to follow-up has been previously reported[29]. Briefly, participants from baseline who died between ages 75 – 80 (n=189) were more likely to be men, renters, institutionalized, have lower income, and predominantly sedentary (all p-values ≤0.01).

Men who rented were more likely to die as compared to those who owned (33% renters versus 23% owners, p=0.02) and were more likely not to participate at the follow-up assessment five years later (16% renters versus 10% owners, p=0.05). There were no significant differences in the proportion of men who died or did not participate between levels of income. The proportion of women who died or did not participate was not significantly different between renters and owners. However, a higher proportion of women with low income were significantly more likely to die than those with high income (30% low versus 16% high, p<0.01).

At follow-up (age 80), 362 participants underwent a medical evaluation and agreed to have blood drawn. Of these, 32 participants had missing data on at least one of the ten physiological markers (n=330) that were used to create the allostatic load measure. There were no significant differences in the distribution of social and behavioral characteristics between participants with complete and incomplete data on physiological markers (**table 2.2**).

2.3.5. Statistical Analyses

Exploratory factor analyses

A descriptive summary of the physiological markers is provided on **table 2.3**. Most marker values exhibited a normal distribution. Women had significantly higher average levels of HDL (p<0.0001), systolic blood pressure (p=0.04), and significantly lower levels of blood glucose (p=0.03) than men (**supplemental Table 2.2**). No other significant differences were observed by sex. Physiological markers with skewed distributions, such as TNF-α and IL-6, were log-transformed prior to analyses.

Because physiological markers often tend to be correlated and may contribute to multi-collinearity in multivariable modeling, we performed principal components analysis (PCA). PCA

is a summarization technique that reduces the dimensionality of the data by producing a maximally weighted linear combination of uncorrelated variables called principal components[33]. The principal axis method was used to initially extract the components, followed by a varimax (orthogonal) rotation. To determine the number of principal components to retain, we considered components with eigenvalues ≥ 1.0, and applied the Scree test[34]. To facilitate interpretation of the rotated factor pattern, a physiological marker was said to load on a given component if the factor loading was 0.40 or greater for that component, and less than 0.40 for the others. Factor loadings can be interpreted as correlation coefficients between the marker and each physiological pattern. All PCA analyses were performed using the FACTOR procedure in SAS (version 9.2: SAS institute Inc, Cary NC).

Factor loadings were used to compute factor scores, which can be described as linear combinations or composite measures of uncorrelated and optimally weighted markers for each of the physiological pattern derived. To obtain factor scores, physiological markers for HDL and albumin were reverse coded such that higher levels depicted higher risk. Each participant received a factor score for every physiological pattern derived from PCA. In preliminary analyses, we evaluated associations between factor scores with social and behavioral characteristics.

Derivation of allostatic load

Operationalization of allostatic load followed the original summary score formulation of Seeman et al.[35]. Physiological markers whose levels reached a certain high-risk threshold were assigned a score of one (1), and those values in other percentiles were assigned a score of zero (0). High-risk cut-points were defined at the upper quartile for physiological markers: BMI, ratio

of total cholesterol/HDL, blood glucose, systolic and diastolic blood pressure, IL-6, and TNF-α.

High-risk cut-points were defined at the lower quartile for HDL and albumin. Allostatic load

scores were computed by summing the number of physiological markers that cross the high-risk

threshold.

Evaluation of predictors of allostatic load

Analysis of variance was used to examine whether there were differences in allostatic

load scores across categories of social and behavioral characteristics. All independent variables

were categorical according to the original scale in the questionnaire. The distribution of allostatic

load scores was skewed (**figure 2.3**), so a simple dichotomization would have lost much of the

information about the wide range of values, which in effect would have introduced measurement

error[36]. Summary measures of allostatic load that preserve the continuous properties of the

construct have been shown to yield stronger associations with a broader range of health

outcomes at older age[37]. Therefore, to capture more of the dispersion of scores, allostatic load

was categorized into quintiles of equal sample sizes within each level, on the basis of its

distribution in the overall study population. To evaluate the associations of allostatic load with

social and behavioral characteristics, we used cumulative logistic models to estimate cumulative

odds ratios (ORs) for having a higher level (quintile) of allostatic load versus having a lower

level in relation to the independent variables (i.e., social and behavioral characteristics).

Multivariable models were constructed with serial adjustment of social and behavioral

characteristics in order to understand the patterns of potential confounding. Score test results

indicated that the assumption of proportional odds was not violated for any of the variables

examined.

2.4. Results

2.4.1. Associations between allostatic load and measures of social position

The distribution of allostatic load scores is depicted in **figure 2.3.** In the overall

population, allostatic load scores ranged from 0 to 9, with a mean of 2.45 ± 1.82 (standard

deviation). When we evaluated mean differences in allostatic load scores by sex, we found that

men had higher average scores than women (2.53 versus 2.28, *P*-value for t-test=0.20). In men,

average allostatic load scores were highest among those with no vocational training (mean= 3.34,

table 2.4), lower income (mean=3.29), unskilled occupation (mean=3.37), and renters

(mean=3.08**)**. Compared to participants with any vocational training, male participants with no

training were significantly more likely to have a higher allostatic load (crude OR=2.08, 95% CI:

1.04 – 4.14; **table 2.5**). In analyses with 3 levels of occupation (i.e., managerial, skilled/other,

and unskilled), having an unskilled occupation was significantly associated with higher allostatic

load as compared to managerial occupations (crude OR=2.63, 95% CI: 1.16 – 5.97), and

exhibited a significant trend (*P* for trend=0.03 for increased level of skill).

Similar non-significant patterns of mean allostatic load scores were observed among

women participants, with few exceptions; participants with "other" occupations (mostly

housewives) and those who owned homes had higher mean allostatic load scores than their

counterparts, albeit not significantly **(table 2.4)**. The mean allostatic load scores for women

participants with unskilled occupations were fairly similar to those who reported "other", and

were therefore combined in subsequent analyses. Women who had unskilled/other occupations

had higher odds of allostatic load than those who were in managerial occupations (**table 2.6,

crude model**). After adjustment for income, housing status, smoking and alcohol consumption,

the association had diminished completely (**model 3**). In contrast, crude models showed no

relationship between income and allostatic load. After serial adjustment for vocational training, occupation, housing, smoking, alcohol, and physical activity, women with low income had non-significant higher odds of allostatic load than those with higher income.

2.4.2. Associations between allostatic load and behavioral characteristics

With regard to smoking behavior, a higher proportion of women (38.6%) than men (14.1%) reported never smoking. Mean allostatic load scores were highest among never smokers in both men and women, albeit not significantly. In subgroup analyses, we investigated associations between changes in smoking behavior (ages 70 – 80) and allostatic load. We found that current smokers at age 75 who had quit smoking by age 80 had significantly lower allostatic load scores than those who continued as current smokers, adjusting for sex and physical activity (OR=0.35, 0.12 – 0.98; **supplemental table 2.3**). Similar protective effects were found among those who were current smokers at age 70 and 75, and had quit by age 80 as compared to current smokers throughout the 10-year period.

Mean allostatic load scores generally followed a U-shaped relationship with alcohol consumption; this relationship was most apparent in women. For both men and women, mean allostatic load scores increased with 3x per week - daily alcohol consumption. Women who consumed one drink per week were significantly less likely to have a higher allostatic load than rare/never drinkers (OR=0.29, 0.09 – 0.97; **table 2.6**). Although the data are not shown, women who were current smokers and frequent drinkers (daily-once per week) exhibited significantly lower mean allostatic load scores than those who never or rarely drank (1.56 versus 3.07, respectively; *P* for t-test= 0.01). These differences in allostatic load scores by alcohol consumption were not found with ex/never women smokers or in men.

In the overall population, mean allostatic load scores were higher among individuals with lower levels of physical activity. This inverse association was observed in men (2.96 versus 2.14 comparing light exercise/mostly sedentary to moderate/rigorous exercise, respectively; *P* for t-test= 0.04), but not among females. In 194 participants with complete data on physical activity, we evaluated whether the association between physical activity and allostatic load differed by sex. **Supplementary table 2.4** shows that men with moderate/rigorous levels of physical activity exhibit lower allostatic load scores than women with mostly sedentary activity after adjustment for measures of social position, smoking status, and alcohol consumption (OR=0.61, 0.29 – 1.31).

Serial adjustments in multivariable models restricted to men showed an attenuation of the association between vocational training and allostatic load (**table 2.5**; **crude model**), which was most apparent after adjustment for last occupation (**model 1**). Associations between other measures of material wealth (i.e., last occupation, income and housing status) and allostatic load were not appreciably changed after adjustments and remained elevated across models. We found that protective effects of never smoking only strengthened with additional adjustments, reaching statistical significance with full adjustment (OR= 0.24, 0.07 – 0.82; **model 4**). Similarly, associations between lower levels of physical activity and allostatic load also strengthened somewhat and remained significant even after fully adjusting for material wealth, vocational training, last occupation, smoking and alcohol consumption (OR= 2.71, 1.21 – 6.05; **model 4**).

In women, serial adjustments strengthened the relationship between low income and high allostatic load, although the estimates did not reach statistical significance (**table 2.6**; **crude model – model 4**). The positive association between unskilled/other occupation and higher levels of allostatic load strengthened after adjustment for vocational training (**model 1**), but

precipitately attenuated after adjustment for smoking and alcohol consumption (**model 3**). The U-shaped relationship between alcohol consumption and allostatic load persisted with serial adjustments.

2.4.3. Exploratory Factor Analysis

Principal component analysis with the ten physiological markers revealed four factors with eigenvalues greater than one. Evidence from the scree plot suggested that the first four factors were meaningful, and these were subsequently retained for orthogonal rotation (**figure 2.4**). Combined, these factors accounted for approximately 63% of the total variance.

Factor loadings for the four retained factors are presented in **table 2.7**. Physiological markers BMI, HDL, and the ratio of total cholesterol/HDL were found to load strongly onto the first factor. These markers are related to metabolic lipid profiles and were subsequently labeled "lipid pattern". Markers that loaded heavily onto the second factor, resting systolic and diastolic blood pressure, were characterized as the "blood pressure pattern". Inflammatory markers TNF-alpha, IL-6, and albumin loaded strongly onto the third factor and were described as the "inflammation pattern". Lastly, heart rate and non-fasting glucose loaded strongly onto the fourth factor and are related with glucose metabolism.

Table 2.8 shows the Spearman correlations between each physiological marker and the retained factor scores. The lipid pattern is negatively correlated with HDL but positively correlated with total cholesterol/HDL. The lipid pattern correlation with BMI was positive but less pronounced. The blood pressure pattern showed strong positive correlations with systolic and diastolic blood pressures. The inflammation pattern showed strong positive correlations with

IL6 and TNF-α and a negative correlation with albumin. The glucose metabolism pattern exhibits strong positive correlations with blood glucose and pulse rate.

Associations between social/behavioral characteristics and components of allostatic load

To evaluate the relationship between social and behavioral characteristics and factor scores, we categorized each score into quintiles and estimated cumulative odds ratios. In men, measures of social disadvantage (no vocational training, lower skilled [and unskilled] occupation, low income, and renting) were generally positively associated with lipid, blood pressure, inflammation, and glucose metabolism patterns (**supplemental table 2.5**). There were similar patterns of associations between factor scores and most measures of social position in women, although the effects were much less pronounced (**supplemental table 2.6**).

Secondary analyses examining associations between smoking with components of allostatic load showed that levels of IL-6 were significantly higher in ever smokers as compared to non-smokers (p-value for t-test=0.01), although no differences in levels of the other two biomarkers of inflammation (TNF-α and albumin) were found. Inverse associations between smoking and factor scores were evident in both sexes, with the exception of the lipid pattern in men. In most instances, these relationships were weak and non-significant. With the exception of the blood pressure pattern, associations were observed between alcohol consumption and factor scores in men. In women, increased consumption of alcohol was associated with decreased lipid and inflammation patterns (lipid pattern *P* for trend=0.08; inflammation pattern *P* for trend=0.004). In contrast, alcohol consumption was positively associated with blood pressure patterns in both men and women. Associations between physical activity (moderate/rigorous exercise vs. light exercise and mostly sedentary) and factor scores were consistently inversely

associated in men, with the strongest association observed with glucose metabolism pattern (OR=4.01 comparing sedentary to moderate/rigorous exercise, 95% CI: 1.11 – 14.5). In women, only lipid and inflammation patterns were elevated with lower levels of physical activity.

2.5. Discussion

Over the past few decades, a small but emerging body of evidence from cross-sectional and longitudinal studies[17,19,21,23,38,39] have shown that socioeconomic disadvantage, captured by low levels of education, income, and occupational status across the life course, are associated with greater accumulation of allostatic load. To our knowledge, ours is the first birth cohort to comprehensively examine these relationships in an older adult population. Our results confirm previous studies that report socioeconomic gradients in allostatic load at older age and provide further evidence to support the "weathering"[40] of physiological systems as a consequence of life long chronic adversity and early old age risky behavioral factors.

Overall, our results showed a higher level of allostatic load among men than women, consistent with findings from previous cohorts of older populations[16,41]. Using NHANES data (1988 – 2006), Yang and Kozloski found that men exhibited higher mean levels of metabolic syndrome but lower levels of inflammatory markers than women[13]. These differences were greatest at younger ages, but leveled off with increasing age. Indeed, rates of accumulation of allostatic load appear to be more rapid in older women, helping to close the gap in the overall male disadvantage. Some evidence suggests that estrogens can down-regulate pro-inflammatory cytokines in women and suppress inflammation[42-44], but the advantage is lost as women reach postmenopausal age. However, estrogen can exert both pro- and anti-inflammatory effects on the HPA axis, highlighting the complicated nature of these biological interactions[45].

Social Position

Our data show that measures of cumulative/current wealth and education are determinants of allostatic load at older age, and the stronger and more consistently observed associations are evident in men. Additionally, in the present cohort, we previously reported strong significant associations between lower levels of material wealth and multiple health outcomes at older age including risk of having two or more chronic diseases, decline in functional ability, and lower numbers of natural teeth present[46]. These findings support the hypothesis that lower levels of wealth lead to faster accumulation of physiological dysregulation, and further contribute to emerging evidence that suggests allostatic load may be a pathway through which socioeconomic adversity affects health at older age. Kahn and Pearlin found that persistant financial strain over the life course rather than episodic occurrences has more detrimental influence on health at older age[47]. More recently, Gruenewald et al reported a dose-response relationship between socioeconomic disadvantage, measured at three separate time periods (one at childhood and two in adulthood) and allostatic load in midlife in the US (MIDUS) cohort. Seemen et al similarly reported dose-response relationships in the MacArthur Study of Successful Aging (MAC) cohort of older adults[39]. The Northern Swedish study of young adults aged 16 – 43 similarly reported associations between cumulative levels socioeconomic disadvantage in early adulthood and allostatic load in midlife (age 43)[16]. Taken together, these findings suggest that socioeconomic-related gradients in allostatic load persist throughout the life course.

Of the various measures of social position evaluated, we found that occupation was the strongest determinant of allostatic load in our study. Having an unskilled occupation was

positively associated with allostatic load, independent of income, vocational training, and risky behaviors. Earlier studies that explored stress-related pathways associated with occupation focused primarily on HPA axis dysregulation and found that work stress influenced neuroendocrine reactivity or recovery[48,49]. As the focus shifted to multisystem dysregulation, a growing body of evidence has emerged demonstrating positive associations between high workplace stress, work demands, and burnout symptoms with allostatic load[50-53]. Findings from the Northern Swedish study showed that having lower skilled jobs was significantly associated with higher allostatic load scores at adulthood even after controlling for cumulative socioeconomic position[54]. Taken together, these data strongly suggest that work-related stress has a strong influence on allostatic load accumulation over the life course.

Behavioral characteristics

In the overall population, we found that smoking in early old age was not a significant predictor of allostatic load. In men, current smoking appeared to exhibit a protective effect in fully adjusted models. The cross-sectional study of older Chicagoan adults (CHASRS) similarly showed that smoking was not significantly associated with allostatic load after controlling for gender and race[38]. Healthy survivor effects could in part explain our finding given that a greater proportion of smokers than non-smokers had died between ages 75 – 80. Smokers who reached the age of 80 might be less likely to exhibit physiological dysregulation, since the detrimental effects of smoking would have manifested earlier in life and susceptible smokers would have died at earlier ages. Therefore, the remaining cohort may disproportionately represent smokers with a resilient phenotype. Alternatively, smoking may represent self-medication for stress, as smokers report that cigarettes mitigate aversive affective states[55]. Nonetheless, the finding that

IL-6 is significantly higher in ever versus never smokers is consistent with several studies of older adult populations that report a sustained elevation in levels of IL-6 among those who actively smoke as compared to non-smokers[56,57].

In exploratory analyses, we found that current smokers at early old age (i.e., ages 70 and 75) who had quit by age 80 were more likely to exhibit lower levels of allostatic load than those who continued smoking. This suggests that smoking cessation at old age could potentially reduce allostatic load accumulation. It is also possible that sicker persons may have quit smoking from ages 75 – 80. In a longitudinal study of older adult Chinese men, smoking cessation led to significant reductions in levels of inflammation[58]. In a recent systematic review, Gellert et. al. demonstrated that smoking cessation imparts an overall survival benefit even at older age[59]. More research is needed to replicate our findings in other populations to more comprehensively elucidate the role smoking cessation on physiological dysregulation.

Our results showed that alcohol consumption had a U-shaped relationship with allostatic load, particularly in women. Although this is a novel finding, similarly shaped associations have been reported between levels of alcohol intake and selected cardiovascular outcomes and all-cause mortality[60,61]. These studies show that moderate levels of alcohol consumption, defined as one or two drinks per day, exhibit protective effects on the cardiovascular system. There is evidence that moderate amounts of alcohol exhibit positive influences on lipoprotein activity and reduces inflammation, although the mechanisms are not fully understood[62]. Consistent with this literature, our study found that increased alcohol consumption was associated with lower lipid and inflammation patterns. A greater and more detailed understanding of these effects is needed with studies that investigate different types of alcoholic beverages consumed in relation to allostatic load at older ages.

We found that physical inactivity is a strong predictor of allostatic load, independent of social position, smoking and alcohol consumption. The CHASRS study reported similar inverse associations between levels of physical activity and allostatic load. Results from the Northern Swedish study similarly found a strong inverse association after controlling for cumulative socioeconomic status, smoking and alcohol consumption[16]. Taken together, these data support a growing body of evidence that suggest a protective role of physical activity on stress system dysregulation over the life course[63]. Evidence from these studies has shown that exercise has positive influences on depression[64], anxiety[65], and lead to improvements in cognitive functioning[66]. Several potential mechanisms have been proposed to explain how exercise could exhibit protective effects, the most common of which involves a decrease in HPA axis and sympathetic nervous system activity with increased exercise, resulting in reductions in levels of glucorticocoids and catecholamines[63,67]. Another proposed pathway suggests that exercise promotes neurogenesis in the hippocampus area of the brain through up-regulation of brain-derived neurotrophic factor, which may subsequently decrease stress reactivity and allostatic load[68].

The strong inverse relationship between physical activity and allostatic load was most apparent in men than in women. These sex differences were also reported in the CHASRS study after adjustment of cumulative socioeconomic status. When we examined associations with components of allostatic load in women, we found that lower levels of physical activity were associated with higher lipid and inflammation patterns, consistent with the notion that stress promotes central adiposity and leads to increased secretion of proinflammatory cytokines, such as TNF-α and IL-6[69]. Our results suggest that physical activity in older age is critical for reduction of inflammation related to allostatic load in both men and women. Although men were

significantly more active than women during early middle age (ages 50 – 60), these differences diminished at older ages[70]. We previously reported that sustained physical activity in our cohort was associated with a reduction in all-cause mortality[71], myocardial infarction[70], hip fractures[72], and functional decline[73], and our present study suggest an allostatic load pathway to explain these relationships.

We should note that our study had several limitations. First, our study did not measure neuroendocrine markers such as dopamine, epinephrine, norepinephrine, cortisol and dehydroepiandrosterone (DHEA-S), which have been previously used in studies of allostatic load and are known to act as primary mediators of the stress response[7]. Not including these markers in general and makes comparisons across studies difficult. However, some have argued that baseline levels of neuroendocrine biomarkers in older adults may be an inaccurate representation of neuroendocrine dysregulation because they can greatly fluctuate and not truly reflect long-term biological processes over the life course[74,75]. Secondly, we have measured allostatic load cross-sectionally and this precludes any definitive statement regarding causal association. However, most of our measures of social position were obtained retrospectively thus preserving a longitudinal component. Third, our small sample sizes limited our power to find statistically significant associations when stratified by sex. Lastly, we did not measure other social or behavioral determinants of allostatic load that have been previously linked with high levels of allostatic load, such as poor dietary habits[76], weak social relationships[77], and other aspects of the social environment.

Our study had several strengths. Inclusion of inflammation markers to our allostatic load construct strengthened our findings because inflammatory burden has been previously associated with socioeconomic adversity at multiple stages of life[24,25]. Secondly, our study is a birth cohort,

which minimizes the influence of any potential cohort and age effects. Lastly, our study population is homogenous, and this allows us better control over factors that may confound relationships between social and behavioral characteristics and allostatic load.

2.6. Conclusions

We found that aspects of socioeconomic position over the life course and selected modifiable behaviors in early old age were determinants of allostatic load at older age. These findings open avenues of intervention at different points in time throughout the life course and with the overarching goal of reducing the burden of allostasis and promoting successful aging. Future research should consider moderating factors that may interact with social and behavioral characteristics to accelerate allostatic load accumulation even further. We also need a better understanding of pathways that link socioeconomic adversity with disease through maladaptive behaviors across the lifespan.

2.7. References

1. Masoro EJ, American Physiological Society (1887-). Aging. New York: Published for the American Physiological Society by Oxford University Press; 1995.

2. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev 2007;87:873-904.

3. Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev 1984;5:25-44.

4. Sterling P, Eyer J. Handbook of life stress, cognition, and health. New York: John Wiley and Sons; 1988.

5. Schulkin J. Allostasis, homeostasis and the costs of physiological adaptation. New York: Cambridge University Press; 2004.

6. Sterling P. Allostasis: A model of predictive regulation. Physiol Behav 2011.

7. McEwen BS. Protective and damaging effects of stress mediators. N Engl J Med 1998;338:171-9.

8. Juster RP, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci Biobehav Rev 2010;35:2-16.

9. Goldman N, Turra CM, Glei DA, Lin YH, Weinstein M. Physiological dysregulation and changes in health in an older population. Exp Gerontol 2006;41:862-70.

10. Crimmins EM, Johnston M, Hayward M, Seeman T. Age differences in allostatic load: an index of physiological dysregulation. Exp Gerontol 2003;38:731-4.

11. Kuh D, Ben-Shlomo Y, Lynch J, Hallqvist J, Power C. Life course epidemiology. J Epidemiol Community Health 2003;57:778-83.

12. Ben-Shlomo Y, Kuh D. A life course approach to chronic disease epidemiology: conceptual models, empirical challenges and interdisciplinary perspectives. Int J Epidemiol 2002;31:285-93.

13. Yang Y, Kozloski M. Sex differences in age trajectories of physiological dysregulation: inflammation, metabolic syndrome, and allostatic load. J Gerontol A Biol Sci Med Sci 2011;66:493-500.

14. Dowd JB, Simanek AM, Aiello AE. Socio-economic status, cortisol and allostatic load: a review of the literature. Int J Epidemiol 2009;38:1297-309.

15. Seeman T, Epel E, Gruenewald T, Karlamangla A, McEwen BS. Socio-economic differentials in peripheral biology: cumulative allostatic load. Ann N Y Acad Sci 2010;1186:223-39.

16. Gustafsson PE, Janlert U, Theorell T, Westerlund H, Hammarstrom A. Socioeconomic status over the life course and allostatic load in adulthood: results from the Northern Swedish Cohort. J Epidemiol Community Health 2011;65:986-92.

17. Gruenewald TL, Karlamangla AS, Hu P, et al. History of socioeconomic disadvantage and allostatic load in later life. Soc Sci Med 2012;74:75-83.

18. Kubzansky LD, Kawachi I, Sparrow D. Socioeconomic status, hostility, and risk factor clustering in the Normative Aging Study: any help from the concept of allostatic load? Ann Behav Med 1999;21:330-8.

19. Hu P, Wagle N, Goldman N, Weinstein M, Seeman TE. The associations between socioeconomic status, allostatic load and measures of health in older Taiwanese persons: Taiwan social environment and biomarkers of aging study. J Biosoc Sci 2007;39:545-56.

20. Bird CE, Seeman T, Escarce JJ, et al. Neighbourhood socioeconomic status and biological 'wear and tear' in a nationally representative sample of US adults. J Epidemiol Community Health 2010;64:860-5.

21. Seeman T, Merkin SS, Crimmins E, Koretz B, Charette S, Karlamangla A. Education, income and ethnic differences in cumulative biological risk profiles in a national sample of US adults: NHANES III (1988-1994). Soc Sci Med 2008;66:72-87.

22. Seeman TE, Singer BH, Rowe JW, Horwitz RI, McEwen BS. Price of adaptation--allostatic load and its health consequences. MacArthur studies of successful aging. Arch Intern Med 1997;157:2259-68.

23. Singer B, Ryff CD. Hierarchies of life histories and associated health risks. Ann N Y Acad Sci 1999;896:96-115.

24. Loucks EB, Pilote L, Lynch JW, et al. Life course socioeconomic position is associated with inflammatory markers: the Framingham Offspring Study. Soc Sci Med 2010;71:187-95.

25. Pollitt RA, Kaufman JS, Rose KM, Diez-Roux AV, Zeng D, Heiss G. Cumulative life course and adult socioeconomic status and markers of inflammation in adulthood. J Epidemiol Community Health 2008;62:484-91.

26. Pollitt RA, Kaufman JS, Rose KM, Diez-Roux AV, Zeng D, Heiss G. Early-life and adult socioeconomic status and inflammatory risk markers in adulthood. Eur J Epidemiol 2007;22:55-66.

27. Adler NE, Boyce T, Chesney MA, et al. Socioeconomic status and health. The challenge of the gradient. Am Psychol 1994;49:15-24.

28. Bruunsgaard H, Ladelund S, Pedersen AN, Schroll M, Jorgensen T, Pedersen BK. Predicting death from tumour necrosis factor-alpha and interleukin-6 in 80-year-old people. Clin Exp Immunol 2003;132:24-31.

29. Avlund K. Disability in old age. Longitudinal population-based studies of the disablement process. Dan Med Bull 2004;51:315-49.

30. Rose GA, Blackburn H. Cardiovascular survey methods. Monogr Ser World Health Organ 1968;56:1-188.

31. Schroll M. A ten-year prospective study, 1964-1974, of cardiovascular risk factors in men and women from the Glostrup population born in 1914. Dan Med Bull 1982;29:213-52.

32. Schroll M, Jorgensen T, Ingerslev J. The Glostrup Population Studies, 1964-1992. Dan Med Bull 1992;39:204-7.

33. Joliffe IT, Morgan BJ. Principal component analysis and exploratory factor analysis. Stat Methods Med Res 1992;1:69-95.

34. Hatcher L. A step-by-step approach to using the SAS system for factor analysis and structural equation modeling. Cary, NC: SAS Institute; 1994.

35. Seeman TE, Singer BH, Rowe JW, Horwitz RI, McEwen BS. Price of adaptation--allostatic load and its health consequences. MacArthur studies of successful aging. Arch Intern Med 1997;157:2259-68.

36. Lagakos SW. Effects of mismodelling and mismeasuring explanatory variables on tests of their association with a response variable. Stat Med 1988;7:257-74.

37. Seplaki CL, Goldman N, Glei D, Weinstein M. A comparative analysis of measurement approaches for physiological dysregulation in an older population. Exp Gerontol 2005;40:438-49.

38. Hawkley LC, Lavelle LA, Berntson GG, Cacioppo JT. Mediators of the relationship between socioeconomic status and allostatic load in the Chicago Health, Aging, and Social Relations Study (CHASRS). Psychophysiology 2011;48:1134-45.

39. Seeman TE, Crimmins E, Huang MH, et al. Cumulative biological risk and socio-economic differences in mortality: MacArthur studies of successful aging. Soc Sci Med 2004;58:1985-97.

40. Geronimus AT, Hicken M, Keene D, Bound J. "Weathering" and age patterns of allostatic load scores among blacks and whites in the United States. Am J Public Health 2006;96:826-33.

41. Seeman TE, Singer BH, Ryff CD, Dienberg Love G, Levy-Storms L. Social relationships, gender, and allostatic load across two age cohorts. Psychosom Med 2002;64:395-406.

42. Novella S, Heras M, Hermenegildo C, Dantas AP. Effects of estrogen on vascular inflammation: a matter of timing. Arterioscler Thromb Vasc Biol 2012;32:2035-42.

43. Sunday L, Tran MM, Krause DN, Duckles SP. Estrogen and progestagens differentially modulate vascular proinflammatory factors. Am J Physiol Endocrinol Metab 2006;291:E261-7.

44. Stork S, von Schacky C, Angerer P. The effect of 17beta-estradiol on endothelial and inflammatory markers in postmenopausal women: a randomized, controlled trial. Atherosclerosis 2002;165:301-7.

45. Straub RH. The complex role of estrogens in inflammation. Endocr Rev 2007;28:521-74.

46. Avlund K, Holstein BE, Osler M, Damsgaard MT, Holm-Pedersen P, Rasmussen NK. Social position and health in old age: the relevance of different indicators of social position. Scand J Public Health 2003;31:126-36.

47. Kahn JR, Pearlin LI. Financial strain over the life course and health among older adults. J Health Soc Behav 2006;47:17-31.

48. Sluiter JK, Frings-Dresen MH, van der Beek AJ, Meijman TF, Heisterkamp SH. Neuroendocrine reactivity and recovery from work with different physical and mental demands. Scand J Work Environ Health 2000;26:306-16.

49. Schlotz W, Hellhammer J, Schulz P, Stone AA. Perceived work overload and chronic worrying predict weekend-weekday differences in the cortisol awakening response. Psychosom Med 2004;66:207-14.

50. Juster RP, Sindi S, Marin MF, et al. A clinical allostatic load index is associated with burnout symptoms and hypocortisolemic profiles in healthy workers. Psychoneuroendocrinology 2011;36:797-805.

51. Schnorpfeil P, Noll A, Schulze R, Ehlert U, Frey K, Fischer JE. Allostatic load and work conditions. Soc Sci Med 2003;57:647-56.

52. Sun J, Wang S, Zhang JQ, Li W. Assessing the cumulative effects of stress: the association between job stress and allostatic load in a large sample of Chinese employees. Work & Stress 2007;21:333-47.

53. Bellingrath S, Weigl T, Kudielka BM. Chronic work stress and exhaustion is associated with higher allostastic load in female school teachers. Stress 2009;12:37-48.

54. Westerlund H, Gustafsson PE, Theorell T, Janlert U, Hammarstrom A. Social adversity in adolescence increases the physiological vulnerability to job strain in adulthood: a prospective population-based study. PLoS One 2012;7:e35967.

55. Khantzian EJ. The self-medication hypothesis of substance use disorders: a reconsideration and recent applications. Harv Rev Psychiatry 1997;4:231-44.

56. Helmersson J, Larsson A, Vessby B, Basu S. Active smoking and a history of smoking are associated with enhanced prostaglandin F(2alpha), interleukin-6 and F2-isoprostane formation in elderly men. Atherosclerosis 2005;181:201-7.

57. Bermudez EA, Rifai N, Buring JE, Manson JE, Ridker PM. Relation between markers of systemic vascular inflammation and smoking in women. Am J Cardiol 2002;89:1117-9.

58. Lao XQ, Jiang CQ, Zhang WS, et al. Smoking, smoking cessation and inflammatory markers in older Chinese men: The Guangzhou Biobank Cohort Study. Atherosclerosis 2009;203:304-10.

59. Gellert C, Schottker B, Brenner H. Smoking and All-Cause Mortality in Older People: Systematic Review and Meta-analysisSmoking and All-Cause Mortality in Older People. Archives of internal medicine 2012;172:837-44.

60. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali WA. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. Bmj 2011;342:d671.

61. Corrao G, Rubbiati L, Bagnardi V, Zambon A, Poikolainen K. Alcohol and coronary heart disease: a meta-analysis. Addiction 2000;95:1505-23.

62. Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies. Bmj 2011;342:d636.

63. Tsatsoulis A, Fountoulakis S. The protective role of exercise on stress system dysregulation and comorbidities. Ann N Y Acad Sci 2006;1083:196-213.

64. Lawlor DA, Hopker SW. The effectiveness of exercise as an intervention in the management of depression: systematic review and meta-regression analysis of randomised controlled trials. Bmj 2001;322:763-7.

65. Petruzzello SJ, Landers DM, Hatfield BD, Kubitz KA, Salazar W. A meta-analysis on the anxiety-reducing effects of acute and chronic exercise. Outcomes and mechanisms. Sports Med 1991;11:143-82.

66. Chang YK, Labban JD, Gapin JI, Etnier JL. The effects of acute exercise on cognitive performance: a meta-analysis. Brain Res 2012;1453:87-101.

67. Leal-Cerro A, Gippini A, Amaya MJ, et al. Mechanisms underlying the neuroendocrine response to physical exercise. J Endocrinol Invest 2003;26:879-85.

68. Sylvia LG, Ametrano RM, Nierenberg AA. Exercise treatment for bipolar disorder: potential mechanisms of action mediated through increased neurogenesis and decreased allostatic load. Psychother Psychosom 2010;79:87-96.

69. Coppack SW. Pro-inflammatory cytokines and adipose tissue. Proc Nutr Soc 2001;60:349-56.

70. Schroll M. Physical activity in an ageing population. Scand J Med Sci Sports 2003;13:63-9.

71. Andersen LB, Schnohr P, Schroll M, Hein HO. All-cause mortality associated with physical activity during leisure time, work, sports, and cycling to work. Archives of internal medicine 2000;160:1621-8.

72. Hoidrup S, Sorensen TI, Stroger U, Lauritzen JB, Schroll M, Gronbaek M. Leisure-time physical activity levels and changes in relation to risk of hip fracture in men and women. Am J Epidemiol 2001;154:60-8.

73. Schroll M, Avlund K, Davidsen M. Predictors of five-year functional ability in a longitudinal survey of men and women aged 75 to 80. The 1914-population in Glostrup, Denmark. Aging (Milano) 1997;9:143-52.

74. Gersten O, Dow WH, Rosero-Bixby L. Stressors over the life course and neuroendocrine system dysregulation in Costa Rica. J Aging Health 2010;22:748-71.

75. Gersten O. Neuroendocrine biomarkers, social relations, and the cumulative costs of stress in Taiwan. Soc Sci Med 2008;66:507-19; discussion 20-35.

76. Mattei J, Noel SE, Tucker KL. A meat, processed meat, and French fries dietary pattern is associated with high allostatic load in Puerto Rican older adults. J Am Diet Assoc 2011;111:1498-506.

77. Seeman TE, Lusignolo TM, Albert M, Berkman L. Social relationships, social support, and patterns of cognitive aging in healthy, high-functioning older adults: MacArthur studies of successful aging. Health Psychol 2001;20:243-55.

Table 2.1: Summary list of social and behavioral characteristics at age 75

Social and Behavioral Characteristics	Measure	Categories
Social position	Vocational training	**(1)** no training, **(2)** < 3 years, **(3)** ≥ 3 years
	Occupation	**(1)** unskilled, **(2)** skilled, **(3)** managerial, **(5)** other
	Income	**(1)** low, **(2)** high
	Housing status	**(1)** rent, **(2)** own, **(3)** other (mostly institutionalized)
Modifiable behaviors	Smoking status	**(1)** current (daily or casual), **(2)** ex/former (daily or casual), **(3)** never
	Alcohol consumption	**(1)** daily, **(2)** 2-3 times per week, **(3)** once per week, **(4)** 1-2 times per month **(5)** rarely, **(6)** never
	Physical activity	**(1)** no physical activity, **(2)** mostly sitting, **(3)** light exercise (< 1 hr/wk), **(4)** moderate exercise (1-2 hrs/wk), **(5)** moderate exercise (3-4 hrs/wk), **(6)** rigorous exercise (>4 hrs/wk)

Table 2.2: Distribution of social and behavioral characteristics among those who participated at baseline (age 75), those who died between ages 75 and 80, and those who had complete and incomplete data on physiological markers at age 80

Social and Behavioral Characteristics	Overall cohort (age 75) N (%)	Deaths (ages 75 – 80) N (%)	Deaths (ages 75 – 80) Row Percentage	P-value[a]	Complete (age 80) N (%)	Incomplete (age 80) N (%)	P-value[b]
Overall N	748	189			330	32	
Sex							
Males	337 (45.1%)	104 (55.0%)	30.9%	0.001	160 (48.5%)	12 (37.5%)	0.23
Females	411 (55.0%)	85 (45.0%)	20.7%		170 (51.5%)	20 (62.5%)	
Vocational training							
No training	334 (44.8%)	82 (43.9%)	24.6%	0.90	121 (39.7%)	12 (42.9%)	0.77
<3 years training	111 (14.9%)	27 (14.4%)	24.3%		44 (14.4%)	5 (17.9%)	
≥3 years training	300 (40.3%)	78 (41.7%)	26.0%		140 (45.9%)	11 (39.3%)	
Income							
Low	234 (31.3%)	74 (39.2%)	31.6%	0.01	74 (22.4%)	8 (25.0%)	0.73
High	514 (68.7%)	115 (60.9%)	22.4%		256 (77.6%)	24 (75.0%)	
Last occupation							
Unskilled	272 (36.5%)	72 (38.1%)	26.5%	0.31	90 (29.7%)	10 (35.7%)	0.78
Skilled	195 (26.1%)	54 (28.6%)	27.7%		74 (24.4%)	5 (17.9%)	
Managerial	121 (16.2%)	32 (16.9%)	26.5%		65 (21.5%)	5 (17.9%)	
Other	158 (21.2%)	31 (16.4%)	19.6%		74 (24.4%)	8 (28.6%)	
Housing status							
Renter	389 (52.0%)	101 (53.4%)	26.0%	<0.0001	143 (46.9%)	13 (46.4%)	0.47
Owner	303 (40.5%)	59 (31.2%)	19.5%		147 (48.2%)	15 (53.6%)	
Other	56 (7.5%)	29 (15.3%)	51.8%		15 (4.9%)	0	
Smoking status							
Never smoker	98 (24.0%)	13 (14.1%)	13.3%	0.04	52 (26.9%)	4 (33.3%)	0.31
Ex-smoker	137 (33.5%)	34 (37.0%)	24.8%		74 (38.4%)	2 (16.7%)	
Current smoker	174 (42.5%)	45 (48.9%)	25.9%		67 (34.7%)	6 (50.0%)	
Alcohol consumption							
Rarely/never	134 (33.0%)	30 (33.3%)	22.4%	0.99	59 (30.6%)	5 (41.7%)	0.27

				P-value[a]			P-value[b]
1-2 times per month	48 (11.8%)	10 (11.1%)	20.8%		28 (14.5%)	0	
Once per week	54 (13.3%)	12 (13.3%)	22.2%		23 (11.9%)	3 (25.0%)	
Daily/3x per week	170 (41.9%)	38 (42.2%)	22.4%		83 (43.0%)	4 (33.3%)	
Physical activity							
None/mostly sitting	96 (23.4%)	39 (42.4%)	40.6%	<0.0001	26 (13.4%)	2 (16.7%)	0.90
Light exercise	190 (46.3%)	73 (37.0%)	17.9%		92 (47.4%)	6 (50.0%)	
Moderate/rigorous	124 (30.2%)	92 (20.7%)	15.3%		76 (39.2%)	4 (33.3%)	

[a] P-value compares differences between those who died (ages 75 – 80) with those who survived and eligible for the follow-up assessment at age 80

[b] P-value compares differences between those who had complete data with those who had incomplete data on all 10 physiological markers at age 80.

Table 2.3: Summary statistics and high-risk cut-points for physiological markers, n=330

Domain	Physiological Marker	Range	Median	Mean (SD)	High risk cut-point[a]	Number at high risk
Anthropometric	BMI (kg/m^2)	11.6 – 37.0	24.5	24.9 (3.8)	≥ 27.2	82
Metabolic	Ratio of total cholesterol/HDL	1.9 – 14.6	4.3	4.6 (1.5)	≥ 5.3	82
	HDL cholesterol (mg/dL)	17.8 – 122.6	52.0	55.3 (16.6)	≤ 44.5	82
	Blood glucose (mg/dL)	54.0 – 392.4	90.0	101.9 (39.8)	≥ 106.2	82
Cardiovascular	Resting DBP (mmHg)	59.0 – 120.0	83.0	84.2 (11.4)	≥ 93.0	78
	Resting SBP (mmHg)	103.0 – 224.0	150.0	150.6 (21.2)	≥ 164.0	83
	Resting heart rate (bpm)	48.0 – 124.0	72.0	72.7 (11.8)	≥ 84.0	69
Inflammation	IL-6 (pg/ml)	0.5 – 412.0	3.2	7.5 (26.2)	≥ 5.4	82
	TNF-α (pg/ml)	1.6 – 32.0	4.1	4.4 (2.5)	≥ 5.0	82
	Albumin (g/dL)	2.6 – 5.0	4.1	4.1 (0.3)	≤ 3.9	80

BMI= Body Mass Index, HDL= High Density Lipoprotein, SD= Standard Deviation, DBP= Diastolic blood pressure, SBP= Systolic blood pressure
[a] High risk cut-points are based on the upper or lower 25th percentile of the marker's distribution in the study population.

Table 2.4: Mean allostatic load scores measured at age 80 across levels of population characteristics measured at age 75 and stratified by sex

Social and Behavioral Characteristics	Males, N=160			Females, N=170		
	n	Mean (SE)	P-value[a]	n	Mean (SE)	P-value[a]
Vocational Training						
No training	35	3.34 (0.31)	0.04	86	2.36 (0.20)	0.92
<3 years training	14	2.14 (0.49)		30	2.20 (0.34)	
≥3 years training	95	2.49 (0.19)		45	2.33 (0.28)	
Income						
Low	35	3.29 (0.31)	0.02[b]	39	2.54 (0.30)	0.40[b]
High	125	2.47 (0.17)		131	2.25 (0.17)	
Last Occupation						
Unskilled	35	3.37 (0.30)	0.06	55	2.38 (0.25)	0.83
Skilled	39	2.49 (0.29)		35	2.20 (0.31)	
Managerial	41	2.31 (0.28)		24	2.08 (0.38)	
Other	27	2.44 (0.35)		47	2.47 (0.27)	
Housing Status						
Renter	60	3.08 (0.23)	0.01[b]	83	2.22 (0.20)	0.52[b]
Owner	80	2.30 (0.20)		67	2.42 (0.22)	
Smoking status						
Never smoker	13	2.92 (0.53)	0.64	39	2.82 (0.31)	0.53
Ex-smoker	43	2.63 (0.29)		31	2.65 (0.35)	
Current smoker	36	2.36 (0.32)		31	2.29 (0.35)	
Alcohol consumption						
Rarely/never	15	2.20 (0.47)	0.50	44	2.70 (0.29)	0.40
1-2 times per month	11	1.91 (0.55)		17	2.82 (0.47)	
Once per week	13	2.46 (0.51)		10	1.60 (0.62)	
Daily/3x per week	52	2.73 (0.25)		31	2.65 (0.35)	
Physical activity						
Moderate-rig. exercise	44	2.14 (0.28)	0.11	32	2.75 (0.23)	0.81
Light exercise	38	3.00 (0.30)		54	2.48 (1.95)	
Mostly sedentary	10	2.80 (0.59)		16	2.69 (1.71)	

[a] P for group difference (ANOVA)
[b] P for t-test

Table 2.5: Odds ratios for high allostatic load scores in men, N= 160

Social and Behavioral Characteristics	n	Crude Model ORs (95% CI)	Model 1[a] ORs (95% CI)	Model 2[b] ORs (95% CI)	Model 3[c] ORs (95% CI)	Model 4[d] ORs (95% CI)
Vocational Training						
Training	109	1.00	1.00	1.00	1.00	1.00
No training	35	2.08 (1.04 – 4.14)	1.31 (0.47 – 3.64)	1.24 (0.44 – 3.45)	1.08 (0.37 – 3.09)	0.99 (0.34 – 2.84)
Last occupation						
Managerial	41	1.00	1.00	1.00	1.00	1.00
Skilled/other	66	1.29 (0.65 – 2.58)	1.27 (0.64 – 2.55)	1.20 (0.59 – 2.43)	1.34 (0.65 – 2.74)	1.34 (0.65 – 2.75)
Unskilled	35	2.63 (1.16 – 5.97)	2.18 (0.70 – 6.79)	1.88 (0.59 – 6.03)	2.43 (0.74 – 7.94)	2.50 (0.76 – 8.18)
Income						
High	125	1.00	---	1.00	1.00	1.00
Low	35	1.76 (0.89 – 3.50)		1.35 (0.66 – 2.77)	1.63 (0.77 – 3.46)	1.75 (0.82 – 3.73)
Housing status						
Owner	80	1.00	---	1.00	1.00	1.00
Renter	60	1.92 (0.98– 3.78)		1.81 (0.98 – 3.33)	2.09 (1.11 – 3.94)	1.67 (0.86 – 3.25)
Smoking status						
Never smoker	13	1.00	---	---	1.00	1.00
Ex-smoker	43	0.70 (0.23 – 2.13)			0.42 (0.13 – 1.37)	0.35 (0.11 – 0.16)
Current smoker	36	0.49 (0.16 – 1.53)			0.30 (0.09 – 1.03)	0.24 (0.07 – 0.82)
Alcohol consumption						
Never/rarely	26	1.00	---	---	1.00	1.00
Daily – 1x/ week	65	1.43 (0.64 – 3.21)			1.40 (0.61 – 3.20)	1.34 (0.58 – 3.07)
Physical activity						
Moderate/rigorous	44	1.00	---	---	---	1.00
Light/none	48	2.58 (1.23 – 5.41)				2.71 (1.21 – 6.05)

[a] Model 1 adjusted for vocational training and occupation
[b] Model 2 additionally adjusted for income and housing status
[c] Model 3 additionally adjusted for smoking and alcohol consumption
[d] Model 4 additionally adjusted for physical activity

Table 2.6: Odds ratios[a] for high allostatic load scores in women, N= 170

Social and Behavioral Characteristics	n	Crude Model ORs (95% CI)	Model 1[a] ORs (95% CI)	Model 2[b] ORs (95% CI)	Model 3[c] ORs (95% CI)	Model 4[d] ORs (95% CI)
Vocational Training						
Training	75	1.00	1.00	1.00	1.00	1.00
No training	86	1.06 (0.61 – 1.83)	0.86 (0.40 – 1.89)	0.82 (0.37 – 1.84)	0.93 (0.40 – 2.17)	0.93 (0.40 – 2.16)
Last occupation						
Managerial	24	1.00	1.00	1.00	1.00	1.00
Skilled	35	1.11 (0.44 – 2.77)	1.10 (0.44 – 2.76)	1.12 (0.56 – 3.82)	0.81 (0.35 – 2.38)	0.81 (0.31 – 2.13)
Unskilled/other	102	1.29 (0.59 – 2.83)	1.42 (0.55 – 3.68)	1.46 (0.56 – 2.23)	1.09 (0.42 – 3.01)	1.08 (0.40 – 2.92)
Income						
High	131	1.00	---	1.00	1.00	1.00
Low	39	1.09 (0.57 – 2.05)		1.14 (0.57 – 2.29)	1.26 (0.63 – 2.54)	1.26 (0.63 – 2.55)
Housing Status						
Owner	67	1.00	---	1.00	1.00	1.00
Renter	83	0.86 (0.48 – 1.51)		0.84 (0.47 – 1.50)	0.88 (0.49 – 1.58)	0.88 (0.49 – 1.58)
Smoking						
Never smoker	39	1.00	---	---	1.00	1.00
Ex-smoker	31	0.96 (0.41 – 2.21)			1.07 (0.45 – 2.55)	1.08 (0.45 – 2.56)
Current smoker	31	0.80 (0.35 – 1.84)			0.91 (0.38 – 2.17)	0.90 (0.37 – 2.17)
Alcohol consumption						
Never/rarely	61	1.00	---	---	1.00	1.00
Once per week	10	0.29 (0.09 – 0.97)			0.28 (0.08 – 0.95)	0.28 (0.08 – 0.97)
Daily/3x per week	31	0.72 (0.34 – 1.56)			0.74 (0.33 – 1.65)	0.76 (0.33 – 1.72)
Physical activity						
Moderate/rigorous	32	1.00	---	---	---	1.00
Light/none	70	1.18 (0.56 – 2.48)				1.09 (0.49 – 2.44)

[a] Model 1 adjusted for vocational training and occupation
[b] Model 2 additionally adjusted for income and housing status
[c] Model 3 additionally adjusted for smoking and alcohol consumption
[d] Model 4 additionally adjusted for physical activity

Table 2.7: Rotated factor pattern matrix derived from principal component analysis of physiological markers, n=330

Physiological Markers	Rotated factor loadings[a]			
	Lipid pattern	Blood Pressure pattern	Inflammation pattern	Glucose metabolism pattern
BMI (kg/m2)	0.57	0.08	0.06	0.13
Total cholesterol/HDL	0.88	0.02	-0.09	0.03
HDL cholesterol	-0.86	0.11	-0.16	-0.01
Resting SBP	-0.02	0.88	-0.05	0.04
Resting DBP	0.04	0.89	-0.11	0.12
Heart rate	-0.07	0.19	0.09	0.73
Blood glucose	0.31	-0.04	-0.02	0.72
Albumin	0.07	0.15	-0.69	-0.06
TNF-α	0.34	0.12	0.64	-0.25
IL-6	0.03	-0.07	0.75	0.15
Proportion explained[b] (%)	*21.9*	*18.3*	*13.1*	*10.5*

[a] Factor loadings were obtained using the varimax (orthogonal) rotation and represent correlations between factor scores and each of the physiological markers.

[b] Proportion of the variability that each principal component explains

Table 2.8. Spearman correlations between factor scores and physiological markers at age 80

Physiological Markers	Factor scores			
	Lipid pattern	Cardiovascular pattern	Inflammation pattern	Glucose metabolism pattern
BMI (kg/m^2)	0.27	0.04	-0.01	0.06
Total cholesterol/HDL	0.45	-0.01	-0.14	-0.04
HDL cholesterol (mg/dL)	-0.42	0.06	-0.03	0.05
Resting SBP (mmHg)	-0.01	0.54	0.05	-0.06
Resting DBP (mmHg)	0.02	0.54	0.00	0.00
Heart rate (bpm)	-0.11	0.04	0.07	0.63
Blood glucose (mg/dL)	0.10	-0.11	-0.06	0.62
Albumin (mg/dL)	0.10	0.03	-0.47	-0.06
TNF-α (pg/ml)	0.14	0.16	0.43	-0.28
IL-6 (pg/ml)	-0.06	0.01	0.50	0.12
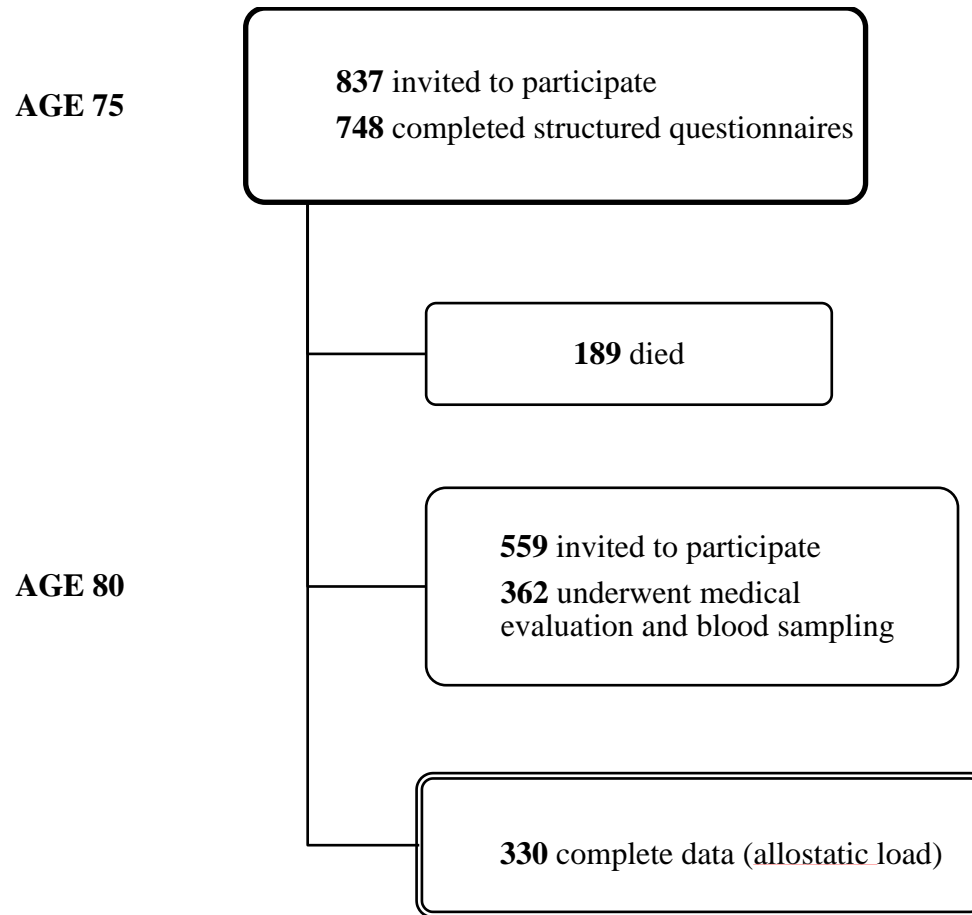
Figure 2.1. Flow chart of study population in the current study
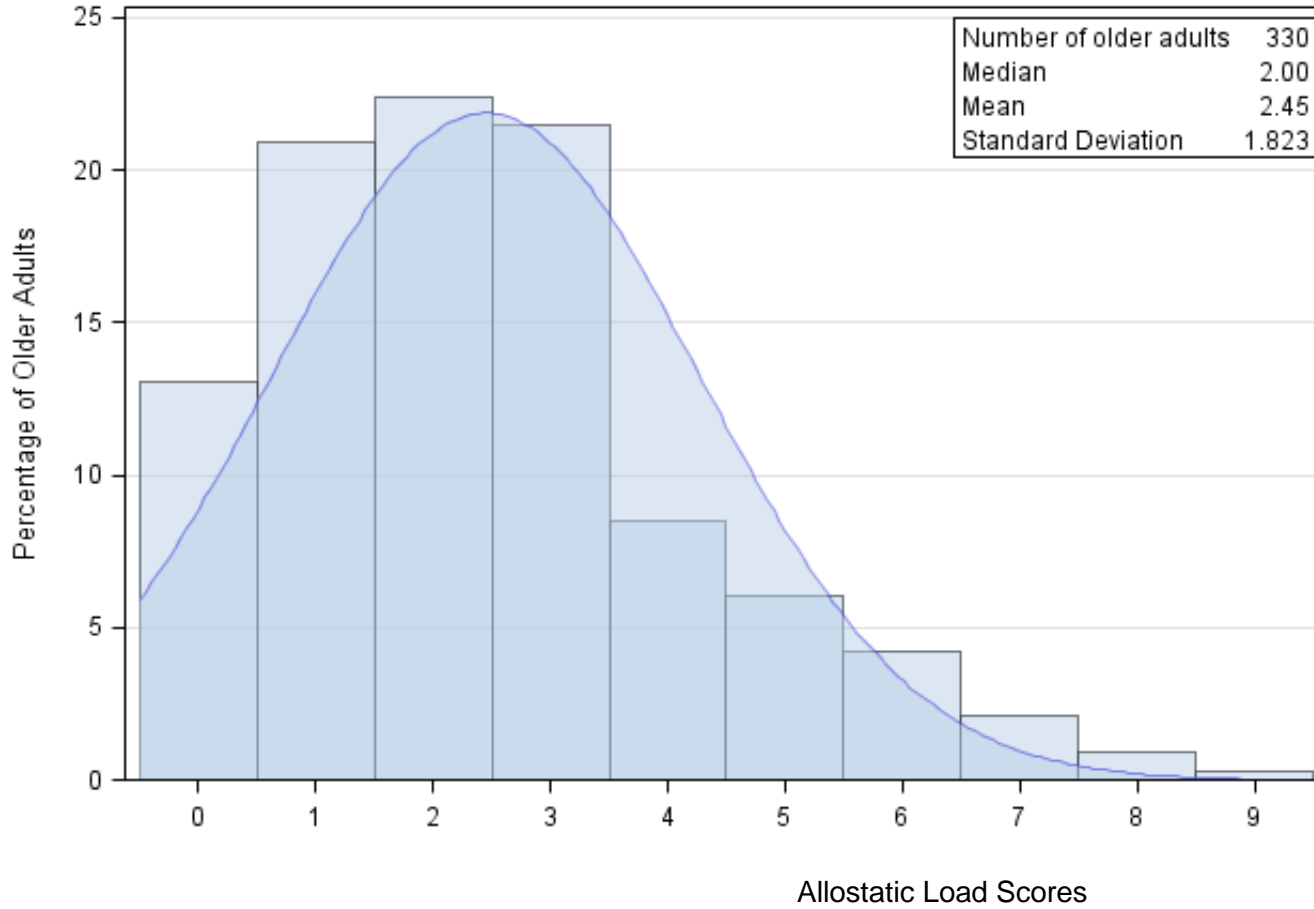
Figure 2.2: Distribution of allostatic load scores in the overall study population. Each participant received a score summing the number of physiological markers that cross the high-risk threshold.

Figure 2.3a.

Scree Plot
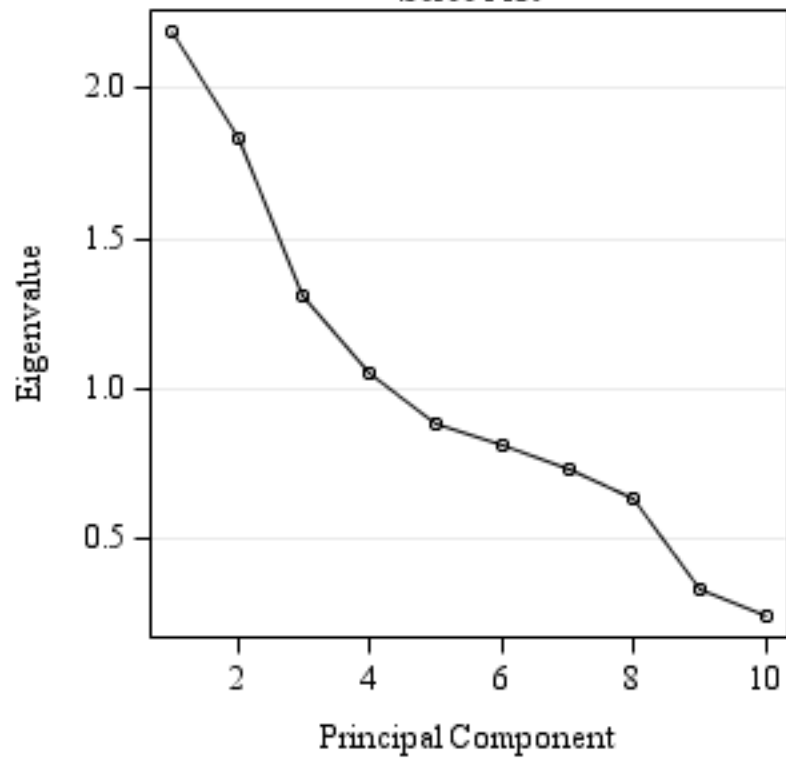
Figure 2.3b.

Variance Explained
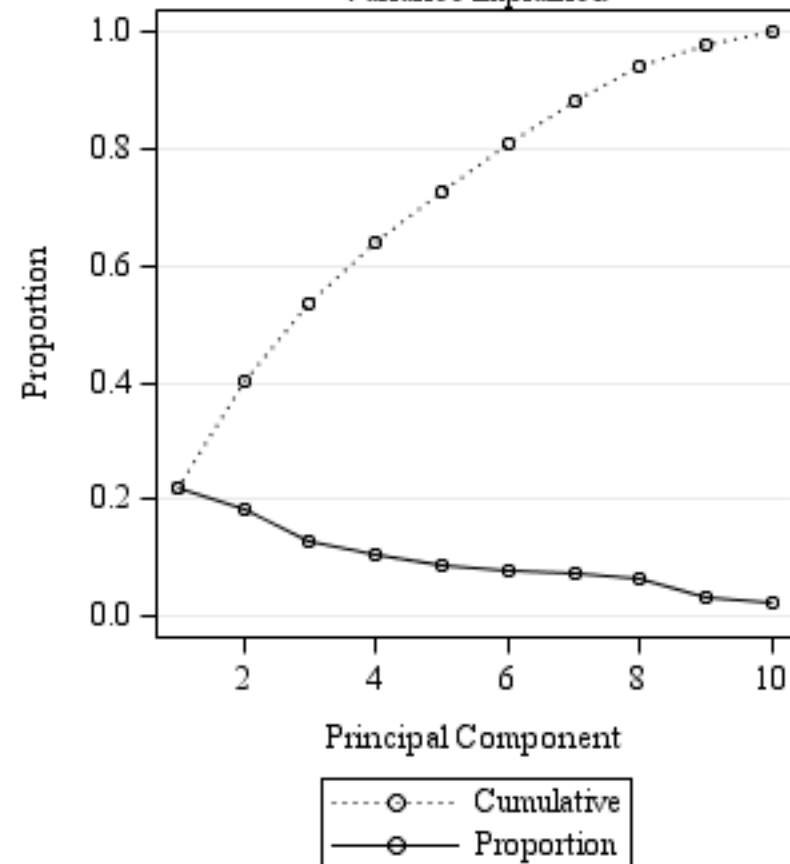
Figure 2.3: Scree plot and proportion of variance explained. The left panel (2.3a) is a scree plot of principal components against eigenvalues derived from PCA. The right panel (2.3b) depicts the percent of total variance explained by each principal component. Each component was derived from a maximally weighted linear combination of 10 physiological markers assessed at age 80, n=330.

CHAPTER 3:

PROSPECTIVE EVALUATION OF ASSOCIATIONS BETWEEN ALLOSTATIC LOAD

AND PERIODONTAL DISEASE IN A COHORT OF OLDER DANISH ADULTS

3.1. ABSTRACT

Background: While recent cross-sectional studies suggest an association between allostatic load and chronic infection-induced inflammation such as periodontal disease, longitudinal studies are needed to better understand the temporal association of the relationship.

Objective: Using prospective data from the 70-year old cohort of the 1914 Glostrup Aging Study with 15 years of follow-up, we examined the bidirectional relationship between allostatic load and periodontal disease in a birth cohort of 453 older adults residing in Glostrup, Denmark.

Methods: Periodontal disease was assessed using a modified version of the Community Periodontal Index at age 70 and with measures of periodontal pocket depths (PD) and clinical attachment loss (CAL) at age 85. Summary measures of allostatic load were constructed using a count of inflammatory, metabolic and anthropometric markers at ages 70 and 80. Structured questionnaires were administered at each follow-up assessment to ascertain social and behavioral characteristics. We used proportional-odds and linear regression models to evaluate the following: i) cross sectional associations between allostatic load and periodontal inflammation at age 70; ii) prospective associations between allostatic load at age 70 and periodontal disease at age 85; and iii) prospective associations periodontal disease at age 70 and allostatic load at age 80.

Results: In cross sectional analyses we found nonlinear associations between allostatic load and periodontal inflammation, controlling for sex, smoking, and physical activity (adjusted odds ratio: 1.85 comparing allostatic load quartiles 2 – 4 vs. 1, 95% confidence interval [CI]: 1.01 – 3.42). In the prospective analyses, positive nonlinear associations were observed between allostatic load at age 70 and periodontal disease at age 85 (the absolute increase in percentage of tooth sites with PD$\geq$ 3 mm and CAL$\geq$ 3 mm was 16% and 12%, respectively, when comparing

the participants in the upper 25th percentile of allostatic load to those in the lower 75th percentile). No longitudinal association was observed between periodontal inflammation at age 70 and allostatic load at age 80.

Conclusions: Our results show that physiological dysregulation in early old age may influence the course of periodontal disease, and supports emerging evidence that suggests a role of chronic stress on infection-induced inflammation.

3.2. Introduction

Epidemiological evidence largely supports the role of stress as a contributing factor in the susceptibility and progression of periodontal disease[1-3]. While the mechanism(s) are not entirely understood, the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS) are thought to be central in mediating the cellular and molecular response to stressors[4]. One theoretical model that underscores the role of the HPA axis and SNS activity in influencing disease trajectories is allostatic load, a measure of cumulative biological damage that results from excessive cycles of response across physiological systems (e.g., neuroendocrine, immune, metabolic, cardiovascular, and respiratory systems) as they adapt to environmental challenges[5,6]. According to the model, over-exposure to stress hormones, such as glucocorticoids, catecholamines and pro-inflammatory cytokines, leads to cumulative physiological dysregulation over the life course. As such, allostatic load serves as an appropriate framework for examining the cumulative biological burden of stress on periodontal disease at older age[7,8].

Older adults experience the highest prevalence of periodontal disease, which may in part reflect a cumulative burden of prolonged exposure to stress, along with other risk factors that promote sustained inflammatory activity over the life course[9,10]. Epidemiological studies of older adults have shown that high scores on measures of allostatic load predict decline in physical and cognitive functioning[11], frailty[12,13], and a multitude of chronic age-related diseases including cardiovascular events[6], type-2 diabetes[14], and mortality[15-17]. In a large sample of adults aged 18 years and over, Sabbah et al. reported positive associations of allostatic load with both periodontal and ischaemic heart disease suggesting a potential common stress pathway; their measure of allostatic load included metabolic, inflammatory, and anthropometric markers from NHANES III (1989 – 1994)[18]. With more recent NHANES data (1999-2004), Borrell and

Crawford reported a similar positive association between allostatic load and prevalence of periodontal disease, independent of smoking and selected demographic characteristics[19]. While these cross-sectional studies have provided the first epidemiological evidence in support of a relationship, no study to our knowledge has explored this association using longitudinal data among older adults.

Since periodontal disease is an inflammatory condition characterized by chronic low-grade infection with specific oral pathogens, chronic exposure to bacterial products could act as a physiological stressor with the potential for chronically activating the HPA axis[20]. Persistent infection with periodontal microorganisms could thereby exacerbate allostatic load burden. Given that negative emotions and other psychological stressors also trigger over-activity of the HPA axis/SNS and may contribute to prolonged infection with sustained proinflammatory cytokine production[21], a bidirectional relationship between allostatic load and periodontal disease may exist. Longitudinal studies are warranted to examine this potential relationship.

The main objective of this study is to examine bidirectional associations between allostatic load and periodontal disease in a cohort of older Danish adults using measures obtained at multiple time points. To achieve this, we evaluate a series of five associations using cross-sectional and longitudinal data summarized in **figure 3.1**: **(1a and 1b)** cross-sectional bidirectional associations between allostatic load and periodontal disease measured at age 70; **(2)** longitudinal association between periodontal disease at age 70 and allostatic load at age 80; and **(3a and 3b)** longitudinal associations between: (a) allostatic load at age 70 and periodontal disease at age 85, and (b) allostatic load at age 80 and periodontal disease at age 85.

3.3. Methods

3.3.1. Study Population

 The present study uses data from the Glostrup Aging Study; a full description of the parent study has been previously described elsewhere (**see Methods appendix**). The study population includes assessments at ages 70 (baseline), 75, 80, and 85. The cohort was supplemented at age 75 with additional subjects to increase the geographic representation of the sample as well as improve statistical power. Follow-up assessments at the Copenhagen County Hospital (CCH) in Glostrup at ages 70 and 80 involved physical examinations, collection of blood samples, and completion of a health questionnaire. In addition, information regarding social and behavioral factors was collected through study questionnaires and in-person interviews during home visits at each follow-up period. Clinical oral health examinations were performed at ages 70 and 85.

 Separate subpopulations were chosen to examine cross-sectional and longitudinal associations between allostatic load and periodontal disease. To examine cross-sectional relationships at age 70, we began with the 804 individuals who participated in the medical evaluation at CCH (**figure 3.2**). A total of 535 participants underwent blood sampling and 453 had complete data for allostatic load evaluation. A final sample of 339 underwent an oral examination; 199 were dentate (i.e., at least one natural tooth present) and 140 were edentate (no natural teeth present). To examine longitudinal relationships, we chose three samples. The first includes 652 participants who underwent an oral health examination at age 70 and were followed for 10 years (**figure 3.3**). At follow-up, 248 participants had died and a final sample of 228 had complete data for allostatic load evaluation. The second sample was comprised of 453 individuals with complete allostatic load data at baseline who were followed for 15 years

(**Figure 3.4a**). At follow-up, 282 participants had died and a final sample of 104 participated in the oral health examination; 64 were dentate and 40 edentate. The third sample comprises 330 individuals with complete data on allostatic load at age 80 who were followed for 5 years (**Figure 3.4b**). At follow-up, 107 individuals had died. A final sample of 155 participated in the oral health examination; 98 were dentate and 57 edentate.

3.3.2. Oral Examinations

Periodontal disease measure at age 70

Periodontal inflammation at age 70 was clinically assessed by inspection, probing, and scored based on the Community Periodontal Index[22], but modified to include recordings for all teeth present rather than indicator teeth alone as was intended in the original index. Each tooth received a score according to predefined criteria (**supplemental table 3.1**). This modified Community Periodontal Index (MCPI) offers an indication of current periodontal inflammation. The index does not, however, measure the cumulative manifestations of the disease such as clinical attachment loss, recession or alveolar bone loss.

Periodontal disease measure at age 85

At age 85, approximately 58% of surviving Glostrup participants had retained some natural teeth. The prevalence of edentulism in this study population is similar to that found among the oldest old populations in developed nations of the same general geographic location[23,24]. Periodontal assessments were carried out in the home of each participant using mobile dental equipment that included a fiber optic light source, mouth mirrors, and periodontal probe. Teeth were dried using an air syringe and cotton rolls. Standardized and calibrated dental

hygienists examined all existing teeth, following procedures recommended by Hunt and Beck[25].

Periodontal probing depth (PD) was defined as the distance in millimeters (mm) from the free

gingival margin to the bottom of the gingival sulcus/pocket while clinical attachment loss (CAL)

was defined as the distance from the cemento-enamel junction to the bottom of the pocket in

mm. Both PD and CAL were recorded for four surfaces per tooth, excluding root tips. Levels of

PD and CAL objectively measure presence of periodontitis and extent of periodontal destruction

resulting from the disease[26].

3.3.3. Physiological markers

At age 70, physiological markers were derived from blood collected at CCH while other

markers comprised clinical measurements recorded by a clinician at the scheduled CCH visit.

Prior to this visit, participants were asked to fast for at least 13 hours. Venous blood was drawn

with participants in a supine position employing the least stasis[27,28]. Whole blood samples were

analyzed on the same day of collection. Blood sera were snap-frozen for later analyses of lipids.

Assays for evaluation of lipid levels were performed at CCH. Standard techniques were used to

determine levels of total cholesterol, high-density lipoprotein [HDL], and triglycerides[28].

Clinical markers

Hospital staff assessed height and weight during the medical evaluation visit.

Measurements were obtained with participants wearing light clothing and no shoes. Height was

measured to the nearest 0.5 cm and weight to the nearest 100 g. Body mass index (BMI) was

computed as weight in kg divided by height in m^2. Resting heart rate was determined from the R-

R interval on an ECG^{28}. Diastolic and systolic blood pressures were measured in a supine

position after 10 minutes of rest using a Doppler technique. Forced expiratory volume in the first

second (FEV$_1$) was measured three times on participants using a Godart bell respirograph and Wright peak flowmeter[28].

Procedures for assessing physiological markers at age 80 have been previously described (**Chapter 2**). Briefly, participants underwent blood sampling and clinical evaluation at CCH, following a similar protocol used for those at age 70. However, participants did not fast prior to blood collection, and additional physiological markers were included at age 80 such as inflammatory cytokines IL-6 and TNF-α, albumin, and blood glucose.

3.3.4. Statistical Analyses

Derivation of allostatic load

Operationalization of allostatic load at ages 70 and 80 followed the original summary score formulation of Seeman et al.[6]. Physiological markers whose levels reached a certain high-risk threshold were assigned a score of one (1), while values in other percentiles were assigned a score of zero (0). High-risk cut-points were defined at the upper quartile for physiological markers: BMI, ratio of total cholesterol/HDL, triglycerides, blood glucose, systolic and diastolic blood pressure, IL-6, and TNF-α. High-risk cut-points were defined at the lower quartile for HDL, albumin, and FEV$_1$. Allostatic load scores were computed by summing these values for each participant. In analyses, we categorized allostatic load scores into quartiles, whether treated as the principal exposure or outcome.

Evaluation of associations

A summary of all periodontal disease measures is presented in **supplemental Table 3.2**. We calculated mean MCPI scores and the percentage of teeth with MCPI scores ≥ 3 and ≥ 6 for

each participant. When treated as outcomes (association 1a), we categorized these measures into quartiles and used cumulative logistic models to estimate cumulative odds ratios (ORs) for having a higher level (quartile) of periodontal inflammation versus having a lower level in relation to allostatic load. When treated as exposures (associations 1b and 2), we categorized these measures into quartiles and additionally included a separate edentulous category to evaluate the influence of edentulism on allostatic load. Score test results indicated that the assumption of proportional odds was not violated for any of the models examined.

To evaluate longitudinal relationships between allostatic load and periodontitis at age 85, we utilized continuous outcome measures such as percentages of tooth sites with PD and CAL $\geq$ 3, 4, and 5 mm depths per participant. We first excluded 11 dentate participants, who had $\geq$ 10 teeth present, at age 85 for a final sample size of 53 and 87 (associations 3a and 3b, respectively); 9 had no valid scores for PD or CAL and 2 had PD and CAL scores measured on only 1 tooth. We used linear regression models to examine these relationships prospectively. In addition, we chose a case definition for advanced periodontal disease, depicted as "deep" periodontal pockets, suggested by the Center for Disease Control Working Group[29] and modified for use in older adult populations: $\geq$ 2 sites with PD $\geq$ 5 mm (with at least 2 teeth present). Studies show that high levels of colonization with oral microorganisms etiologically linked to periodontal disease are strongly correlated with deep periodontal pockets, as well as with active gingival bleeding[30-32]. Odds ratios for periodontal disease were calculated using unconditional logistic regression.

All multivariable models were constructed with serial adjustment of covariates in order to understand the patterns of potential confounding. Covariates included measures of social and behavioral factors assessed with structured questionnaires at baseline (age 70) and included

income (low, high), housing status (renters, owners, other), education (< 9 years, ≥ 9 years),

smoking status (ever smokers, never smokers), drinking status (never drinkers, ever drinkers),

and physical activity (sedentary, light exercise, moderate exercise, rigorous exercise).

Exploratory Analyses

Because physiological markers often tend to be correlated and may contribute to multi-collinearity in multivariable modeling, we performed principal components analysis (PCA). PCA is a summarization technique that reduces the dimensionality of the data by producing a maximally weighted linear combination of uncorrelated variables called principal components[33]. The principal axis method was used to initially extract the components, followed by a varimax (orthogonal) rotation. To determine the number of principal components to retain, we considered components with eigenvalues ≥ 1.0, and applied the scree test[34]. To facilitate interpretation of the rotated factor pattern, a physiological marker was said to load on a given component if the factor loading was 0.40 or greater for that component, and less than 0.40 for the others. Factor loadings can be interpreted as correlation coefficients between the marker and each physiological pattern.

Factor loadings were used to compute factor scores, which can be described as linear combinations or composite measures of uncorrelated and optimally weighted markers for each of the physiological pattern derived. To obtain factor scores, physiological markers HDL, albumin, and FEV_1 were reverse coded such that higher levels depicted higher risk. Each participant received a factor score for every physiological pattern derived from PCA. In exploratory analyses, we evaluated associations between factor scores with periodontal disease at ages 70 and 85. All tests were 2-sided, and all analyses were performed in SAS (version 9.2: SAS institute Inc, Cary NC).

3.4. Results

<u>3.4.1. Description of the study cohort</u>

At baseline (age 70), the study population had almost equal proportions of men and women; roughly three quarters of participants reported having a high income (**table 3.1a**). A majority (59%) had 9 or more years of education and owned their home. Approximately 78% of participants reported ever smoking and an overwhelming majority (94%) reported low levels of current physical activity (< 1 hr/week). Overall, the mean MCPI score was 2.39 (SD=1.10) and the distribution of scores was slightly positively skewed (**supplemental Figure 3.1**). As expected, the proportion of participants who were men, those with low education, low income, renters, ever smokers, and those with lower levels of physical activity increased across categories of periodontal inflammation (all *P*-values <0.05). Among dentate participants, the proportion of individuals with fewer than 16 teeth increased with quartiles of MCPI scores (p<0.0001). Mean allostatic load score at baseline was 1.96 (SD=1.70) and was also positively skewed (**supplemental figure 3.2**). The proportion of participants with low education and those who were sedentary increased across quartiles of allostatic load (all *P*-values <0.05; **table 3.1b**).

To evaluate missing data patterns attributable to death and non-participation in the study sample involved with assessment of association 2, we compared distributions of population characteristics at baseline among those who underwent a clinical oral examination, those who died between ages 70 – 80, and those with complete allostatic load data at follow-up (age 80). **table 3.2a** shows that a higher proportion of men versus women, ever versus never smokers, and edentate versus dentate individuals died between ages 70 – 80 (all *P*-values < 0.05). Of those who survived to age 80, a greater proportion of men, those with higher income, owners of homes and dentulous individuals had complete data to evaluate for allostatic load at follow-up

compared to their counterparts (all *P*-values < 0.05). Deceased individuals and survivors with missing data due to non-participation or missing information on allostatic load (age 80) had a significantly higher mean allostatic load at baseline than those who survived and participated (*P*-values ≤ 0.02). In contrast, no significant differences were noted in mean MCPI scores between deceased and survivors or between individuals with missing and complete data at follow-up.

Patterns of missing data followed similar trends in the study samples involved with assessment of associations (3a) and (3b), as described in **tables 3.2b and 3.2c**. However, most differences in population characteristics were observed among those who died rather than those who did not participate or had incomplete outcome data. Most notably, deceased individuals consistently exhibited higher baseline allostatic load scores than survivors. In the overall cohort, mean levels of allostatic load markers were generally similar at ages 70 and 80, except for higher systolic blood pressures at age 80 (**supplemental table 3.3**). To examine changes in allostatic load across time, we compared quartile-quartile proportions in a subsample of 164 individuals with complete allostatic load data at ages 70 and 80 and found strong correlations (*P*<0.0001; data not shown). Similarly, we found modest positive correlations between mean MCPI scores at age 70 and periodontal disease indices at age 85 (pearson correlations ranged 0.34 – 0.41, *P*-values <0.05; **supplemental figure 3.3**).

3.4.2. Cross-sectional relationships

Association (1a): Cross-sectional association of allostatic load with periodontal disease at age 70

Unadjusted mean MCPI scores and mean percentages of MCPI scores ≥ 3 tended to increase linearly across quartiles of allostatic load, with the largest increase from the $1^{st} – 2^{nd}$

quartile (**table 3.3a,** crude models; *P*-values < 0.05 for trend). When we used a higher severity threshold (mean percentage of MCPI scores ≥ 6), no clear linear pattern was evident. Adjustment for number of teeth, sex, income, education, and housing status, did not change the inference (models 2 – 4).

Table 3.3b shows that participants at the 2nd quartile of allostatic load had 2-fold higher odds of periodontal inflammation than those in the 1st quartile after adjustment for number of teeth (OR=2.06, 95% CI: 0.99 – 4.27; model 1). The effect estimates remained elevated after quartile 2 and in some instances strengthened after serial adjustment for social and behavioral characteristics (models 2 – 4). When quartiles 2 – 4 were combined, their odds of periodontal inflammation was significantly higher than the 1st quartile in the final model adjusted for number of teeth, sex, smoking, and physical activity (OR=1.85, 95% CI: 1.01 – 3.42).

Association (1b): Cross-sectional association of periodontal disease with allostatic load at age 70

Using various periodontal disease definitions, **table 3.4a** shows that mean allostatic load scores increased linearly across levels of periodontal inflammation in crude models (all *P*-values for trend <0.05). In adjusted models, mean allostatic load scores were highest at more severe levels (quartiles 3 or 4) of periodontal inflammation, but the pattern was no longer linear. Edentate participants exhibited among the highest mean allostatic load scores. Serial adjustments by social and behavioral characteristics did not appear to change this overall pattern.

Given similar trends in associations across different definitions of periodontal inflammation, we chose mean MCPI scores to evaluate magnitude of the relationship. **Table 3.4b** shows that participants with a mean MCPI score of approximately 3 or higher (4th quartile)

exhibited an 84% higher odds of having a higher versus lower level of allostatic load as compared to the first quartile, after controlling for number of teeth (OR= 1.84, 0.83 – 4.11; model 1). Adjustment for covariates did not change the estimates appreciably (models 2 – 3), except for a modest attenuation after adjusting for physical activity (model 4).

3.4.3. Longitudinal relationships

Association (2): No association of periodontal disease at age 70 with allostatic load at age 80

Table 3.5 compares mean allostatic load scores at age 80 by levels of periodontal inflammation at age 70. Crude models showed no discernable pattern of association regardless of the periodontal inflammation definition used. Moreover, serial adjustments by sex, income, education, and housing status, smoking, alcohol consumption and physical activity did not change any of the estimates. Model 4 shows that after adjustment for baseline allostatic load scores (age 70) as a continuous variable, mean allostatic load scores at age 80 slightly attenuated across levels of periodontal inflammation, except for the edentulous category, whose adjusted mean values all strengthened.

Association (3a): Association of allostatic load at age 70 with periodontal disease at age 85

We first compared periodontal disease level at age 85 across quartiles of allostatic load at age 70 using multiple definitions of periodontal disease (**table 3.6a**). Means of PD were generally similar at quartiles 1 – 3, but substantially increased at quartile 4 (after adjustment for number of teeth) regardless of cut-point (*P*-values ranged from 0.003 – 0.03 comparing quartiles 1 – 3 vs. 4). Similar trends were observed with CAL; however, they were not statistically significant.

Crude linear regression models showed that individuals in the 4th quartile of allostatic load (AL scores 4 – 8) had 22% higher sites with PD ≥ 3 mm and 19% higher sites with PD ≥ 4 mm as compared to those in the lower 3 quartiles, after controlling for number of teeth at age 85 (*P*-values < 0.01; **table 3.6b**). After adjustment for sex and income, which are strong predictors of periodontal disease at age 85, estimates slightly attenuated but remained significant (15.9% and 13.9%, respectively; model 2). Additional adjustment for current smoking at older age did not change the estimates appreciably (model 3). Similar positive associations were observed for CAL, although the effects did not reach statistical significance. When adjusted for past periodontal inflammation (age 70), as measured by mean MCPI scores, associations remained positive for all measures, but only statistically significant for PD at ≥ 3 and ≥ 4 mm thresholds.

Association (3b): Association of allostatic load at age 80 with periodontal disease at age 85

Table 3.7a compares mean percentage of sites with PD and CAL ≥ 3, 4 and 5 mm across quartiles of allostatic load scores at age 80. Mean PD at the highest quartile of allostatic load was always higher than the first 3 categories, although these differences were not statistically significant. No discernable pattern was evident with CAL. Multivariable regression models showed positive, non-significant associations between allostatic load at 80 and % of sites with PD ≥ 3 and 4 mm (**table 3.7b**). The associations attenuated after adjustment for number of teeth, sex, income, and current smoking (model 3).

Similar results were obtained when we used the case definition for periodontal disease at age 85; that is, allostatic load at age 70, but not age 80, was significantly associated with periodontal disease (adjusted OR= 1.99, for every one-score difference in allostatic load at 70, 95% CI: 1.13 – 3.52; **supplemental table 3.4a**). In subgroup analysis of 50 participants, we

explored whether there was a longitudinal association between allostatic load (ages 70 and 80) and periodontal disease (age 85); allostatic load was categorized into high and low categories depending on whether values fell on the upper or lower 2 quartiles. Participants with high allostatic load scores at ages 70 and 80 had significantly higher odds of periodontal disease than those with low scores at both ages, after adjustment for number of teeth at age 85 (OR=6.80, 95% CI% 1.20 – 38.4; **supplementary table 3.4b**).

3.4.4. Associations between allostatic load and edentulism

In secondary analyses, we compared mean allostatic load scores between dentate and edentate participants to evaluate the association of edentulism on allostatic load. In cross-sectional analysis at age 70, edentulous participants exhibited significantly higher allostatic load scores than those with teeth (2.18 vs. 1.80, p=0.04; Supplemental Table 3.5). Multivariable models showed that edentulism was significantly associated with allostatic load after adjustment of physical activity (adjusted OR=1.49, 95% CI: 1.01 – 2.20). Similar positive associations were found between edentulism and allostatic load at age 80 after adjustment for sex, and physical activity; however, the OR did not reach statistical significance (adjusted OR=1.43, 95% CI: 0.70 – 2.93).

To examine the association of allostatic load with edentulism, we first examined cross-sectional associations at age 70. Supplemental Table 3.6 shows that the odds of edentulism increased across quartiles of allostatic load (crude model, P-value for trend= 0.04). Adjustment for strong predictors of edentulism attenuated the association modestly, but the estimates remained elevated at quartiles 3 and 4 as compared to quartile 1. A total of thirteen individuals became edentulous from age 70 – 85, five of whom had information on allostatic load at age 70.

Exploratory analysis showed that the mean allostatic load score among those who became edentulous was higher than that among those who remained dentate or edentate over the 15 years of follow-up (means= 2.53, 1.31, 1.49, respectively; P-values NS, data not shown). When we examined longitudinal associations between allostatic load at age 70 and age 80 with edentulism at age 85, no consistent pattern emerged.

3.4.5. Exploratory analyses of associations between components of allostatic load and periodontal disease

Principal component analysis with the eight physiological markers revealed three factors with eigenvalues greater than one. Evidence from the scree plot suggested that the first three factors were meaningful, and these were subsequently retained for orthogonal rotation (**supplemental Figure 3.5**). Combined, these factors accounted for approximately 70% of the total variance. Factor loadings for the three retained factors are presented in Supplemental Table 3.7. Physiological markers BMI, HDL, and ratio of total cholesterol/HDL, and triglycerides were found to load strongly onto the first factor and are related to metabolic lipid profiles. Markers that loaded heavily onto the second factor, systolic and diastolic blood pressure, were characterized as the "blood pressure pattern". Those that loaded strongly with the cardio-pulmonary pattern were heart rate and FEV_1. Factor scores and their strongly loaded physiological markers were highly correlated in magnitude and direction, as expected (**supplemental Table 3.8**).

To evaluate the cross-sectional associations between factor scores and periodontal disease at age 70, we categorized mean MCPI scores and % MCPI scores ≥ 3 into quartiles and estimated cumulative odds ratios. **Supplemental Table 3.9** shows that lipid and cardio-

pulmonary factor scores at the 4th quartile had higher odds of periodontal inflammation as compared to the 1st quartile in multivariable models, although not statistically significant. Similar patterns of associations were observed between "lipid" and "cardio-pulmonary" factor scores with edentulism. To examine longitudinal associations between factor scores at 70 and 80 with periodontal disease at age 85, we estimated linear regression models. **Supplemental Table 3.10** shows that participants at the 4th quartile of factor scores generally had a higher percentage of sites with PD $\geq$ 3, 4, and 5 mm than those at the lower quartiles of scores, albeit not statistically significant. When we considered factor scores at age 80, this pattern of association was only evident for the lipid pattern. Although the inflammatory factor pattern was not associated with any of periodontal disease measures at age 85, we did observe higher mean levels of IL-6 and TNF-alpha in cases with deep periodontal pockets compared to non-cases when analyses were restricted to women and in men who were current smokers (*P*-values ranged 0.20 – 0.30; data not shown).

3.5. Discussion

Recent cross-sectional studies suggest associations between allostatic load and periodontal disease. Ours is the first study to comprehensively investigate the bidirectional relation of this association in a prospective birth cohort of older Danish adults. Overall, we found bidirectional positive cross-sectional associations in early old age, but only unidirectional positive associations of allostatic load predicting periodontal disease longitudinally. These data are in accord with results from previous studies and further support a growing body of evidence that highlights the role of stress in modulating chronic infection-induced inflammation.

Cross-sectional findings at age 70 showed positive nonlinear associations of allostatic load with periodontal disease evident at low levels of allostasis, independent of potential confounding factors such as sex, smoking, physical activity, and socio-economic characteristics. These effects were consistent across different measures of periodontal inflammation, although a higher severity threshold generally yielded a weaker association. We observed these associations despite our use of a crude measure of allostatic load, comprised largely of metabolic markers with exclusion of inflammatory markers such as those used in prior NHANES studies, namely fibrinogen and C-reactive protein (CRP). These findings are consistent with those observed by Sabbah et al.[18], Borrell and Crawford[19], and with several large cross-sectional studies of diverse populations that show positive associations between metabolic syndrome or their component markers and severity of periodontal disease[35-37]. Evidence shows that metabolic syndrome markers are also linked with proinflammatory cytokines[38,39], and may collectively suggest presence of underlying chronic systemic inflammation.

Longitudinally, our results generally showed that allostatic load was positively associated with extent of PD and CAL after 15 years of follow-up, and persisted after adjustment for baseline periodontal inflammation. Our finding of a strong positive association between high versus low allostatic load scores at ages 70 and 80 in relation to "deep" periodontal pocket depths at age 85 further strengthens the evidence for a longitudinal relationship. Moreover, we found a non-significant positive association between levels of IL-6 and TNF-alpha at age 80 and deep periodontal pockets at age 85 in women and amongst men who were current smokers. This is consistent with a large cross-sectional study of older adults in which inflammatory markers IL-6 and TNF-alpha were independently positively associated with advanced periodontitis defined as >10% of sites with PD $\geq$ 6 mm[40]. Using NHANES data (1991 – 1994), Dye et al. reported an

independent positive association between serum antibodies against *Porphyromonas gingivalis*, a known oral pathogen etiologically related to periodontal disease, and levels of CRP and fibrinogen in adults aged 40 years and older[41], further supporting the role of chronic systemic inflammation. However, future studies that incorporate inflammatory markers into allostatic load at multiple time points are necessary to further elucidate this relationship.

The lack of association between periodontal disease at age 70 and allostatic load at age 80 can be explained in part by the mitigating effect of selection bias. First, Table 3.2a showed evidence of differential survival among older adults who were female versus male, non-smoker versus ever smoker, and dentulous versus edentulous. In addition, survivors had lower mean baseline allostatic load scores than deceased individuals, suggesting a preferential selection for a healthier older cohort at follow-up. Secondly, survivors who participated and had complete allostatic load data at follow-up were more likely to have high versus low income, were owners versus renters of homes, and had lower mean baseline allostatic load scores. These effects would tend to differentially bias our observed estimates towards the null.

While several models have been proposed to explain mechanistic pathways between stressors and periodontal disease onset and progression[42], the model of allostatic load focuses on stress-induced over or under-activity of the HPA axis and SNS. On the one hand, perceived stressors or chronic psychological distress may heighten the immune response towards pathogenic antigens through chronic over-activation of the HPA axis and SNS. For instance, several studies suggest that exposure to anxiety and depression can modulate antibody and T-cell responses to antiviral vaccines[43-45]. In a more recent study of older adults receiving immunological challenge with influenza vaccine, Glaser et al. reported a prolonged elevation in plasma levels of IL-6 among participants with mild depressive symptoms as compared to those

who reported fewer symptoms[46]. On the other hand, exposure to endotoxins and lipopolysaccharides from the outer membrane of Gram-negative bacteria have been shown to activate the HPA axis through the action of inflammatory mediators, which lead to an increase in levels of glucocorticoids a general suppression of the immune response[20,47]. Therefore, over-activity of HPA functioning from exposure to chronic infection with periodontal disease related pathogens might potentially accelerate the rate of physiological dysregulation in older adults. Studies that incorporate these oral pathogens are necessary to further explore this relationship.

This study has several important limitations. First, we had limited sample sizes at ages 80 and 85 to fully discern the dose-response relationship of each longitudinal association. Second, we did not have data regarding oral hygiene, dental care utilization patterns and complete dietary intake. Since exposure to stress can lead to changes in periodontal disease through health impairing behaviors such as oral hygiene neglect, poor dietary modification, and reduction in compliance with dental care, we lacked the ability to control for these potential confounders. Finally, we did not have information regarding neuroendocrine markers thought to be important primary mediators of the stress response such as cortisol, dehydroepiandrosterone-sulphate (DHEAS), norepinephrine and epinephrine. These markers were included in Seeman et al's original index of allostatic load, and there is some limited evidence to suggest that these may also be correlated with periodontal disease[48].

Our study had important strengths worth highlighting. As a birth cohort of a relatively homogeneous population, our findings were not prone to the effects of age. The clinical periodontal examinations were full-mouth assessments and reduced the potential for misclassification. Finally, the long follow-up period allowed us to study the effects of allostatic load at early old age on periodontal disease in the oldest old.

3.6. Conclusions

In summary, we found positive nonlinear associations between allostatic load and periodontal disease both cross-sectionally and longitudinally in a cohort of fully functioning older adults. While bidirectional communication exists between neuroendocrine/CNS and immune systems, we did not observe an association between periodontal inflammation and allostatic load prospectively, although associations might have been diluted by differential survival effects. Taken together, our findings support a growing body of evidence that suggest chronic exposure to stress contributes to the pathophysiology of inflammatory diseases. Future longitudinal studies with larger sample sizes that evaluate changes in colonization patterns of specific oral pathogens etiologically linked to periodontal disease in relation to allostatic load may allow a more complete understanding and confirmation of the allostatic load-periodontal disease relationship.

3.7. References

1.   Rosania AE, Low KG, McCormick CM, Rosania DA. Stress, depression, cortisol, and periodontal disease. J Periodontol 2009;80:260-6.

2.   Peruzzo DC, Benatti BB, Ambrosano GM, et al. A systematic review of stress and psychological factors as possible risk factors for periodontal disease. J Periodontol 2007;78:1491-504.

3.   da Silva AM, Newman HN, Oakley DA. Psychosocial factors in inflammatory periodontal diseases. A review. J Clin Periodontol 1995;22:516-26.

4.   Miller DB, O'Callaghan JP. Neuroendocrine aspects of the response to stress. Metabolism 2002;51:5-10.

5.   McEwen BS. Stress, adaptation, and disease. Allostasis and allostatic load. Ann N Y Acad Sci 1998;840:33-44.

6.   Seeman TE, Singer BH, Rowe JW, Horwitz RI, McEwen BS. Price of adaptation--allostatic load and its health consequences. MacArthur studies of successful aging. Arch Intern Med 1997;157:2259-68.

7.   Ben-Shlomo Y, Kuh D. A life course approach to chronic disease epidemiology: conceptual models, empirical challenges and interdisciplinary perspectives. Int J Epidemiol 2002;31:285-93.

8.   Seeman TE, McEwen BS, Rowe JW, Singer BH. Allostatic load as a marker of cumulative biological risk: MacArthur studies of successful aging. Proc Natl Acad Sci U S A 2001;98:4770-5.

9.   Papapanou PN. Periodontal diseases: epidemiology. Ann Periodontol 1996;1:1-36.

10. Papapanou PN, Lindhe J, Sterrett JD, Eneroth L. Considerations on the contribution of ageing to loss of periodontal tissue support. J Clin Periodontol 1991;18:611-5.

11. Karlamangla AS, Singer BH, McEwen BS, Rowe JW, Seeman TE. Allostatic load as a predictor of functional decline. MacArthur studies of successful aging. J Clin Epidemiol 2002;55:696-710.

12. Szanton SL, Allen JK, Seplaki CL, Bandeen-Roche K, Fried LP. Allostatic load and frailty in the women's health and aging studies. Biol Res Nurs 2009;10:248-56.

13. Gruenewald TL, Seeman TE, Karlamangla AS, Sarkisian CA. Allostatic load and frailty in older adults. J Am Geriatr Soc 2009;57:1525-31.

14. Crews DE. Composite estimates of physiological stress, age, and diabetes in American Samoans. Am J Phys Anthropol 2007;133:1028-34.

15. Gruenewald TL, Seeman TE, Ryff CD, Karlamangla AS, Singer BH. Combinations of biomarkers predictive of later life mortality. Proc Natl Acad Sci U S A 2006;103:14158-63.

16. Karlamangla AS, Singer BH, Seeman TE. Reduction in allostatic load in older adults is associated with lower all-cause mortality risk: MacArthur studies of successful aging. Psychosom Med 2006;68:500-7.

17. Goldman N, Turra CM, Glei DA, Seplaki CL, Lin YH, Weinstein M. Predicting mortality from clinical and nonclinical biomarkers. J Gerontol A Biol Sci Med Sci 2006;61:1070-4.

18. Sabbah W, Watt RG, Sheiham A, Tsakos G. Effects of allostatic load on the social gradient in ischaemic heart disease and periodontal disease: evidence from the Third National Health and Nutrition Examination Survey. J Epidemiol Community Health 2008;62:415-20.

19. Borrell LN, Crawford ND. Social disparities in periodontitis among US adults: the effect of allostatic load. J Epidemiol Community Health 2011;65:144-9.

20. Webster JI, Sternberg EM. Role of the hypothalamic-pituitary-adrenal axis, glucocorticoids and glucocorticoid receptors in toxic sequelae of exposure to bacterial and viral products. J Endocrinol 2004;181:207-21.

21. Glaser R, Kiecolt-Glaser JK. Stress-induced immune dysfunction: implications for health. Nat Rev Immunol 2005;5:243-51.

22. Ainamo J, Barmes D, Beagrie G, Cutress T, Martin J, Sardo-Infirri J. Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). Int Dent J 1982;32:281-91.

23. Thorstensson H, Johansson B. Oral health in a population-based sample of the oldest-old: findings in twins 80 years and older in Sweden. Swed Dent J 2003;27:49-57.

24. Lundgren M, Osterberg T, Emilson G, Steen B. Oral complaints and utilization of dental services in relation to general health factors in a 88-year-old Swedish population. Gerodontology 1995;12:81-8.

25. Hunt RJ, Beck JD. Methodological considerations in a dental epidemiologic survey of an elderly population. J Public Health Dent 1985;45:257-60.

26. Robertson PB, Buchanan SA, Armitage GC, Newbrun E, Taggart EJ, Hoover CI. Evaluation of clinical and microbiological measures to predict treatment response in severe periodontitis. J Periodontal Res 1987;22:230-2.

27. Schroll M, Jorgensen T, Ingerslev J. The Glostrup Population Studies, 1964-1992. Dan Med Bull 1992;39:204-7.

28. Schroll M. A ten-year prospective study, 1964-1974, of cardiovascular risk factors in men and women from the Glostrup population born in 1914. Dan Med Bull 1982;29:213-52.

29. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. J Periodontol 2007;78:1387-99.

30. Papapanou PN, Teanpaisan R, Obiechina NS, et al. Periodontal microbiota and clinical periodontal status in a rural sample in southern Thailand. Eur J Oral Sci 2002;110:345-52.

31. Papapanou PN, Baelum V, Luan WM, et al. Subgingival microbiota in adult Chinese: prevalence and relation to periodontal disease progression. J Periodontol 1997;68:651-66.

32. Demmer RT, Papapanou PN, Jacobs DR, Jr., Desvarieux M. Bleeding on probing differentially relates to bacterial profiles: the Oral Infections and Vascular Disease Epidemiology Study. J Clin Periodontol 2008;35:479-86.

33. Joliffe IT, Morgan BJ. Principal component analysis and exploratory factor analysis. Stat Methods Med Res 1992;1:69-95.

34. Hatcher L. A step-by-step approach to using the SAS system for factor analysis and structural equation modeling. Cary, NC: SAS Institute; 1994.

35. Fukui N, Shimazaki Y, Shinagawa T, Yamashita Y. Periodontal status and metabolic syndrome in middle-aged Japanese. J Periodontol 2012;83:1363-71.

36. D'Aiuto F, Sabbah W, Netuveli G, et al. Association of the metabolic syndrome with severe periodontitis in a large U.S. population-based survey. J Clin Endocrinol Metab 2008;93:3989-94.

37. Kwon YE, Ha JE, Paik DI, Jin BH, Bae KH. The relationship between periodontitis and metabolic syndrome among a Korean nationally representative sample of adults. J Clin Periodontol 2011;38:781-6.

38. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation 2003;107:391-7.

39. Esposito K, Ciotola M, Sasso FC, et al. Effect of a single high-fat meal on endothelial function in patients with the metabolic syndrome: role of tumor necrosis factor-alpha. Nutr Metab Cardiovasc Dis 2007;17:274-9.

40. Bretz WA, Weyant RJ, Corby PM, et al. Systemic inflammatory markers, periodontal diseases, and periodontal infections in an elderly population. J Am Geriatr Soc 2005;53:1532-7.

41. Dye BA, Choudhary K, Shea S, Papapanou PN. Serum antibodies to periodontal pathogens and markers of systemic inflammation. J Clin Periodontol 2005;32:1189-99.

42. Genco RJ, Ho AW, Kopman J, Grossi SG, Dunford RG, Tedesco LA. Models to evaluate the role of stress in periodontal disease. Ann Periodontol 1998;3:288-302.

43. Glaser R, Sheridan J, Malarkey WB, MacCallum RC, Kiecolt-Glaser JK. Chronic stress modulates the immune response to a pneumococcal pneumonia vaccine. Psychosom Med 2000;62:804-7.

44. Morag M, Morag A, Reichenberg A, Lerer B, Yirmiya R. Psychological variables as predictors of rubella antibody titers and fatigue--a prospective, double blind study. J Psychiatr Res 1999;33:389-95.

45. Glaser R, Kiecolt-Glaser JK, Bonneau RH, Malarkey W, Kennedy S, Hughes J. Stress-induced modulation of the immune response to recombinant hepatitis B vaccine. Psychosom Med 1992;54:22-9.

46. Glaser R, Robles TF, Sheridan J, Malarkey WB, Kiecolt-Glaser JK. Mild depressive symptoms are associated with amplified and prolonged inflammatory responses after influenza virus vaccination in older adults. Arch Gen Psychiatry 2003;60:1009-14.

47. Pollmacher T, Mullington J, Korth C, et al. Diurnal variations in the human host response to endotoxin. J Infect Dis 1996;174:1040-5.

48. Ishisaka A, Ansai T, Soh I, et al. Association of cortisol and dehydroepiandrosterone sulphate levels in serum with periodontal status in older Japanese adults. J Clin Periodontol 2008;35:853-61.

Table 3.1a: Distribution of population characteristics at baseline (age 70) by periodontal inflammation, N=339

| Population characteristics at baseline (age 70) | n (%) | MCPI scores at age 70 in quartiles | | | | Edentulous | P-value[a] |
		Quartile 1 (0.40 – 1.54)	Quartile 2 (1.56 – 2.10)	Quartile 3 (2.11 – 2.90)	Quartile 4 (2.94 – 6.00)		
Total (n)	339	46	51	47	55	140	
Sex							
Males	166 (49.0%)	37.0%	41.2%	51.1%	56.4%	52.1%	0.04
Females	173 (51.0%)	63.0%	58.8%	49.9%	43.6%	47.9%	
Education in years							
< 9	138 (40.7%)	23.9%	21.6%	38.3%	38.2%	55.0%	<0.0001
≥ 9	201 (59.3%)	76.1%	78.4%	61.7%	61.8%	45.0%	
Income[b]							
Low	76 (22.4%)	6.5%	9.8%	17.0%	12.7%	37.9%	<0.0001
High	263 (77.6%)	93.5%	90.2%	83.0%	87.3%	62.1%	
Housing status							
Renter/other	138 (40.7%)	45.7%	41.2%	57.5%	50.9%	70.0%	0.0002
Owner	201 (59.3%)	54.4%	58.8%	42.6%	49.1%	30.0%	
Smoking status							
Never smoker	75 (22.1%)	34.8%	27.5%	23.4%	18.2%	17.1%	0.007
Ever smoker	264 (77.9%)	65.2%	72.6%	76.6%	81.8%	82.9%	
Alcohol consumption							
Never drinker	121 (35.7%)	32.6%	31.4%	31.9%	27.3%	42.8%	0.12
Ever drinker	218 (64.3%)	67.4%	68.6%	68.1%	72.7%	57.1%	
Physical activity							
Sedentary/light	319 (94.1%)	82.6%	96.1%	93.6%	96.4%	96.4%	0.01
Moderate/rigorous	20 (5.9%)	17.4%	3.9%	6.4%	3.6%	3.5%	
Number of teeth[c]							
< 16	97 (48.8%)	28.3%	37.3%	48.9%	76.4%	NA	<0.0001
≥ 16	102 (51.3%)	71.7%	62.8%	51.1%	23.6%	NA	

[a] *P*-value for Cochran-Armitage trend test

[b] Low income: compensation from old-age pension alone; high income: additionally supplemented with income from other sources

[c] Cut-point for number of teeth is at the median of the sample and excludes edentulous individuals

Table 3.1b: Distribution of population characteristics at baseline by allostatic load scores at age 70 in quartiles, N=339

| Population characteristics at baseline (age 70) | Allostatic load scores at age 70 in quartiles | | | | P-value[a] |
	AL quartile 1 (score= 0)	AL quartile 2 (score= 1)	AL quartile 3 (scores= 2 – 3)	AL quartile 4 (scores= 4 – 8)	
Total (n)	75	85	122	57	
Sex					
Males	52.0%	47.1%	47.5%	50.9%	0.84
Females	48.0%	52.9%	52.5%	49.1%	
Education in years					
< 9	29.3%	40.0%	44.3%	49.1%	0.01
≥ 9	70.7%	60.0%	55.7%	50.9%	
Income					
Low	13.3%	27.1%	23.8%	24.6%	0.16
High	86.7%	72.9%	76.2%	75.4%	
Housing status					
Renter/other	49.3%	69.4%	50.8%	64.9%	0.49
Owner	50.7%	30.6%	49.2%	35.1%	
Smoking status					
Never smoker	25.3%	20.0%	23.0%	19.3%	0.55
Ever smoker	74.7%	80.0%	77.1%	80.7%	
Alcohol consumption					
Never drinker	24.0%	43.5%	39.3%	31.6%	0.34
Ever drinker	76.0%	56.5%	60.7%	68.4%	
Physical activity					
Sedentary	4.0%	9.4%	12.3%	24.6%	0.001
Light - rigorous exercise	96.0%	90.6%	87.7%	75.4%	

AL= Allostatic load

[a] *P*-value for Cochran-Armitage trend test

Table 3.2a. Distribution of population characteristics at baseline among those who underwent a clinical oral examination at age 70, those who died between ages 70 – 80, and those with complete data on allostatic load at follow-up (age 80)

Population Characteristics	Baseline (age 70) N (column %)	Deaths (ages 70-80) N (row %)	Survivors (age 80) N (row %)	P-value[a]	Missing data[b] (age 80) N (row %)	Complete data (age 80) N (row %)	P-value[a]
Overall N	652	248	404		176	228	
Sex							
Males	322 (49.4%)	148 (46.0%)	174 (54.0%)	<0.0001	64 (36.8%)	110 (63.2%)	0.02
Females	330 (50.6%)	100 (30.3%)	230 (69.7%)		112 (48.7%)	118 (51.3%)	
Education in years							
< 9	264 (44.6%)	102 (38.6%)	162 (61.4%)	0.60	77 (47.5%)	85 (52.5%)	0.07
≥ 9	328 (55.4%)	120 (36.6%)	208 (63.4%)		79 (38.0%)	129 (62.0%)	
Income							
Low	123 (18.9%)	52 (42.3%)	71 (57.7%)	0.28	40 (56.3%)	31 (43.7%)	0.02
High	529 (81.1%)	196 (37.1%)	333 (62.9%)		136 (40.8%)	197 (59.2%)	
Housing status							
Renter	304 (51.3%)	119 (39.1%)	185 (60.9%)	0.42	90 (48.6%)	95 (51.4%)	0.04
Owner	255 (43.0%)	89 (34.9%)	166 (65.1%)		60 (36.1%)	106 (63.9%)	
Other	34 (5.7%)	15 (44.1%)	19 (55.9%)		6 (31.6%)	13 (68.4%)	
Smoking status							
Never smoker	143 (22.5%)	40 (28.0%)	103 (72.0%)	0.01	42 (40.8%)	61 (59.2%)	0.52
Ever smoker	493 (77.5%)	196 (39.8%)	297 (60.2%)		132 (44.4%)	165 (55.6%)	
Edentulism							
Edentulous	277 (42.5%)	129 (46.6%)	148 (53.4%)	0.0001	81 (54.7%)	67 (45.3%)	0.0006
Dentulous	375 (57.5%)	119 (31.7%)	256 (68.3%)		95 (37.1%)	161 (62.9%)	
MCPI scores[c]							
Mean (SD)	2.39 (1.16)	2.41 (1.09)	2.38 (1.20)	0.74	2.53 (1.28)	2.29 (1.14)	0.12
Allostatic load at 70[c]							
Mean (SD)	1.96 (1.70)	2.27 (1.74)	1.78 (1.66)	0.01	2.10 (1.67)	1.54 (1.62)	0.02

[a] P-value for chi-square test
[b] Missing data from non-participation at follow-up and incomplete data on allostatic load markers
[c] MCPI scores among dentulous participants (n=375). Overall sample size for evaluation of allostatic load at 70 is 339.

Table 3.2b. Distribution of population characteristics at baseline among those with allostatic load measures at age 70, those who died between ages 70 – 85, and those who underwent a clinical oral examination at follow-up (age 85)

Population Characteristics	Baseline (age 70) N (column %)	Deaths (ages 70-85) N (row %)	Survivors (age 85) N (row %)	P-value[a]	Missing data[b] (age 85) N (row %)	Complete data (age 85) N (row %)	P-value[a]
Overall N	453	282	171		67	104	
Sex							
Males	230 (50.8%)	160 (69.6%)	70 (30.4%)	0.001	25 (35.7%)	45 (64.3%)	0.44
Females	223 (49.2%)	122 (54.7%)	101 (45.3%)		42 (41.6%)	59 (58.4%)	
Education in years							
< 9	179 (39.6%)	120 (67.0%)	59 (33.0%)	0.08	23 (39.0%)	36 (61.0%)	0.96
≥ 9	273 (60.4%)	161 (59.0%)	112 (41.0%)		44 (39.3%)	68 (60.7%)	
Income							
Low	93 (20.5%)	26 (28.0%)	67 (72.0%)	0.03	9 (13.4%)	17 (86.6%)	0.60
High	360 (79.5%)	145 (40.3%)	215 (59.7%)		58 (27.0%)	87 (73.0%)	
Housing status							
Renter	288 (50.3%)	152 (52.8%)	76 (47.2%)	0.08	35 (46.1%)	41 (53.9%)	0.11
Owner	200 (44.2%)	113 (56.5%)	87 (43.5%)		31 (35.6%)	56 (64.4%)	
Other	25 (5.5%)	17 (68.0%)	8 (32.0%)		1 (12.5%)	7 (87.5%)	
Smoking status							
Never smoker	105 (23.2%)	54 (51.4%)	51 (48.6%)	0.009	21 (41.2%)	30 (58.8%)	0.73
Ever smoker	348 (76.8%)	228 (65.5%)	120 (34.5%)		46 (38.3%)	74 (61.7%)	
Edentulism							
Edentulous	140 (41.3%)	103 (73.6%)	37 (26.4%)	<0.0001	14 (37.8%)	23 (62.2%)	0.72
Dentulous	199 (58.7%)	102 (51.3%)	97 (48.7%)		40 (41.2%)	57 (58.8%)	
MCPI scores[c]							
Mean (SD)	2.39 (1.10)	2.51 (1.10)	2.26 (1.06)	0.11	2.28 (1.13)	2.25 (1.01)	0.88
Allostatic load at 70							
Mean (SD)	2.01 (1.71)	2.20 (1.71)	1.69 (1.67)	0.002	1.73 (1.57)	1.66 (1.73)	0.80

[a] P-value for chi-square test
[b] Missing data from non-participation at follow-up and incomplete data on oral health examination
[c] MCPI scores among dentulous participants (n=199).

Table 3.2c. Distribution of population characteristics among those with allostatic load measures at age 80, those who died between ages 80 − 85, and those who underwent a clinical oral examination at follow-up (age 85)

Social and Behavioral Characteristics	Age 80	Deaths (ages 80-85)	Survivors (age 85)	P-value[a]	Missing data[b] (age 85)	Complete data (age 85)	P-value[a]
	N (column %)	N (row %)	N (row %)		N (row %)	N (row %)	
Overall N	330	107	223		68	155	
Sex							
Males	160 (48.5%)	64 (40.0%)	96 (60.0%)	0.004	31 (32.3%)	65 (67.7%)	0.61
Females	170 (51.2%)	43 (25.3%)	127 (74.7%)		37 (29.1%)	90 (70.9%)	
Education in years							
< 9	123 (39.8%)	39 (31.7%)	84 (68.3%)	0.69	29 (34.5%)	55 (65.5%)	0.24
≥ 9	186 (60.2%)	63 (33.9%)	123 (66.1%)		33 (26.8%)	90 (73.2%)	
Income							
Low	110 (33.3%)	31 (28.2%)	79 (71.8%)	0.24	31 (39.2%)	48 (60.8%)	0.04
High	220 (66.7%)	76 (34.5%)	144 (65.5%)		37 (25.7%)	107 (74.3%)	
Housing status							
Renter	154 (46.8%)	58 (37.7%)	96 (62.3%)	0.16	64 (66.7%)	32 (33.3%)	0.30
Owner	160 (48.6%)	44 (27.5%)	116 (72.5%)		82 (70.7%)	34 (29.3%)	
Other	15 (4.6%)	5 (33.3%)	10 (66.7%)		9 (90.0%)	1 (10.0%)	
Smoking status							
Never smoker	92 (27.9%)	20 (21.7%)	72 (78.3%)	0.01	21 (29.2%)	51 (70.8%)	0.77
Ever smoker	238 (72.1%)	87 (36.6%)	151 (63.4%)		47 (31.1%)	104 (68.9%)	
MCPI scores							
Mean (SD)	2.29 (1.14)	2.37 (1.12)	2.25 (1.15)	0.53	2.29 (1.01)	2.24 (1.20)	0.83
Allostatic load at 70							
Mean (SD)	1.60 (1.60)	1.78 (1.55)	1.52 (1.62)	0.33	1.51 (1.14)	1.55 (1.07)	0.91
Allostatic load at 80							
Mean (SD)	2.45 (1.82)	2.82 (1.90)	2.27 (1.76)	0.01	2.26 (1.72)	2.28 (1.87)	0.95

[a] P-value for chi-square test
[b] Missing data from non-participation at follow-up and incomplete data on oral health examination
[c] MCPI scores among dentulous participants (n=199).

Table 3.3a. Mean levels of periodontal inflammation at age 70 in relation to allostatic load scores at age 70

Allostatic load scores at 70		Serial adjustments of mean MCPI scores (SE), N=199				
	n	Unadjusted	Model 1[a]	Model 2[b]	Model 3[c]	Model 4[d]
AL quartile 1 (score= 0)	50	2.03 (0.15)	2.18 (0.14)	2.14 (0.17)	2.14 (0.19)	2.11 (0.19)
AL quartile 2 (score= 1)	52	2.58 (0.15)	2.52 (0.14)	2.51 (0.16)	2.69 (0.18)	2.48 (0.18)
AL quartile 3 (scores= 2 − 3)	68	2.44 (0.13)	2.39 (0.12)	2.34 (0.15)	2.51 (0.17)	2.29 (0.17)
AL quartile 4 (scores= 4 − 8)	29	2.58 (0.20)	2.55 (0.18)	2.49 (0.21)	2.59 (0.23)	2.40 (0.23)
P for linear trend		0.04	0.18	0.23	0.11	0.35
		Serial adjustments of mean percentage of MCPI scores ≥ 3 (SE), N=199				
AL quartile 1 (score= 0)	50	25.87 (4.90)	31.46 (4.31)	30.28 (5.20)	29.60 (5.81)	28.70 (5.81)
AL quartile 2 (score= 1)	52	41.68 (4.80)	39.35 (4.18)	39.15 (5.02)	45.34 (5.68)	37.59 (5.40)
AL quartile 3 (scores= 2 − 3)	68	38.47 (4.20)	36.52 (3.66)	35.82 (4.60)	41.32 (5.31)	33.35 (5.25)
AL quartile 4 (scores= 4 − 8)	29	46.49 (6.43)	45.57 (5.59)	44.16 (6.29)	47.22 (7.25)	39.92 (6.85)
P for linear trend		0.02	0.10	0.11	0.05	0.23
		Serial adjustments of mean percentage of MCPI scores ≥ 6 (SE), N=78				
AL quartile 1 (score= 0)	15	2.75 (0.21)	2.94 (0.17)	3.02 (0.22)	3.24 (0.29)	3.19 (0.26)
AL quartile 2 (score= 1)	23	2.95 (0.17)	2.86 (0.14)	2.92 (0.20)	3.33 (0.24)	3.06 (0.22)
AL quartile 3 (scores= 2 − 3)	30	2.87 (0.15)	2.82 (0.12)	2.90 (0.18)	3.34 (0.23)	3.08 (0.21)
AL quartile 4 (scores= 4 − 8)	10	3.06 (0.26)	3.15 (0.21)	3.22 (0.27)	3.47 (0.33)	3.35 (0.30)
P for linear trend		0.49	0.69	0.74	0.57	0.75

[a] Adjusted for the number of retained teeth at age 70 (excluding root tips) as a continuous measure
[b] Additionally adjusted for sex, income, education, and housing status
[c] Additionally adjusted for smoking and alcohol consumption
[d] Additionally adjusted for physical activity

Table 3.3b. Association between allostatic load scores at age 70 and periodontal inflammation[a] at age 70, N=199

Multivariable models	n	Odds Ratios (95% CI) for a higher quartile of mean MCPI scores				
		Model 1[b]	Model 2[c]	Model 3[d]	Model 4[e]	Final model[f]
AL quartile 1	50	1.00	1.00	1.00	1.00	1.00
AL quartile 2	52	2.06 (0.99–4.27)	2.30 (1.09–4.84)	2.23 (1.05–4.74)	2.35 (1.10–5.01)	2.28 (1.08–4.79)
AL quartile 3	68	1.47 (0.74–2.91)	1.46 (0.73–2.94)	1.35 (0.66–2.75)	1.31 (0.64–2.68)	1.41 (0.70–2.82)
AL quartile 4	29	2.71 (1.15–6.43)	2.68 (1.12–6.43)	2.49 (1.03–6.01)	2.31 (0.95–5.59)	2.32 (0.97–5.53)
AL quartiles 2–4 vs. 1	149	1.87 (1.02–3.43)	1.94 (1.05–3.59)	1.84 (0.98–3.45)	1.82 (0.97–3.41)	1.85 (1.01–3.42)

[a] Periodontal inflammation was measured as mean MCPI scores
[b] Adjusted for the number of retained teeth at age 70 (excluding root tips) as a continuous measure
[c] Additionally adjusted for sex, income, education, and housing status
[d] Additionally adjusted for smoking and alcohol consumption
[e] Additionally adjusted for physical activity
[f] Final model adjusted by number of retained teeth, sex, smoking and physical activity

Table 3.4a. Mean allostatic load scores at age 70 in relation to periodontal inflammation at age 70, N=339

Analysis of variance	n	Serial adjustments of mean allostatic load scores at age 70 (SE)				
		Crude	Model 1[a]	Model 2[b]	Model 3[c]	Model 4[d]
Mean MCPI scores						
Q1 (0.40 – 1.54)	46	1.39 (0.25)	1.52 (0.31)	1.56 (0.33)	1.56 (0.33)	1.75 (0.34)
Q2 (1.56 – 2.10)	51	1.65 (0.23)	1.74 (0.27)	1.79 (0.28)	1.79 (0.29)	1.91 (0.32)
Q3 (2.11 – 2.90)	47	2.06 (0.24)	2.13 (0.27)	2.17 (0.28)	2.17 (0.28)	2.27 (0.30)
Q4 (2.94 – 6.00)	55	2.05 (0.22)	2.07 (0.23)	2.12 (0.25)	2.13 (0.26)	2.14 (0.28)
Edentulous	140	2.18 (0.14)	2.07 (0.21)	2.11 (0.22)	2.12 (0.23)	2.18 (0.27)
P for linear trend		0.003	0.14	0.15	0.14	0.27
% MCPI scores ≥ 3						
Q1 (0 – 5.0%)	50	1.36 (0.24)	1.48 (0.30)	1.52 (0.31)	1.51 (0.32)	1.69 (0.33)
Q2 (6.3 – 25.0%)	48	1.77 (0.24)	1.87 (0.29)	1.92 (0.30)	1.92 (0.31)	2.07 (0.32)
Q3 (26.1 – 62.5%)	49	1.94 (0.24)	2.00 (0.26)	2.05 (0.27)	2.05 (0.28)	2.12 (0.30)
Q4 (63.6 – 100%)	52	2.12 (0.23)	2.12 (0.23)	2.18 (0.25)	2.18 (0.26)	2.20 (0.28)
Edentulous	140	2.18 (0.14)	2.08 (0.21)	2.12 (0.22)	2.12 (0.23)	2.19 (0.27)
P for linear trend		0.003	0.13	0.13	0.12	0.24
% MCPI scores ≥ 6						
0%	121	1.77 (0.15)	1.90 (0.19)	1.95 (0.20)	1.94 (0.21)	2.02 (0.24)
3.6 – 18.2%	36	1.58 (0.28)	1.78 (0.32)	1.82 (0.33)	1.81 (0.35)	1.86 (0.37)
19.1 – 80%	38	2.07 (0.26)	2.13 (0.27)	2.18 (0.28)	2.18 (0.29)	2.23 (0.30)
Edentulous	140	2.18 (0.14)	2.00 (0.20)	2.03 (0.21)	2.03 (0.22)	2.11 (0.26)
P for linear trend		0.03	0.68	0.69	0.68	0.71

[a] Mean allostatic load scores adjusted for the number of retained teeth at age 70 (excluding root tips) as a continuous measure
[b] Additionally adjusted for sex, income, education, and housing status
[c] Additionally adjusted for smoking and alcohol consumption
[d] Additionally adjusted for physical activity

Table 3.4b. Association between periodontal inflammation at age 70 and allostatic load at age 70, N=339

Periodontal Inflamation	n	ORs for having a higher level (quartile) of allostatic load scores				
		Model 1[a]	Model 2[b]	Model 3[c]	Model 4[d]	Final model[e]
Mean MCPI scores						
Q1 (0.40 – 1.54)	50	1.00	1.00	1.00	1.00	1.00
Q2 (1.56 – 2.10)	48	1.25 (0.60 – 2.59)	1.26 (0.60 – 2.63)	1.25 (0.60 – 2.62)	1.17 (0.55 – 2.46)	1.15 (0.55 – 2.49)
Q3 (2.11 – 2.90)	49	1.67 (0.78 – 3.58)	1.64 (0.76 – 3.54)	1.63 (0.76 – 3.52)	1.47 (0.68 – 3.21)	1.47 (0.68 – 3.17)
Q4 (2.94 – 6.00)	52	1.84 (0.83 – 4.11)	1.86 (0.83 – 4.15)	1.83 (0.82 – 4.10)	1.48 (0.65 – 3.36)	1.47 (0.65 – 3.32)
Edentulous	140	1.68 (0.65 – 4.37)	1.68 (0.65 – 4.38)	1.63 (0.62 – 4.28)	1.43 (0.54 – 3.79)	1.43 (0.54 – 3.76)

[a] Adjusted for the number of retained teeth at age 70 (excluding root tips) as a continuous measure
[b] Additionally adjusted for sex, income, education, and housing status
[c] Additionally adjusted for smoking and alcohol consumption
[d] Additionally adjusted for physical activity
[e] Final model adjusted for number of retained teeth and physical activity

Table 3.5. Mean allostatic load scores at age 80 in relation to periodontal inflammation at age 70, N=228

Analysis of variance		Serial adjustments of mean allostatic load scores at age 80 (SE)				
	n	Crude	Model 1[a]	Model 2[b]	Model 3[c]	Model 4[d]
Mean MCPI scores						
Q1 (0.40 – 1.54)	44	2.68 (0.26)	2.82 (0.32)	2.72 (0.37)	2.62 (0.40)	1.89 (0.46)
Q2 (1.56 – 2.10)	44	2.07 (0.26)	2.17 (0.29)	2.08 (0.34)	2.06 (0.40)	1.80 (0.42)
Q3 (2.11 – 2.90)	39	2.36 (0.28)	2.41 (0.29)	2.28 (0.35)	2.35 (0.40)	2.07 (0.43)
Q4 (2.94 – 6.00)	34	2.32 (0.30)	2.29 (0.30)	2.14 (0.38)	2.11 (0.42)	1.66 (0.44)
Edentulous	67	2.48 (0.21)	2.30 (0.31)	2.25 (0.38)	2.10 (0.40)	2.55 (0.44)
P for linear trend		0.96	0.39	0.39	0.41	0.44
% MCPI scores ≥ 3						
Q1 (0 – 5.0%)	46	2.59 (0.26)	2.71 (0.30)	2.61 (0.36)	2.55 (0.40)	1.89 (0.43)
Q2 (6.3 – 25.0%)	43	2.21 (0.27)	2.34 (0.31)	2.23 (0.36)	2.29 (0.42)	2.15 (0.44)
Q3 (26.1 – 62.5%)	40	2.38 (0.28)	2.42 (0.28)	2.35 (0.34)	2.44 (0.40)	1.86 (0.42)
Q4 (63.6 – 100%)	32	2.22 (0.31)	2.18 (0.32)	2.01 (0.38)	1.99 (0.42)	1.52 (0.46)
Edentulous	67	2.48 (0.21)	2.30 (0.31)	2.26 (0.38)	2.12 (0.40)	2.46 (0.43)
P for linear trend		0.92	0.35	0.37	0.36	0.66
% MCPI scores ≥ 6						
0%	104	2.44 (0.17)	2.48 (0.19)	2.38 (0.26)	2.35 (0.31)	1.91 (0.32)
3.6 – 18.2%	32	2.09 (0.31)	2.17 (0.35)	2.01 (0.40)	1.93 (0.45)	1.66 (0.49)
19.1 – 80%	25	2.36 (0.35)	2.36 (0.35)	2.22 (0.42)	2.26 (0.47)	1.62 (0.47)
Edentulous	67	2.48 (0.21)	2.38 (0.30)	2.36 (0.36)	2.24 (0.39)	2.56 (0.41)
P for linear trend		0.88	0.70	0.77	0.58	0.45

[a] Mean allostatic load scores adjusted for the number of retained teeth at age 70 (excluding root tips) as a continuous measure
[b] Additionally adjusted for sex, income, education, and housing status
[c] Additionally adjusted for smoking and alcohol consumption and physical activity
[d] Additionally adjusted for allostatic load at age 70 as a continuous variable

Table 3.6a. Adjusted[a] mean levels of periodontal disease indices at age 85 by quartiles of allostatic load scores at age 70, N=53

Allostatic load at 70		Pocket Depth Severity and Extent					
		% PD $\geq$ 3 mm		% PD $\geq$ 4 mm		% PD $\geq$ 5 mm	
	n	Mean (SE)	P-value[b]	Mean (SE)	P-value[b]	Mean (SE)	P-value[b]
Quartile 1 (score=0)	18	59.0 (4.8)	0.01	17.8 (3.8)	0.003	9.3 (3.0)	0.03
Quartile 2 (score=1)	16	57.8 (5.1)		15.0 (4.0)		6.7 (3.2)	
Quartile 3 (score=2 – 3)	11	55.8 (6.1)		19.5 (4.8)		9.9 (3.8)	
Quartile 4 (score=4 – 8)	8	79.9 (7.2)		36.1 (5.7)		19.3 (4.5)	

Allostatic load at 70		Clinical attachment loss Severity and Extent					
		% CAL $\geq$ 3 mm		% CAL $\geq$ 4 mm		% CAL $\geq$ 5 mm	
	n	Mean (SE)	P-value[b]	Mean (SE)	P-value[b]	Mean (SE)	P-value[b]
Quartile 1 (score=0)	18	58.4 (5.3)	0.10	45.1 (6.2)	0.07	32.3 (5.7)	0.06
Quartile 2 (score=1)	16	48.1 (5.6)		33.7 (6.4)		23.1 (5.9)	
Quartile 3 (score=2 – 3)	11	56.2 (6.7)		37.4 (7.7)		25.5 (7.1)	
Quartile 4 (score=4 – 8)	8	68.0 (7.9)		57.3 (9.2)		44.5 (8.4)	

[a] Means were adjusted for number of retained teeth
[b] P-value for t-test comparing quartile 4 to quartiles 1 – 3

Table 3.6b. Association (3a): Association of allostatic load scores at age 70 with measures of periodontal disease at age 85, N=53

Multivariable models	Percentage of periodontal pocket depths (PD) at varying thresholds											
	% PD $\geq$ 3 mm				% PD $\geq$ 4 mm				% PD $\geq$ 5 mm			
	Coefficient (ß)	SE	R^2	P-value	Coefficient (ß)	SE	R^2	P-value	Coefficient (ß)	SE	R^2	P-value
AL 70 (Q4 vs. 1–3)												
Model 1[a]	22.1	7.68	0.18	0.006	19.0	6.12	0.30	0.003	10.9	4.79	0.28	0.03
Model 2[b]	15.9	7.41	0.34	0.04	13.9	6.03	0.41	0.03	6.9	4.77	0.38	0.16
Model 3[c]	14.3	7.36	0.37	0.06	13.4	6.12	0.42	0.03	6.3	4.82	0.39	0.20
Model 4[d]	14.6	7.22	0.49	0.05	12.2	6.85	0.50	0.08	5.8	5.79	0.45	0.32
Final Model[e]	15.9	7.41	0.34	0.04	13.9	6.03	0.41	0.03	6.9	4.77	0.38	0.16

Multivariable models	Percentage of clinical attachment loss (CAL) at varying thresholds											
	% CAL $\geq$ 3 mm				% CAL $\geq$ 4 mm				% CAL $\geq$ 5 mm			
	Coefficient (ß)	SE	R^2	P-value	Coefficient (ß)	SE	R^2	P-value	Coefficient (ß)	SE	R^2	P-value
AL 70 (Q4 vs. 1–3)												
Model 1[a]	14.3	8.57	0.34	0.10	18.6	9.92	0.33	0.07	17.5	9.10	0.32	0.06
Model 2[b]	11.9	9.12	0.36	0.20	13.5	10.3	0.37	0.20	11.8	9.27	0.39	0.21
Model 3[c]	10.8	9.23	0.37	0.25	12.0	10.4	0.39	0.26	10.8	9.39	0.40	0.26
Model 4[d]	7.9	10.3	0.39	0.45	10.6	12.3	0.39	0.40	9.6	11.7	0.41	0.42
Final Model[e]	11.9	9.12	0.36	0.20	13.5	10.3	0.37	0.20	11.8	9.27	0.39	0.21

[a] Adjusted for number of retained teeth at age 85 (excluding root tips)
[b] Additionally adjusted for sex and income (low, high)
[c] Additionally adjusted for smoking status (current smokers [ages 70 – 80], never/ex-smokers)
[d] Additionally adjusted for prior periodontal inflammation as measured by mean MCPI scores (continuous variable). _Note_: sample size was reduced to 42 participants
[e] Final model was adjusted by number of retained teeth, sex, and income

Table 3.7a. Association (3b): Adjusted[a] mean levels of periodontal disease indices at age 85 by quartiles of allostatic load scores at age 80, N=87

Allostatic load at 80		Mean periodontal pocket depths (PD) at varying thresholds					
		% PD ≥ 3 mm		% PD ≥ 4 mm		% PD ≥ 5 mm	
	n	Mean (SE)	P-value[b]	Mean (SE)	P-value[b]	Mean (SE)	P-value[b]
Quartile 1 (score=0)	32	59.3 (3.91)	0.12	19.5 (3.34)	0.30	8.3 (2.31)	0.53
Quartile 2 (score=1)	24	56.6 (4.56)		17.9 (3.90)		9.8 (2.70)	
Quartile 3 (score=2 – 3)	15	55.2 (5.72)		17.4 (4.89)		11.0 (3.38)	
Quartile 4 (score=4 – 8)	16	67.1 (5.52)		23.9 (4.72)		11.7 (3.26)	
Allostatic load at 80		Mean clinical attachment loss (CAL) at varying thresholds					
		% CAL ≥ 3 mm		% CAL ≥ 4 mm		% CAL ≥ 5 mm	
	n	Mean (SE)	P-value[b]	Mean (SE)	P-value[b]	Mean (SE)	P-value[b]
Quartile 1 (score=0)	32	58.0 (3.99)	0.50	42.8 (4.43)	0.70	30.4 (4.04)	0.92
Quartile 2 (score=1)	24	62.9 (4.64)		49.3 (5.16)		34.6 (4.71)	
Quartile 3 (score=2 – 3)	15	48.1 (5.82)		37.6 (6.47)		26.7 (5.90)	
Quartile 4 (score=4 – 8)	16	53.3 (5.61)		41.2 (6.24)		30.4 (5.70)	

[a] Means were adjusted for number of retained teeth
[b] P-value for t-test comparing quartile 4 to quartiles 1 – 3

Table 3.7b. Association (3b): Association of allostatic load scores at age 80 with measures of periodontal disease at age 85, N=87

| Multivariable models | Percentage of periodontal pocket depths (PD) at varying thresholds | | | | | | | | | | | |
| | % PD ≥ 3 mm | | | | % PD ≥ 4 mm | | | | % PD ≥ 5 mm | | | |
	Coefficient (ß)	SE	R^2	P-value	Coefficient (ß)	SE	R^2	P-value	Coefficient (ß)	SE	R^2	P-value
AL 80 (Q4 vs. 1 – 3)												
Model 1[a]	9.5	6.05	0.05	0.12	5.4	5.17	0.11	0.30	2.3	3.58	0.15	0.53
Model 2[b]	6.9	6.01	0.13	0.26	3.2	5.07	0.20	0.53	0.9	3.49	0.24	0.80
Model 3[c]	7.7	5.93	0.17	0.20	3.9	5.00	0.24	0.44	1.3	3.46	0.28	0.70
Model 4[d]	9.9	5.99	0.31	0.11	6.3	5.19	0.32	0.23	3.2	3.85	0.32	0.40
Final Model[e]	7.4	5.88	0.16	0.21	3.4	4.97	0.23	0.50	0.8	3.46	0.26	0.81

[a] Adjusted for number of retained teeth at age 85 (excluding root tips)
[b] Additionally adjusted for sex and income (low versus high)
[c] Additionally adjusted for smoking status (current smokers [ages 70 – 80] versus never or ex-smokers)
[d] Additionally adjusted for prior periodontal inflammation as measured by mean MCPI scores (continuous variable). Note: sample size was reduced to 63 participants
[e] Final model was adjusted by number of retained teeth, sex, and smoking status

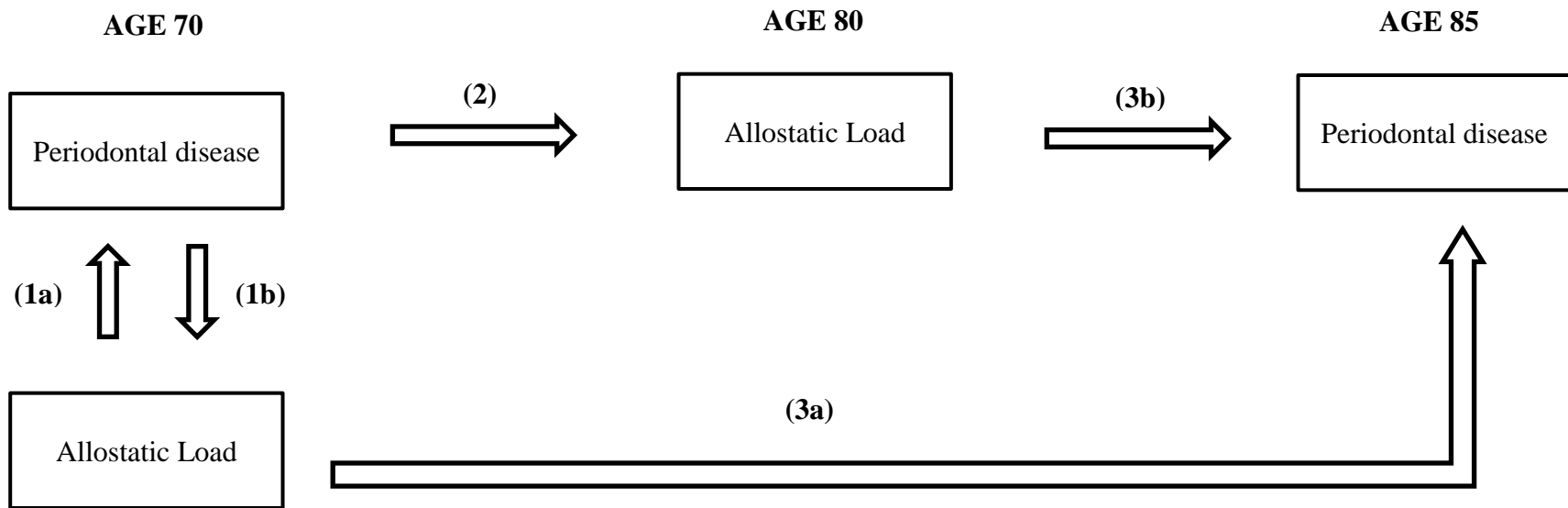**AGE 70** **AGE 80** **AGE 85**

Figure 3.1. Associations (1-3) explored in the current study. Association (1) describes cross-sectional (and bidirectional) relationships between allostatic load and periodontal disease at age 70. Association (2) describes the longitudinal relationship between periodontal disease at age 70 and allostatic load at age 80. Association (3) describes the longitudinal relationships between allostatic load at age 70 and periodontal disease at age 85 (3a) and allostatic load at age 80 and periodontal disease at age 85 (3b)
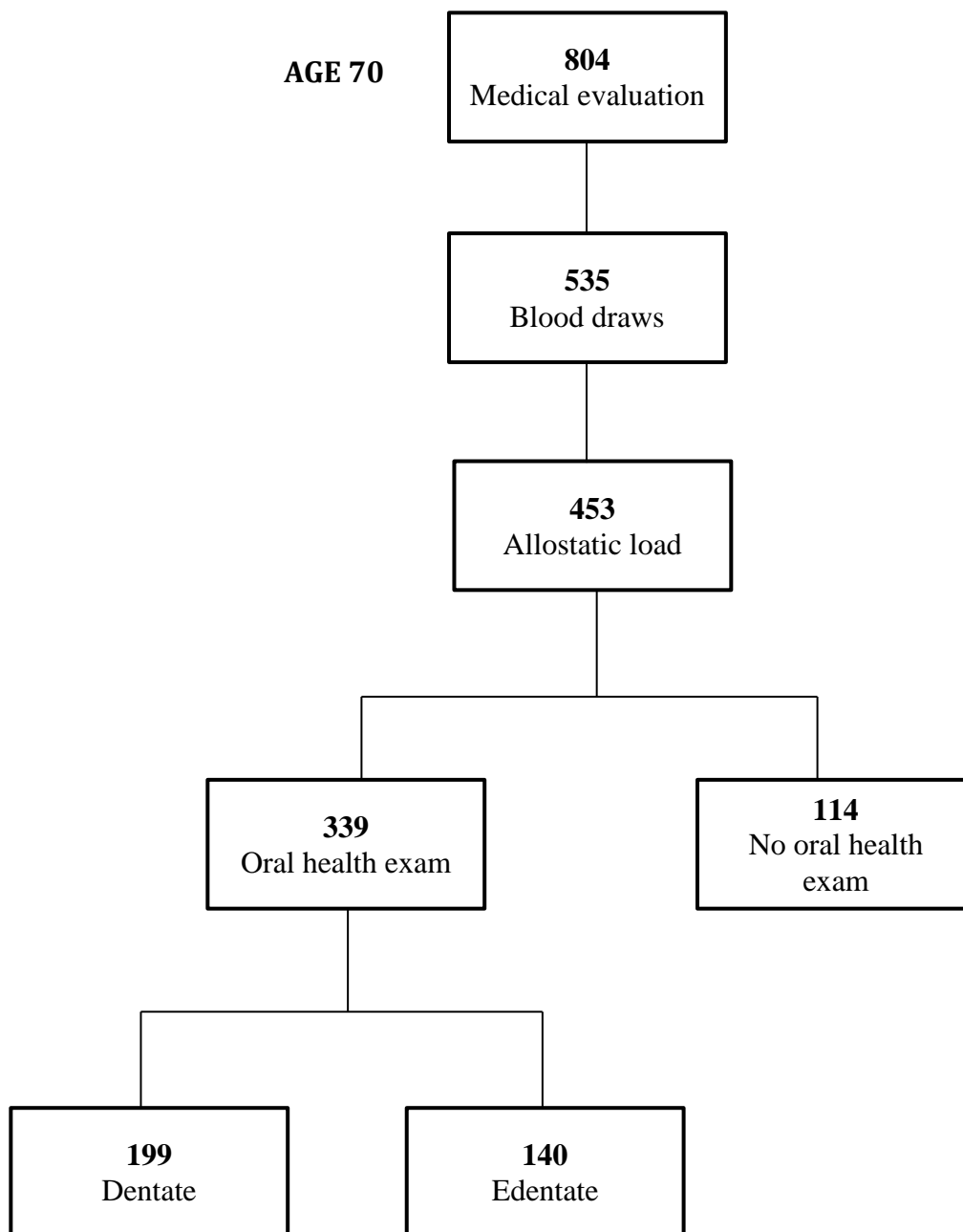
Figure 3.2. Flow chart of study population for assessing the cross-sectional association of allostatic load with periodontal disease at age 70 (*association 1 from figure 3.1*)
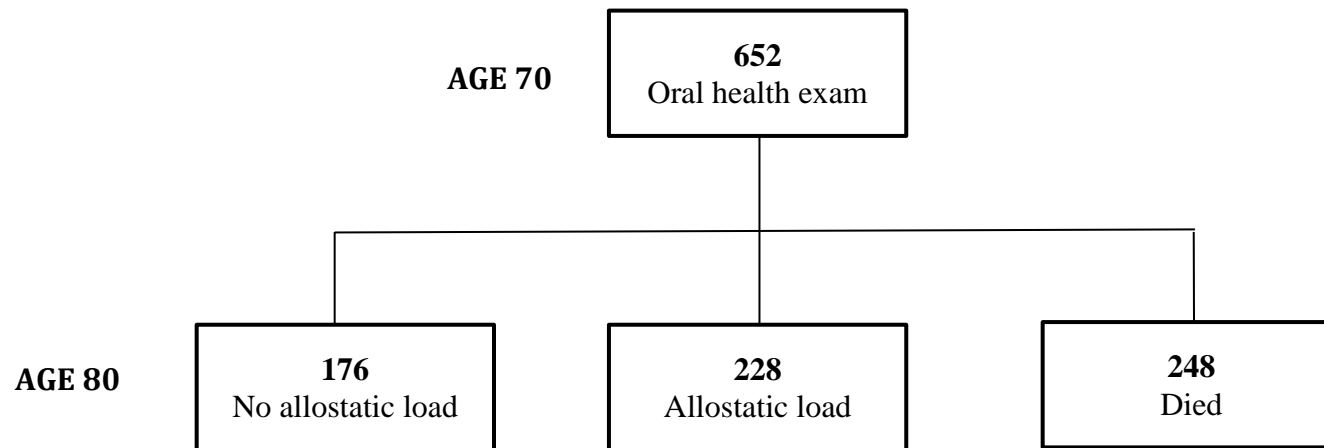
Figure 3.3. Flow chart describing the distribution of study population that was to assess the association of periodontal disease at age 70 with allostatic load at age 80 (*association 2 from figure. 3.1*)
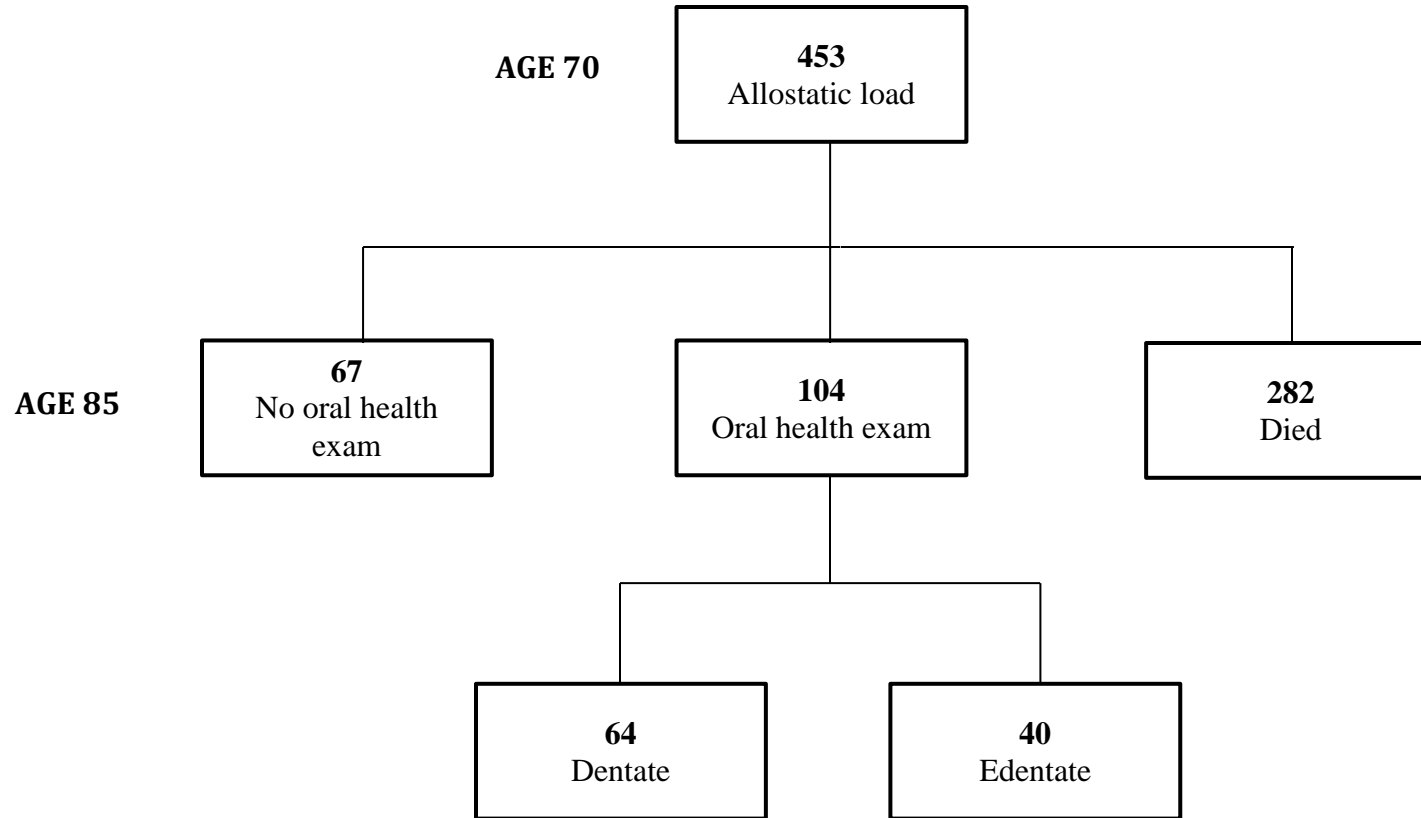
Figure 3.4a.

Figure 3.4b.
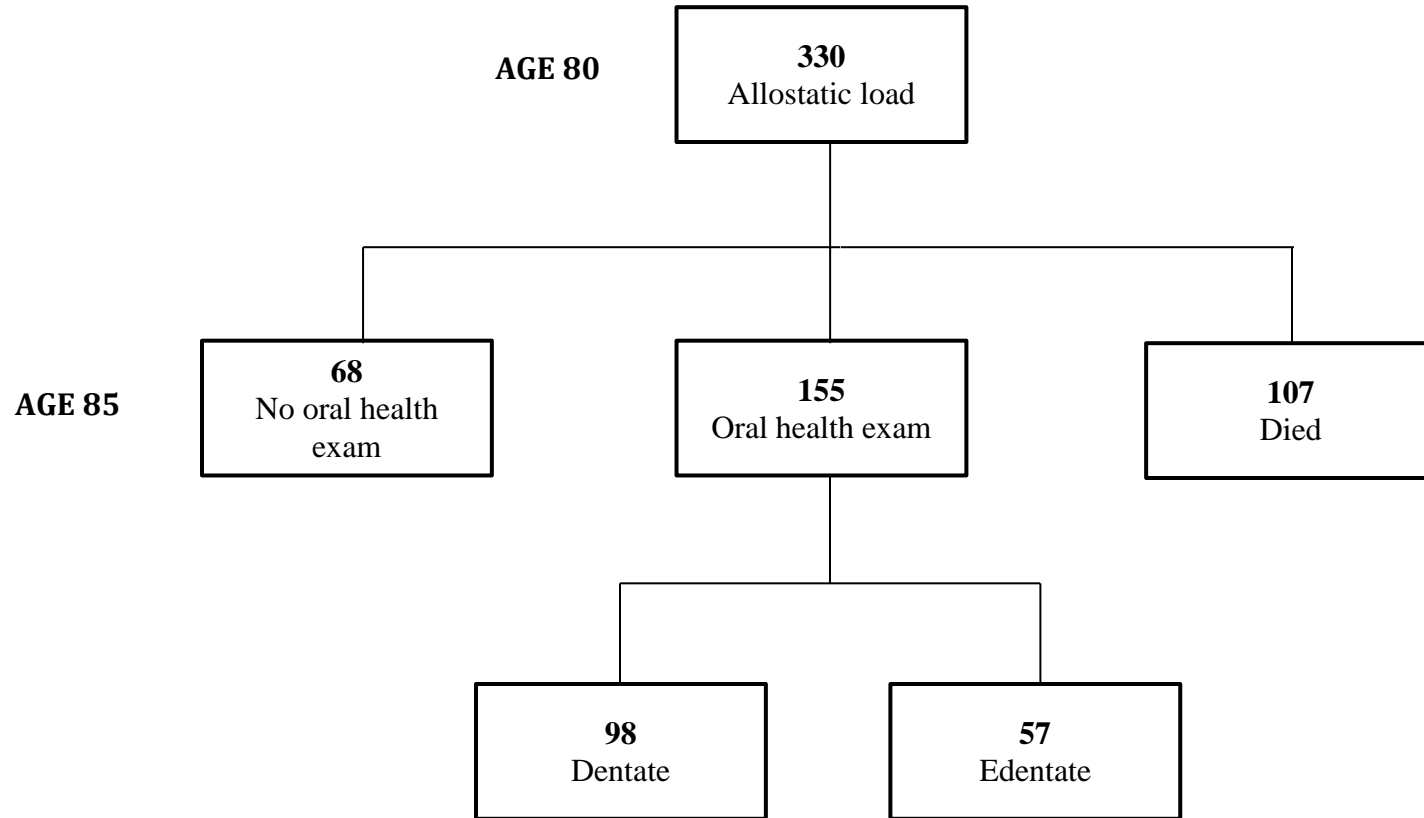
Figure 3.4. Flow charts describing the distribution of the study populations that were used to assess longitudinal associations of allostatic load with periodontal disease [***associations (3a) and (3b) described in Figure 3.1***]. The top panel flowchart **(3.4a)** depicts the association between allostatic load data at age 70 and oral health at age 85. The bottom panel flowchart **(3.4b)** depicts the distribution of participants with allostatic load data at age 80 and oral health at age 85

CHAPTER 4:

ALLOSTATIC LOAD AND MORTALITY RISK IN OLDER DANES: IS THE

ASSOCIATION MODIFIED BY PERIODONTAL INFLAMMATION AND DENTATE

STATUS?

4.1. ABSTRACT

Background: Studies suggest that allostatic load is associated with periodontal disease, tooth loss, and all-cause mortality. Whether periodontal disease and dentate status modifies the relationship between allostatic load and mortality is currently unknown.

Objective: Using prospective data from the 1914 Glostrup aging birth cohort, we explored whether periodontal disease and dentate status modifies the relationship between allostatic load and mortality.

Methods: Seven hundred thirty four participants at baseline (age 70) were followed for 25 years. Our primary end point was all-cause mortality and our secondary endpoint was cardiovascular disease (CVD) mortality. Kaplan Meier curves and Cox proportional hazard models were used to examine associations between periodontal disease, allostatic load and mortality. To assess for effect modification, we stratified allostatic load -mortality models by severity of periodontal disease and edentulism and tested for interaction using likelihood ratio tests in nested models.

Results: Compared to those with low periodontal inflammation, participants with higher periodontal inflammation and those who were edentate had a higher hazard risks of death (adjusted hazard ratio [HR]=1.19, $0.88 - 1.60$ and HR=1.44, $1.06 - 1.94$; respectively). Compared to low allostatic load at age 70 and 80, high AL was consistently positively associated with mortality, with stronger relationships for CVD mortality. Participants with low allostatic load at age 70 but high AL at age 80 exhibited a higher risk of mortality than those who remained low (adjusted HR=2.04, $1.21 - 3.45$). The association between high allostatic load at age 80 and mortality was stronger among the edentate, as compared to the dentate (adjusted HR=2.06, $1.26 - 3.37$ vs. adjusted HR= 1.08, 95% CI: $0.78–1.49$; *P* for interacton=0.02).

Conclusions: Our findings suggest that dentate status modifies the relationship between AL and mortality risk..

4.2. Introduction

Older adults appear to exhibit greater immunological impairments associated with chronic stress and depression than younger adults[1,2]. Chronic stress is thought to accelerate the natural decline of age-related immune function through the action of the hypothalamic-pituitary-axis (HPA) and sympathetic-adrenal-medullary (SAM) pathways, which can modulate inflammatory activity[3]. The theoretical model of allostatic load, defined as cumulative biological damage resulting from over-activation of the HPA and SAM pathways during repeated challenges[4,5], has been a useful framework for evaluating the influence of chronic exposure to stress with morbidity at older age. However, few studies have investigated the potential role of allostatic load on mortality.

To date, only two large prospective studies have assessed the relationship between allostatic load and mortality in older adult populations. Findings from the MacArthur Study of Successful Aging of 70 – 79 year-olds in the US showed a dose-dependent positive association between AL and all-cause mortality after 7.5 years of follow-up[6]. Furthermore, an improvement in markers of allostatic load after only 2.5 years of follow-up yielded a markedly lower risk of overall death[7]. In another cohort of older Taiwanese adults aged 50 – 90 years, Goldman et al reported a positive association between levels of neuroendocrine and inflammatory components of allostatic load and all-cause mortality after 3 years of follow-up[8]. However, more studies incorporating cause-specific deaths and with longer follow-up are necessary in order to establish a causal relationship between changes in allostatic load at older age and mortality.

Inflammatory processes could mediate the potential relationship between allostatic load and mortality. It is possible that dysregulated immune function exacerbated by chronic stress can render older adults more susceptible to infection and immune-mediated illnesses such as

periodontal disease[9,10]. Characterized by bacterially induced inflammation of gingival tissues, periodontal disease is a progressive condition that results in the formation of periodontal pockets, destruction of supporting tissues, and eventual tooth loss[11]. Compared to their younger counterparts, older adults bear the highest prevalence and severity of periodontal disease[12]. Using a measure of allostatic load comprised of physiological markers that span the neuroendocrine, metabolic, cardiovascular, and immune systems, cross-sectional studies have shown positive associations between levels of allostatic load and clinical measures of periodontal disease in adults[13,14]. Furthermore, for the first time, we reported similar associations between allostatic load and periodontal disease in a cohort of older Danish adults using prospective data (chapter 3). While insights into immune modulation and chronic stress among older adults have steadily risen, mostly from studies involving viral infections[15], few studies have investigated whether chronic stress accelerates risk of mortality through inflammatory conditions such as periodontal disease. Understanding the role of periodontal disease in this proposed pathway is of public health importance since chronic infection with oral pathogens is potentially modifiable[16].

The overarching goal of the present study is to examine whether periodontal disease moderates the association between allostatic load and mortality over 25 years of follow-up time. To achieve this, we first examine the association between periodontal disease/tooth loss and mortality using data from the 1914 Glostrup longitudinal study, building on previous work published from our group[17,18]. Secondly, we evaluate the prospective association between allostatic load and mortality using measures of allostatic load collected at ages 70 and 80. Lastly, we explore the potential effect modification of the relationship between allostatic load and mortality by periodontal disease and tooth loss.

4.3. Methods

4.3.1. Study population

A full description of the cohort has been previously provided elsewhere (**chapter 2**). The present study is based on longitudinal data from the 1914 Glostrup Aging studies with 25 years of follow-up time (1984 – 2009). At baseline, a random sample of 1,119 community dwelling adults aged 70 years from Glostrup, Denmark was recruited. A total of 748 older adults participated in-home visits at age 70, and 734 completed a structured questionnaire that ascertained information about social and behavioral characteristics; this comprised our study population at baseline. Of these, 453 participants had complete data for assessment of allostatic load, and 339 had additionally completed a comprehensive oral examination; 199 were dentulous and 140 edentulous. This comprised the study population for the oral health/allostatic load sub-cohort. At age 80, 330 participants had complete data for assessment of allostatic load. When we restricted to those who had complete data regarding allostatic load at ages 70 and 80, there was 164 participants; this comprised our allostatic load sub-cohort.

4.3.2. Periodontal inflammation

A full description of the complete oral examination has been previously described (**chapter 2**). In brief, the periodontal assessment was performed using a modified version of the Community Periodontal Index (MCPI), which measures presence of periodontal inflammation and severity[19]. A dentist calibrated in all assessments recorded periodontal pocket depths for each tooth (excluding root tips) in millimeters using a periodontal probe. Each tooth received one of the following scores: 0= no sign of inflammation or periodontal pockets around the tooth; 1= signs of inflammation partially circumscribing the tooth; no periodontal pockets, 2= signs of

inflammation circumscribing the tooth but no periodontal pockets; 3= signs of inflammation circumscribing the tooth, periodontal pockets 4 – 6 mm; 6= signs of inflammation circumscribing the tooth, periodontal pockets > 6mm; and 8=signs of inflammation circumscribing the tooth, advanced periodontal destruction.

4.3.3. Physiological markers

The majority of physiological markers for assessment of allostatic load were measured in blood collected at the Copenhagen County Hospital (CCH) in Glostrup. Fasting blood samples were collected at age 70 and non-fasting blood samples at age 80. Laboratory methods and quality control procedures are described in detail elsewhere (chapters 2 and 3). Briefly, blood was processed on the same day of collection and snap frozen for same-day analysis of lipids (total cholesterol, high-density lipoprotein [HDL], and triglycerides [age 70 only]), serum albumin (age 80 only) and blood glucose (age 80 only)[20]. Inflammatory cytokines IL-6 and tumor necrosis factor (TNF)-α were assayed from blood sera at age 80 using enzyme-linked immunosorbent assay (ELISA) kits (catalogue numbers HSTA50 and HS600, R&D systems, Minneapolis MN)[21].

Participants also underwent a medical evaluation at CCH. During the visit, diastolic and systolic blood pressures were measured in a supine position after 10 minutes of rest using a Doppler technique[20]. Resting heart rate was determined from the R-R interval on an ECG. At age 70, forced expiratory volume in the first second (FEV_1) was measured using a Godart bell respirograph and Wright peak flowmeter. Height and weight were recorded and body mass index (BMI) calculated as the ratio of weight in kilograms divided by square of height in meters.

4.3.4. Outcome ascertainment

Information regarding mortality was collected on all participants. Deaths were obtained

from the Danish Central National Register. All-cause mortality served as our primary end point,

whereas death from cardiovascular disease was our secondary end point. Participants were

followed for 25 years, from September/October 1984 to October 16, 2009. Deaths from

cardiovascular diseases included International Classification of Diseases, Eighth Revision (ICD-

8) codes 390 – 458 and ICD-10 codes I00 – I99[22].

4.3.5. Statistical Analyses

Measures of allostatic load at age 70 included BMI, HDL, ratio of total cholesterol/HDL,

triglycerides, resting diastolic and systolic blood pressure, resting heart rate, and FEV_1, while

measures of allostatic load at age 80 included: BMI, HDL, ratio of total cholesterol/HDL, non-

fasting blood glucose, resting diastolic and systolic blood pressure, resting heart rate, serum

albumin, IL-6, and TNF-α; a full description of the derivation of allostatic load is provided in the

Methods appendix. We first treated allostatic load as a continuous variable and found that risk

of mortality linearly increased with higher scores Therefore, allostatic load scores were

dichotomized and cut-points were defined at the median; low allostatic load at ages 70 and 80

included scores of 0 – 1 and 0 – 2, and high levels included scores of 2 – 8 and 3 – 9,

respectively. Mean percentage of teeth with MCPI scores ≥ 3 was dichotomized at the median;

low periodontal inflammation was defined as < 25% teeth with MCPI scores ≥ 3 and high

periodontal inflammation as ≥ 25% teeth with MCPI scores ≥ 3. Survival curves at ages 70 and

80 were constructed using the Kaplan-Meier method and compared with the log-rank test.

Univariate Cox proportional hazards models were constructed to examine covariates such as sex,

income, education, smoking, alcohol consumption, and physical activity as predictors of mortality.

To evaluate associations between allostatic load with mortality, we constructed Cox proportional hazards models controlling for strong predictors of mortality and other covariates in serial adjustments in order to examine patterns of potential confounding. We evaluated for multicollinearity by examining intercorrelations between independent variables using variance inflation factors (VIF) or tolerance values[23]. The proportional hazards assumption was confirmed by inspection of log (-log [survival]) curves as well as examination of time-dependent covariates[24].

To evaluate whether the allostatic load -mortality relationship differed by periodontal inflammation, we stratified the models by edentulous status and by severity of periodontal inflammation, with cut-points chosen at the median. We explored an interaction between edentulism and allostatic load using a cross-product term entered into a multivariable Cox model and used a likelihood ratio test to assess statistical significance, comparing models with and without the interaction term. Hazard-risks were additionally illustrated by adjusted survival curves using an inverse probability weighting approach to control for covariates[25]. All analyses were conducted with the SAS 9.2 statistical software package (Cary, NC), and all tests were two-sided.

4.4. Results

The majority of participants at baseline (age 70) had > 9 years of education (57%) and high income (79%; table 4.1). While there were equal proportions of men and women, men were more likely to be current smokers and ever drinkers than women (58% vs. 47% and 81% vs.

47%, respectively; *P*-values<0.0001). Similar distributions in characteristics were evident among participants of the oral health/ allostatic load sub-cohort. There were no differences in mean allostatic load and periodontal inflammation between the baseline and oral health/ allostatic load sub-cohorts. However, the proportions of current smokers and participants with <9 years of education were appreciably lower in the allostatic load sub-cohort as compared to the baseline cohort (*P*-values < 0.05). By the end of the follow-up period, a total of 689 (93.9%) had died from the baseline cohort; 320 (94.4%) died from the oral health/ allostatic load sub-cohort; and 141 (88.4%) died from the AL sub-cohort. Strong baseline predictors of all-cause mortality were being male (unadjusted hazard ratio [HR]= 1.56, 95% confidence interval [CI]= 1.34–1.81), low versus high income (unadjusted HR= 1.22, 95% CI= 1.01–1.47), current versus never smoking (unadjusted HR= 1.63, 95% CI= 1.35–1.98), and sedentary/low exercise versus moderate/vigorous exercise (unadjusted HR= 1.93, 95% CI= 1.24–2.98).

When we evaluated the association between periodontal inflammation and all-cause mortality, participants with lower levels of periodontal inflammation exhibited better overall survival than those with high periodontal inflammation, whereas edentulous participants exhibited the poorest overall survival (log-rank *P*=0.0002; figure 4.1a). Moreover, older adults who lost all their teeth during the first 15 years of follow-up (n=27) exhibited better overall survival than those who remained edentulous (n=277), but worse than those who remained dentulous (n=78) during that same period (log-rank *P*=0.0041; **figure 4.1b**). In multivariable analysis, the HR of overall death was 24% higher among participants with a high level of periodontal inflammation and 50% higher in edentulous participants as compared to those with a low level after adjusting for sex, education, income, and housing status (*P* for trend <0.01; **table 4.2**, model 1). Further adjustment by smoking, alcohol consumption and physical activity only

modestly attenuated the association, and the significant positive trend persisted. Similar associations were evident when we used cardiovascular mortality as the endpoint, and stronger when we restricted these models to high allostatic load at baseline (data not shown).

When we examined the relationship between allostatic load and all-cause mortality, participants with low allostatic load at baseline exhibited better overall survival than those with high allostatic load (log-rank P <0.01; **figure 4.2a**). When stratified by gender, women with low allostatic load had the best survival while men with high allostatic load had the worst (log-rank P <0.0001; **figure 4.2b**). Similar associations were observed when we evaluated the relationship between allostatic load at age 80 and overall survival (**figures 4.2c,d**). In multivariable models, the higher hazard rate of overall death was higher in participants with high allostatic load as compared to those with low allostatic load (HRs=1.29 and 1.34 for allostatic load at ages 70 and 80, respectively; P-values <0.05, table 4.3). Adjustment for sex, income, education, housing status, smoking, alcohol consumption, and physical activity did not change the estimates by an appreciable amount (models 2-3). However, the association was stronger when we used cardiovascular death as the endpoint (HR for high versus low allostatic load at baseline= 1.80, 95% CI: 1.31–2.47; HR for high versus low allostatic load at age 80= 1.90, 95% CI: 1.12–3.23). Moreover, a positive dose response relationship was evident when we evaluated associations between allostatic load in quartiles and cardiovascular death (P-values for trend <0.05; data not shown).

When we restricted to those with complete allostatic load data at ages 70 and age 80 (AL sub-cohort), participants with low allostatic load at age 70 and high allostatic load at age 80 had a significantly higher HR of overall death than those with low allostatic load at both time points (HR=2.28, 95% CI: 1.37–3.78; table 4.3). Adjustment with social and behavioral characteristics

slightly attenuated the relationship, but the estimates remained strong (model 3). The association was even stronger when we evaluated the hazard risk of cardiovascular death (adjusted HR=3.51 comparing low allostatic load at age 70, high allostatic load at age 80 versus low allostatic load at both ages, 95% CI: 1.43 – 8.64). A similar positive association was found comparing high allostatic load with low allostatic load at both ages with all-cause and cardiovascular death, although the estimates were less strong and not statistically significant.

When we evaluated whether associations between allostatic load and mortality differed by edentulous status, high allostatic load at age 80 revealed markedly elevated hazard-risks of overall death among 67 edentulous participants (adjusted HR=2.06, 95% CI: 1.26 – 3.37; **figure 4.3a**). By contrast, no appreciable elevation in hazard risk of overall death was evident among dentulous participants (adjusted HR= 1.08, 95% CI: 0.78–1.49 **figure 4.3b**). We observed a significant interaction when we compared models with and without an interaction term for edentulism and allostatic load (*P* for likelihood ratio test=0.02). When we further restricted the dentulous population by severity of periodontal inflammation, we found a higher HR of overall death comparing high versus low allostatic load at age 80 in participants with ≤16 teeth at baseline and high periodontal inflammation (n=51), after adjustment for sex, income, education, smoking, and physical activity (adjusted HR=1.64, 95% CI: 0.64 – 4.22). The association was stronger when we used cardiovascular death as the endpoint (adjusted HR=2.31, 95% CI: 0.59 – 8.94). By contrast, the association between allostatic load at age 80 and all-cause mortality was attenuated among participants with low inflammation and >16 teeth (n=66, adjusted HR=1.18, 95% CI: 0.74 – 1.86), and no association when we used cardiovascular death as the endpoint (adjusted HR=0.97, 95% CI: 0.40 – 2.39).

4.5. Discussion

      To our knowledge, the present study is the longest prospective investigation to have assessed the relationship between allostatic load and mortality at older age. Our analyses demonstrated a positive association of allostatic load with all-cause and cardiovascular mortality, which appeared to be more pronounced in older adults who were edentate. Our results contribute to a growing body of evidence suggesting that physiological dysregulation as measured by allostatic load at older age increases risk of mortality, and is particularly greater among those exhibiting high levels of periodontal disease/tooth loss [6-8,13,14].

      After 25 years of follow-up, our findings showed that periodontal inflammation and tooth loss is positively associated with cardiovascular and all-cause mortality. This confirms our earlier findings[17,18], and those of other studies that have investigated this relationship in diverse populations[26-29]. Our data shows that the association was fairly weak in participants with high versus low levels of periodontal inflammation but stronger in edentulous versus dentulous participants. It is possible that progression of periodontal inflammation contributed to significant tooth loss by early old age, rendering edentulous individuals increasingly more susceptible to mortality risk. One proposed (indirect) mechanism that helps explain this relationship is systemic inflammation, which can exacerbate both conditions. Studies have shown positive associations between periodontal disease or tooth loss and circulating levels of IL-6, IL-1, IL-18, C-reactive protein, TNF-α and fibrinogen[30-32], many of which are similarly elevated with cardiovascular disease[33,34]. In line with this hypothesis, we previously reported higher mean levels of IL-6 among edentulous as compared to dentulous participants, and in those with higher levels of periodontal inflammation (chapter 3). In addition, our group showed positive associations between inflammatory cytokines IL-6 and TNF-alpha and all-cause mortality after 6 years of

follow-up[21], suggesting that systemic inflammation places older adults at higher risk of mortality. While preliminary, our observation of a stronger periodontal disease-mortality association among those with high compared to low allostatic load is indicative of potential effect modification by stress. Larger studies, however, are needed to confirm this.

Similar to previous studies[6,8], our data suggest that measures of allostatic load at ages 70 and 80 were significantly associated with overall mortality. To further support the allostatic load -mortality association, we found that the mean baseline allostatic load scores for survivors at the end of follow-up was significantly lower than that for the deceased (data not shown). This relationship was even stronger and dose-dependent when cardiovascular mortality was considered, which supports evidence for a common stress pathway for both periodontal and cardiovascular diseases[14]. Our findings also showed that older adults who experienced increases in allostatic load from ages 70 to 80 exhibited the highest risk of mortality as compared to individuals who maintained a low level of allostatic load during this period, consistent with the notion that mortality risk is sensitive to changes in allostatic load scores even in older ages[7]. However, participants with high allostatic load at both time points had an elevated but non-statistically significant mortality risk than those with persistently low allostatic load, perhaps suggesting the presence of a resilient phenotype. Indeed, Danes who reached the age of 80 years would have likely exhibited a survival advantage since they had well surpassed their life expectancy of 56.2 and 59.2 years for Danish men and women born in 1914, respectively[35].

Our results offer preliminary evidence for effect modification of the relationship between AL and mortality risk by edentulism. While novel, this finding is consistent with previous studies that report positive associations between edentulism and chronic stress or depression at old age[36], and other studies that suggest a higher risk of mortality in edentate versus dentate older

adults[18,27,37,38]. While tooth loss during old age is likely to be a consequence of rapidly progressing periodontal disease, we cannot entirely rule out the possibility that it may also be due to dental caries[39]. Nevertheless, our results showed that older adults who became edentate between the age of 70 and 80 had a higher all-cause and cardiovascular mortality than those who retained their teeth during this period. This is consistent with a number of studies that show that tooth loss is associated with systemic inflammation[31,40], and cardiovascular diseases[38,41].

Several limitations in the present study merit consideration. First, our selection of allostatic load markers was governed by what was available at each assessment, so there were differences in the number and type of allostatic load markers used at baseline and age 80. Despite these differences, the associations with mortality were consistent in magnitude and direction. Secondly, while we observed gender specific differences for the allostatic load - mortality associations, we lacked sufficient power to evaluate effect modification by gender in models stratified by periodontal inflammation and tooth loss. Third, we did not have sufficient number of events to sub-categorize cardiovascular mortality into those involving coronary heart disease or stroke. Larger studies are needed to examine whether allostatic load -mortality associations differ across these other CVD-related causes of death.

Despite these limitations, the present study has several noteworthy strengths. Our study population was completely followed for the 25-year period. During that period, information involving mortality was available from the Danish Center National Register for all participants in the study. In addition, exposures of oral health were measured objectively upon clinical examination.

4.6. Conclusions

In conclusion, we found positive associations between allostatic load and all-cause mortality, with an even stronger and dose-dependent association when cardiovascular mortality was considered. Our study provided preliminary evidence of effect modification by edentulism, of the relationship between allostatic load and mortality. These findings open the possibility for the use of number of retained teeth as an additional marker of allostatic load, potentially improving the measure of physiological dysregulation at older age. Taken together, our findings underscore the importance of oral health at older age and the need for prevention measures to potentially influence the rapid decline in immune function that often accompanies the chronic exposure to stress.

4.7. References

1. Graham JE, Christian LM, Kiecolt-Glaser JK. Stress, age, and immune function: toward a lifespan approach. J Behav Med 2006;29:389-400.

2. Butcher SK, Lord JM. Stress responses and innate immunity: aging as a contributory factor. Aging Cell 2004;3:151-60.

3. Gouin JP, Hantsoo L, Kiecolt-Glaser JK. Immune dysregulation and chronic stress among older adults: a review. Neuroimmunomodulation 2008;15:251-9.

4. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 2000;21:55-89.

5. McEwen BS. Stress, adaptation, and disease. Allostasis and allostatic load. Ann N Y Acad Sci 1998;840:33-44.

6. Seeman TE, Crimmins E, Huang MH, et al. Cumulative biological risk and socio-economic differences in mortality: MacArthur studies of successful aging. Soc Sci Med 2004;58:1985-97.

7. Karlamangla AS, Singer BH, Seeman TE. Reduction in allostatic load in older adults is associated with lower all-cause mortality risk: MacArthur studies of successful aging. Psychosom Med 2006;68:500-7.

8. Goldman N, Turra CM, Glei DA, Seplaki CL, Lin YH, Weinstein M. Predicting mortality from clinical and nonclinical biomarkers. J Gerontol A Biol Sci Med Sci 2006;61:1070-4.

9. Heffner KL. Neuroendocrine effects of stress on immunity in the elderly: implications for inflammatory disease. Immunol Allergy Clin North Am 2011;31:95-108.

10. Kiecolt-Glaser JK, Glaser R. Depression and immune function: central pathways to morbidity and mortality. J Psychosom Res 2002;53:873-6.

11. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005;366:1809-20.

12. Albandar JM. Periodontal diseases in North America. Periodontol 2000 2002;29:31-69.

13. Borrell LN, Crawford ND. Social disparities in periodontitis among US adults: the effect of allostatic load. J Epidemiol Community Health 2011;65:144-9.

14. Sabbah W, Watt RG, Sheiham A, Tsakos G. Effects of allostatic load on the social gradient in ischaemic heart disease and periodontal disease: evidence from the Third National Health and Nutrition Examination Survey. J Epidemiol Community Health 2008;62:415-20.

15. Silverman MN, Pearce BD, Biron CA, Miller AH. Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. Viral Immunol 2005;18:41-78.

16. Loesche WJ, Grossman NS. Periodontal disease as a specific, albeit chronic, infection: diagnosis and treatment. Clin Microbiol Rev 2001;14:727-52, table of contents.

17. Avlund K, Schultz-Larsen K, Krustrup U, Christiansen N, Holm-Pedersen P. Effect of inflammation in the periodontium in early old age on mortality at 21-year follow-up. J Am Geriatr Soc 2009;57:1206-12.

18. Holm-Pedersen P, Schultz-Larsen K, Christiansen N, Avlund K. Tooth loss and subsequent disability and mortality in old age. J Am Geriatr Soc 2008;56:429-35.

19. Ainamo J, Barmes D, Beagrie G, Cutress T, Martin J, Sardo-Infirri J. Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). Int Dent J 1982;32:281-91.

20. Schroll M. A ten-year prospective study, 1964-1974, of cardiovascular risk factors in men and women from the Glostrup population born in 1914. Dan Med Bull 1982;29:213-52.

21. Bruunsgaard H, Ladelund S, Pedersen AN, Schroll M, Jorgensen T, Pedersen BK. Predicting death from tumour necrosis factor-alpha and interleukin-6 in 80-year-old people. Clin Exp Immunol 2003;132:24-31.

22. World Health Organization. International statistical classification of diseases and related health problems. 10th revision, 2nd edition. ed. Geneva: World Health Organization; 2004.

23. Fox J, Monette G. Generalized Collinearity Diagnostics. Journal of the American Statistical Association 1992:178-83.

24. Hosmer D, Lemeshow S, May S, eds. Applied Survival Analysis. Regression Modeling of Time to Event Data. New York: John Wiley & Sons Inc; 2008.

25. Cole SR, Hernan MA. Adjusted survival curves with inverse probability weights. Comput Methods Programs Biomed 2004;75:45-9.

26. Xu F, Lu B. Prospective association of periodontal disease with cardiovascular and all-cause mortality: NHANES III follow-up study. Atherosclerosis 2011;218:536-42.

27. Holmlund A, Holm G, Lind L. Number of teeth as a predictor of cardiovascular mortality in a cohort of 7,674 subjects followed for 12 years. J Periodontol 2010;81:870-6.

28. Soder B, Jin LJ, Klinge B, Soder PO. Periodontitis and premature death: a 16-year longitudinal study in a Swedish urban population. J Periodontal Res 2007;42:361-6.

29. Jansson L, Lavstedt S, Frithiof L. Relationship between oral health and mortality rate. J Clin Periodontol 2002;29:1029-34.

30. Meisel P, Wilke P, Biffar R, Holtfreter B, Wallaschofski H, Kocher T. Total tooth loss and systemic correlates of inflammation: role of obesity. Obesity (Silver Spring) 2012;20:644-50.

31. Loos BG. Systemic markers of inflammation in periodontitis. J Periodontol 2005;76:2106-15.

32. Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, van der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. J Periodontol 2000;71:1528-34.

33. Kaptoge S, Di Angelantonio E, Lowe G, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375:132-40.

34. Danesh J, Lewington S, Thompson SG, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. Jama 2005;294:1799-809.

35. Larsen D. Life Table HISB7: Life expectancy for new born babies by sex. In. February 15, 2012 ed: Statistics Denmark; 2012 Accessed in URL: http://www.statbank.dk/HISB7.

36. Sanders AE, Slade GD, Turrell G, Spencer AJ, Marcenes W. Does psychological stress mediate social deprivation in tooth loss? J Dent Res 2007;86:1166-70.

37. Thorstensson H, Johansson B. Does oral health say anything about survival in later life? Findings in a Swedish cohort of 80+ years at baseline. Community Dent Oral Epidemiol 2009;37:325-32.

38. Abnet CC, Qiao YL, Dawsey SM, Dong ZW, Taylor PR, Mark SD. Tooth loss is associated with increased risk of total death and death from upper gastrointestinal cancer, heart disease, and stroke in a Chinese population-based cohort. Int J Epidemiol 2005;34:467-74.

39. Mack F, Mojon P, Budtz-Jorgensen E, et al. Caries and periodontal disease of the elderly in Pomerania, Germany: results of the Study of Health in Pomerania. Gerodontology 2004;21:27-36.

40. Taylor BA, Tofler GH, Carey HM, et al. Full-mouth tooth extraction lowers systemic inflammatory and thrombotic markers of cardiovascular risk. J Dent Res 2006;85:74-8.

41. You Z, Cushman M, Jenny NS, Howard G. Tooth loss, systemic inflammation, and prevalent stroke among participants in the reasons for geographic and racial difference in stroke (REGARDS) study. Atherosclerosis 2009;203:615-9.

Table 4.1. Population characteristics of baseline cohort, oral health/allostatic load (AL) subcohort, and AL only sub-cohort

Population characteristics	Baseline cohort (N=734)	Oral health/AL sub-cohort[a] (N=339)	AL only sub-cohort[b] (N=164)
Sex, n (%)			
Males	366 (49.9%)	166 (49.0%)	80 (48.8%)
Females	368 (50.1%)	173 (51.0%)	84 (51.2%)
Education in years, n (%)			
< 9	318 (43.5%)	138 (40.7%)	56 (34.4%)
≥ 9	413 (56.5%)	201 (59.3%)	107 (65.6%)
Income, n (%)			
Low	151 (20.6%)	76 (22.4%)	22 (13.4%)
High	583 (79.4%)	263 (77.6%)	142 (86.6%)
Housing status, n (%)			
Renter/other	416 (56.7%)	195 (57.5%)	78 (47.6%)
Owner	318 (43.3%)	144 (42.5%)	86 (52.4%)
Smoking status, n (%)			
Current smoker	367 (50.1%)	169 (49.9%)	66 (40.2%)
Ex smoker	202 (27.6%)	95 (28.0%)	53 (32.3%)
Never smoker	164 (22.4%)	75 (22.1%)	45 (27.4%)
Alcohol consumption, n (%)			
Never drinker	175 (36.2%)	121 (35.7%)	62 (38.0%)
Ever drinker	308 (63.8%)	218 (64.3%)	101 (62.0%)
Physical activity, n (%)			
Sedentary	82 (11.2%)	40 (11.8%)	11 (6.7%)
Light exercise	622 (84.7%)	279 (82.3%)	144 (87.8%)
Mod/rig exercise	30 (4.1%)	20 (5.9%)	9 (5.5%)
Number of teeth			
Mean (SD)	8.4 (9.3)	8.9 (9.5)	11.6 (10.1)
AL at baseline			
Mean (SD)	2.0 (1.7)	2.0 (1.7)	1.6 (1.6)

AL= allostatic load

[a] Sub-cohort of participants who underwent an oral examination and had complete allostatic load information at age 70

[b] Sub-cohort of participants with complete allostatic load at ages 70 and 80

Table 4.2. Hazard ratios for all-cause mortality according to severity of periodontal inflammation and edentulism at baseline, N=339

Periodontal Inflamation	n	Hazard ratios for overall death (95% CI)				
		Unadjusted	Model 1[a]	Model 2[b]	Model 3[c]	Final model[d]
< 25% MCPI ≥ 3	94	1.00	1.00	1.00	1.00	1.00
≥ 25% MCPI ≥ 3	105	1.31 (0.98 – 1.75)	1.24 (0.93 – 1.66)	1.22 (0.91 – 1.64)	1.18 (0.88 – 1.59)	1.19 (0.88 – 1.60)
Edentulous	140	1.75 (1.33 – 2.30)	1.50 (1.12 – 2.02)	1.46 (1.08 – 1.97)	1.44 (1.06 – 1.94)	1.44 (1.07 – 1.94)
P for trend		<0.0001	0.007	0.01	0.02	0.01

[a] Model 1 adjusted for sex, education, income, and housing status
[b] Model 2 additionally adjusted for smoking and alcohol consumption
[c] Model 3 additionally adjusted for physical activity
[d] Final model adjusted for sex, income, education, smoking status, and physical activity

Table 4.3. Hazard ratios for all-cause mortality according to levels of allostatic load scores

Allostatic load[a] (AL)	n	Hazard ratios for overall death (95% CI)			
		Unadjusted	Model 1[b]	Model 2[c]	Model 3[d]
AL age 70					
low (scores 0 – 1)	207	1.00	1.00	1.00	1.00
high (scores 2 – 8)	246	1.29 (1.01 – 1.63)	1.20 (0.94 – 1.52)	1.32 (1.09 – 1.60)	1.30 (1.07 – 1.58)
AL age 80					
low (scores 0 – 2)	186	1.00	1.00	1.00	1.00
high (scores 3 – 10)	144	1.34 (1.07 – 1.69)	1.34 (1.06 – 1.69)	1.38 (1.08 – 1.74)	1.33 (1.04 – 1.69)
AL ages 70 and 80					
low AL 70, low AL 80	70	1.00	1.00	1.00	1.00
low AL 70, high AL 80	22	2.28 (1.37 – 3.78)	2.18 (1.30 – 3.67)	2.08 (1.23 – 3.50)	2.04 (1.21 – 3.45)
high AL 70, low AL 80	31	1.40 (0.90 – 2.20)	1.31 (0.82 – 2.07)	1.40 (0.88 – 2.22)	1.49 (0.93 – 2.40)
high AL 70, high AL 80	41	1.24 (0.82 – 1.87)	1.16 (0.76 – 1.77)	1.12 (0.73 – 1.72)	1.13 (0.74 – 1.74)

[a] Cut-point for level of allostatic load scores was defined at the median
[b] Model 1 adjusted for sex, education, income, and housing status
[c] Model 2 additionally adjusted for smoking and alcohol consumption
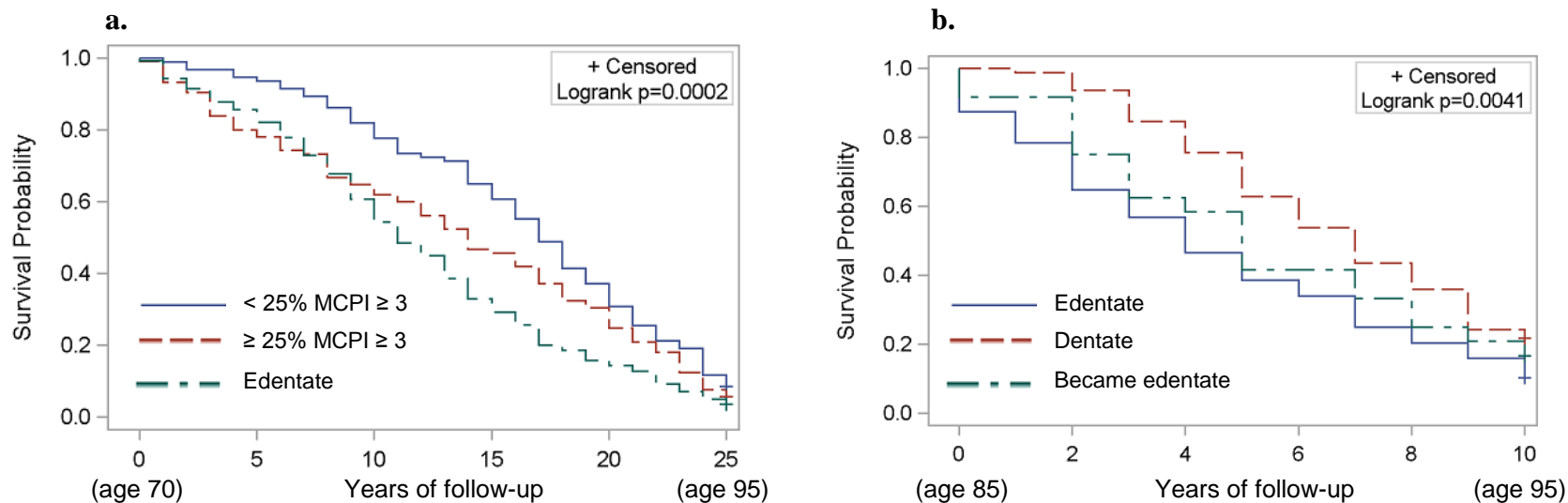[d] Model 3 additionally adjusted for physical activity

Figure 4.1. Kaplan Meier plots of overall survival by severity of periodontal inflammation and dentate status. Left panel (a) shows 25-year overall survival for participants by level of periodontal inflammation at baseline (age 70, n=339). Right panel (b) depicts 10-year overall survival for participants who were edentate (n=277), those who had at least one natural tooth (dentate) by age 85 (n=78), and those who had at least one natural tooth at age 70 but lost all their teeth (became edentate) by age 85 (n=27).
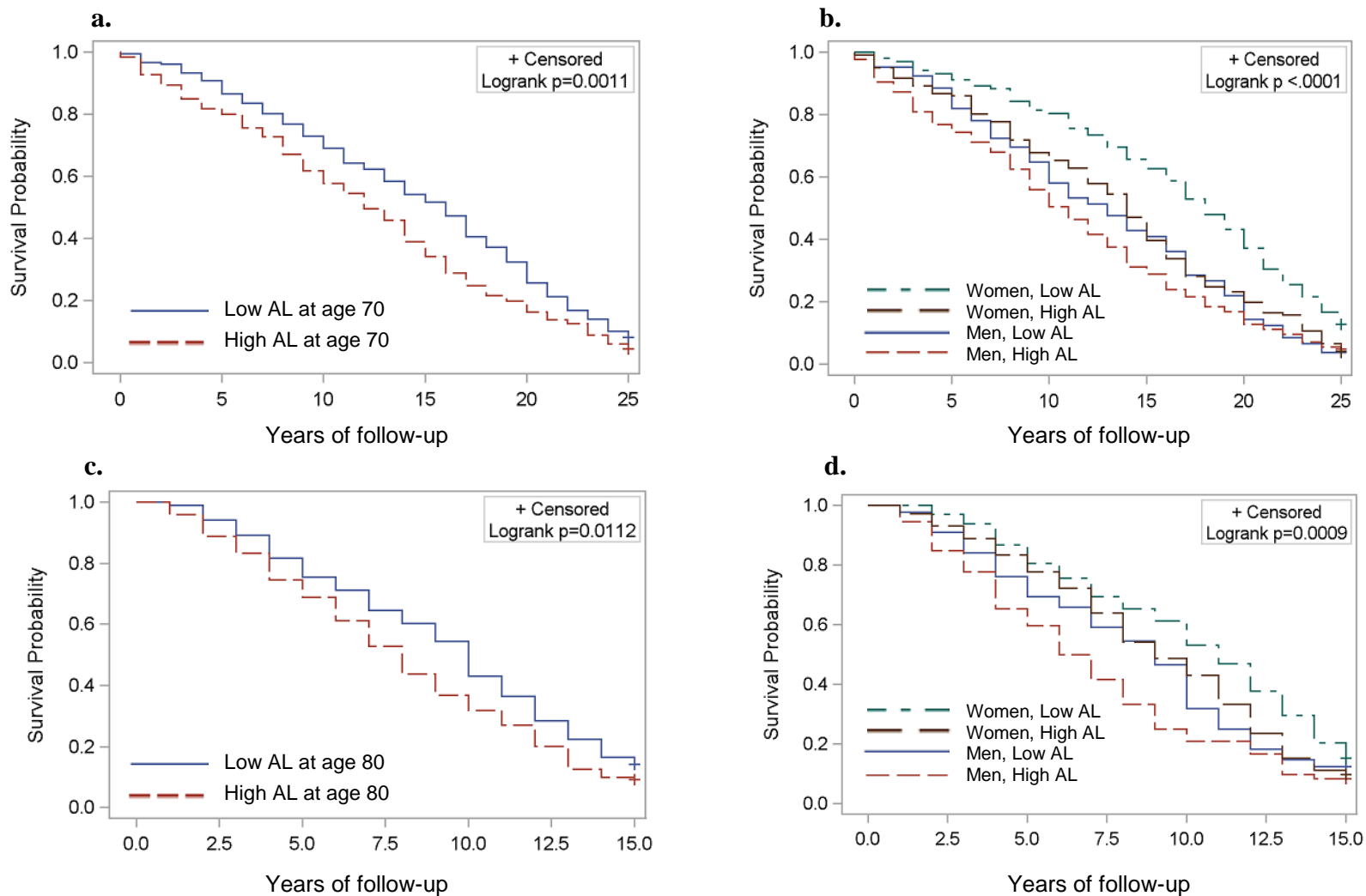
Figure 4.2. Kaplan Meier plots of overall survival. Upper left panel (a) shows 25-year overall survival by allostatic load at baseline (n=453). Upper right panel (b) depicts the allostatic load-associated survival stratified by sex. Lower left panel (c) shows 15-year overall survival by allostatic load at age 80 (n=330). Lower right panel (d) depicts the allostatic load-associated survival stratified by sex. High and low cut-points for allostatic load scores were defined at the median.
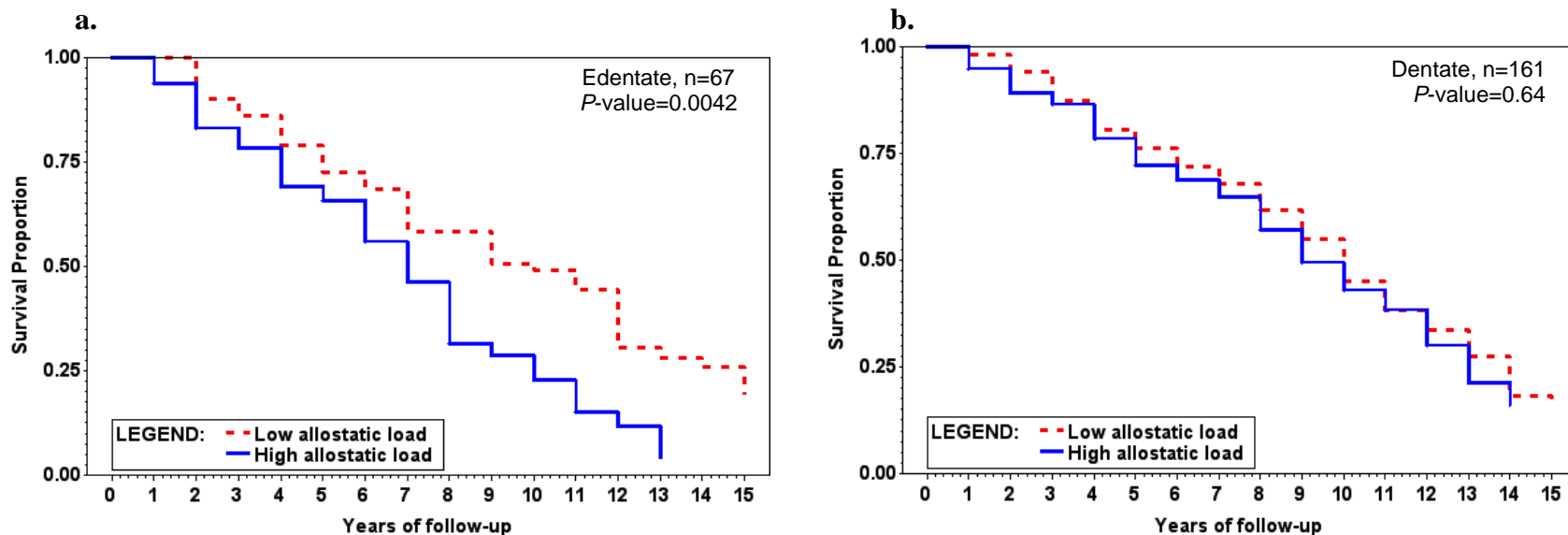
Figure 4.3. Adjusted survival curves for all-cause mortality comparing high and low allostatic load at age 80 and stratified by dentate status. Left panel shows that older adults with high allostatic load had a higher mortality risk than those with low allostatic load, among those who are edentate (HR=2.06, 95% CI: 1.26 – 3.37), but not among those who were dentate (right panel; HR= 1.08, 95% CI: 0.78–1.49). Hazard ratios adjusted by sex, income, education, smoking status, and physical activity and number of teeth (dentate panel) using an inverse probability weighting approach.

CHAPTER 5:

CONCLUSIONS

In the coming decades, the proportion of the world's older adult population (aged 65 years and over) is expected to double in size from 7.9% in 2009 to 16% (1.5 billion persons) by 2050[1,2], where the fastest growing segment of the older adult population is the portion aged 80 years, growing at a rate of 4.0% per year. Paralleling this global upward trend is a steady increase in the number of older adults retaining their natural teeth[3]. As rates of edentulism decline, the number of natural teeth potentially at risk for oral diseases rises; hence, there is widespread concern that older adults may have an increasingly greater prevalence of periodontal diseases[4]. While several risk factors for periodontal disease have been identified, variations in periodontal disease severity in older adults cannot be fully explained by these factors alone. Stress has been implicated in periodontal disease progression.

In chapter 1, I undertook a systematic review of the epidemiological and laboratory evidence in order to better understand the potential role of stress on periodontal disease in older adults. The systematic review showed that the role of stress in the progression of periodontal disease is firmly supported by the weight of the evidence. While the exact mechanism is a matter of debate, the association has a plausible pathophysiological basis. One theoretical model that captures cumulative biological damage resulting from chronic exposure to stress over the life course is allostatic load. While cross-sectional studies showed that allostatic load is associated with periodontitis longitudinal studies are necessary to help establish a causal association.

In chapter 2, I developed an index of allostatic load at 70 and 80. Using allostatic load data at age 80, I evaluated the associations between social and behavioral characteristics of the population at age 75. I found that, in males, high allostatic load was associated with no vocational training, unskilled vs. managerial occupation, low income, renting vs. owning a home and having a mostly sedentary lifestyle. These findings open avenues of intervention at different

points in time throughout the life course and with the overarching goal of reducing the burden of allostasis and promoting successful aging. Future research should consider moderating factors that may interact with social and behavioral characteristics to accelerate AL accumulation even further. We also need a better understanding of pathways that link socioeconomic adversity with disease through maladaptive behaviors across the lifespan.

In chapter 3, I evaluated cross-sectional and longitudinal associations of allostatic load and periodontal disease. I found positive nonlinear relationship between allostatic load and periodontal disease both cross-sectionally and longitudinally. While bidirectional communication exists between neuroendocrine/CNS and immune systems, I did not observe an association between periodontal inflammation and allostatic load prospectively, possibly a consequence of differential survival effects. Taken together, the findings support a growing body of evidence that suggest chronic exposure to stress contributes to the pathophysiology of inflammatory diseases. Future longitudinal studies are needed to further evaluate changes in colonization patterns of specific oral pathogens etiologically linked to periodontal disease in relation to allostatic load. This may allow for a more complete understanding and confirmation of the allostatic load-periodontal disease relationship.

In chapter 4, I found positive associations between allostatic load and all-cause mortality, with an even stronger and dose-dependent association when cardiovascular mortality was considered. The analyses also showed preliminary evidence of effect modification by edentulism, which suggests that tooth loss could modify the effect of allostatic load on mortality. Furthermore, these data open the possibility for the potential use of number of retained teeth as an additional marker of allostatic load that can serve to improve the precision in estimating risk for immune dysregulation at older age. Taken together, the findings underscore the importance

of oral health at older age and the need for prevention measures to potentially influence the rapid decline in immune function that often accompanies the chronic exposure to stress.

However, limitations to the research should be noted. Insofar as the Glostrup cohort is an older adult population with shared experiences unique to a geographic region, the findings may not be generalizable to other more diverse populations of older adults. Secondly, the measures of periodontal disease differed at ages 70 and 85, which may have introduced some degree of misclassification. Lastly, information regarding oral health behaviors was not obtained in the study – particularly at baseline – that would have otherwise helped to identify oral health-related predictors of allostatic load (aim 1). Future research should address these limitations.

While this dissertation addressed important gaps in the literature, there are three general directions in which future studies could focus on remaining questions. First, to confirm the relationship between allostatic load and periodontal disease, an exploration of bacterial colonization patterns is needed. Another direction is an exploration of associations between early developmental adversity and allostatic load in order to more clearly understand immune dysregulation and infectious disease susceptibility later in life. Finally, epigenetics offers a possible mechanism through which chronic adversity over the life course could accelerate allostatic system dysregulation.

5.1. References

1. Lutz W, Sanderson W, Scherbov S. The coming acceleration of global population ageing. Nature 2008;451:716-9.

2. United Nations. Dept. of Economic and Social Affairs. Population Division. World population ageing 2009. New York, N.Y.: United Nations, Dept. of Economic and Social Affairs, Population Division; 2010.

3. Ettinger RL. The unique oral health needs of an aging population. Dent Clin North Am 1997;41:633-49.

4. Joshi A, Douglass CW, Feldman H, Mitchell P, Jette A. Consequences of success: do more teeth translate into more disease and utilization? J Public Health Dent 1996;56:190-7.

APPENDICES

Appendix 1: Methods

The 1914 Glostrup study was initiated in 1964 to investigate risk factors for cardiovascular disease in a cohort of 50-year olds randomly drawn from 11 municipalities around Glostrup University Hospital[1]. Nine hundred seventy five 50-year olds (born in 1914) were asked to participate; 514 men and 461 women. Participants were recruited through the Danish Persons Registration department in Copenhagen. At baseline, 802 agreed to participate (participation rate=82.3%); 436 men (participation rate=84.8%) and 366 women (participation rate= 79.4%). The study population was representative of individuals residing in the suburban areas of Copenhagen County. Participants underwent a complete medical evaluation at the Glostrup University Hospital and completed a structured baseline questionnaire, adapted from the cardiovascular survey methods developed by the WHO[2], to acquire information about lifestyle habits including smoking behaviors, physical activity, consumption of drugs, and morbidity. The cohort was followed for 10 years, and a follow-up assessment was undertaken in 1974. A detailed description of the follow-up procedures has been published elsewhere[3]. In brief, survivors of the 50-year old study were asked to participate; 70 participants of the baseline study died in the interim. A total of 627 (out of 732 survivors) agreed to participate in the follow-up assessment (participation rate=85.7%); 332 men and 295 women. Deaths from cardiovascular disease were ascertained from the Danish Center National Register and death rates from cardiovascular diseases were reported. Participants underwent a medical evaluation and assessed for fasting levels of cholesterol and triglycerides, hypertension, ECG, glucose tolerance, body mass index, and screening for other cardiovascular manifestations. A structured questionnaire was administered to collect follow-up information about lifestyle habits.

Overview of study population for the current dissertation

The study population for this dissertation is the 1914 Glostrup birth cohort, and includes follow-up assessments at ages 70 (baseline 1 for the current dissertation), 75, 80, and 85 (**figure 5.1**). The cohort was supplemented at age 75 (described below) with additional subjects (baseline 2). Follow-up assessments at the Copenhagen County Hospital in Glostrup involved physical examinations, collection of blood samples, and completion of a health questionnaire. In addition, information regarding social and behavioral factors was collected through structured questionnaires administered during home visits at each follow-up period. Oral health examinations were performed at ages 70 and 85, and oral health questionnaires were administered at ages 75 and 80. Vital status and time of death from all causes and for all study subjects were obtained from the Danish Center National Register on October 16, 2009.

Description of sample at each 5-year assessment

In 1984, a total of 1,119 subjects were invited to participate in the 70-year-old baseline study; 736 were survivors of the original 1914 Glostrup cohort initiated in 1964 (50-year-old study), and an additional 383 randomly chosen subjects born in 1914. In total, 804 subjects participated in a medical evaluation that included blood work and completed a health questionnaire. Additionally, a sub-cohort of 652 subjects participated in an oral health examination. Overall, participants and non-participants did not differ significantly with regard to demographic characteristics and prevalence of selected diseases such as cancer, stroke, diabetes mellitus, bronchitis, and ischemic heart diseases[4].

The study of 75-year olds took place in 1989 and was comprised of 2 study samples. The first was from the 5-year follow-up assessment, in which 135 died and 576 (out of 599) surviving

subjects agreed to a home visit (participation rate= 96%). The second was a random sample of 238 men and women (born in 1914) and living in 11 municipalities around Glostrup. They formed part of a larger ongoing cohort study evaluating functional capacity and health among older Nordic urban individuals (Nordic Research on Aging [NORA] study). Out of the 238 invitees, 172 NORA subjects agreed to a home visit (participation rate= 72%) and a total of 748 participants (576 follow-up and 172 newcomers) constituted baseline 2. A sub-cohort of 411 participants underwent a medical evaluation that included blood work and completed questionnaires ascertaining oral and general health status in addition to information on social and lifestyle factors.

During the next follow-up at age 80, 189 had died and 442 out of 559 survivors agreed to a home visit (participation rate= 79%). A sub-cohort of 362 subjects underwent a medical evaluation that included blood work and completion of a general and oral health questionnaire.

In the 85-year-old study administered 5 years later, 165 subjects had died and 277 survivors were invited to participate. Follow-up involved a series of 5 home visits assessing an array of health factors: 1) functional ability and psychosocial factors, 2) physiological factors and general health, 3) psychological factors, 4) oral health, and 5) dietary factors. A total of 242 survivors agreed to at least one home visit (participation rate= 87%), and a sub-cohort of 191 subjects underwent a comprehensive oral health examination. Additionally, a sub-cohort of 189 (out of 242 participating subjects) underwent a medical evaluation that included blood work.

At the end of follow-up (October 16, 2009), a total of 791 all-cause deaths were recorded out of 804 participating subjects from the 70-year-old study (baseline 1). A total of 696 all-cause deaths were recorded out of 748 participating subjects from the 75-year-old study (baseline 2).

Description of methods for each chapter aims (figure 5.2)

Chapter 1: The hypothesis is that stress has a role in periodontal disease progression. To test this, I systematically reviewed the literature for epidemiological and laboratory evidence regarding the association between stress and periodontal disease.

Chapter 2 (aim 1): The hypothesis is that low socio-economic position and maladaptive health behaviors are associated with high scores of allostatic load. To test this, I first developed an index of allostatic load with available physiological markers at ages 70 and 80. Then I evaluated associations between social/behavioral characteristics of participants at age 75 and allostatic load at age 80.

Physiological markers at ages 70 and 80

The majority of physiological markers for assessment of allostatic load were derived from blood collected at the Copenhagen County Hospital (CCH) in Glostrup. Fasting blood samples were collected at age 70 and non-fasting blood samples at age 80. Blood was processed on the same day of collection and snap frozen for same-day analysis of lipids (total cholesterol, high-density lipoprotein [HDL], and triglycerides [age 70 only]), serum albumin (age 80 only) and blood glucose (age 80 only)[3]. Serum TNF-α and IL-6 were measured in serum and detected by commercially available enzyme-linked immunosorbent assay (ELISA) kits (catalogue numbers HSTA50 and HS600, R&D systems, Minneapolis MN)[5]. Detection thresholds for assays were 0.1-0.2 pg/ml. The coefficient for intra-assay and inter-assay variability was 15.7% and 25.0% for TNF-α and 8.7% and 15.1% for IL-6, respectively. All analyses were run within 8 weeks of collection.

Clinical markers

Hospital staff assessed height and weight during the medical evaluation visit.

Measurements were made with participants wearing light clothing and no shoes. Height was

measured to the nearest 0.5 cm and weight to the nearest 100 g. Body mass index (BMI) was

computed as weight in kg/height in m^2. Levels of diastolic and systolic blood pressure were

measured in a supine position after 10 minutes of rest using a Doppler technique[3]. Resting heart

rate was determined from the R-R interval on an ECG administered during the evaluation.

Forced expiratory volume in the first second (FEV$_1$) was measured three times on participants

using a Godart bell respirograph and Wright peak flowmeter.

Derivation of allostatic load

Following Seeman et. al.'s summative scoring approach, I developed an index of

allostatic load at ages 70 and 80[6]. Allostatic load markers that fell within a certain high-risk

percentile (either upper or lower 25th percentile, depending on the marker) were dichotomized

and summed for each participant. The resultant score, which is dependent upon each marker's

sample's distribution, is theorized to capture biological dysregulation. However, the assumption

that each marker has equal weight in the index may not hold true. More complex scoring

methods that incorporate canonical weights or recursive partitioning to account for potential

nonlinearities in risk or interactions between component markers, will be considered.

Nonetheless, it has been suggested that the gains in predictive ability obtained with using more

complex scoring methods are modest at best[7].

To describe the variability among allostatic load markers and reduce them to their

uncorrelated components or factors, I used principal component analysis[8]. This is a well-

established statistical approach for making sense of potentially multi-correlated set of items by

reducing them to smaller dimensions. The objective is to achieve parsimony by uncovering the underlying structure of related variables and ultimately create a set of uncorrelated linear combinations of factors that measure the same thing. Histograms of allostatic load indices reduced to its principal components were be examined to determine their respective distributions.

To evaluate associations between allostatic load and each social/behavioral characteristic, I first compared mean allostatic load scores across social and behavioral characteristic. Then I constructed logistic regression models using cumulative odds ratios to assess potential associations between allostatic load measured at 80 and earlier measures of smoking and alcohol consumption, and socio-economic position at ages 70 and 75.

Chapter 3: (aim 2): The hypothesis is that there are positive bidirectional associations between allostatic load and periodontal disease. To test this, I evaluate a series of cross-sectional and longitudinal associations: (i) cross-sectional associations between allostatic load and periodontal inflammation at age 70; (ii) prospective association between allostatic load at age 70 and periodontal disease at age 85; and (iii) prospective association between periodontal disease at age 70 and allostatic load at age 80 (see figure 3.1).

When periodontal disease was treated as outcomes, I categorized this variable into quartiles and used cumulative logistic models to estimate cumulative odds ratios (ORs). When treated as the exposure, I categorized these measures into quartiles and additionally included a separate edentulous category to evaluate the influence of edentulism on allostatic load. Score tests were used to check for proportionality of models.

Chapter 4 (aim 3): The hypothesis is that allostatic load is associated with mortality risk, and periodontal disease/edentulism mediates this relationship. To test this, I first evaluate the relationship between periodontal disease/edentulism and mortality risk (all-cause and cardiovascular disease mortality). Then I evaluate the relationship between periodontal allostatic load and mortality risk. Lastly, I explore whether periodontal disease/edentulism mediates and/or modifies the relationship between allostatic load and mortality risk.

Information regarding mortality was collected on all participants. Deaths were obtained from the Danish Central National Register. All-cause mortality served as our primary end point, whereas death from cardiovascular disease was our secondary end point. Participants were followed for 25 years, from September/October 1984 to October 16, 2009. Deaths from cardiovascular diseases included International Classification of Diseases, Eighth Revision (ICD-8) codes 390 – 458 and ICD-10 codes I00 – I99[13].

Survival curves at ages 70 and 80 were constructed using the Kaplan-Meier method and compared with the log-rank test. Univariate Cox proportional hazards models were constructed to examine covariates such as sex, income, education, smoking, alcohol consumption, and physical activity as predictors of mortality. To evaluate associations between AL with mortality, we constructed Cox proportional hazards models serially adjusted for strong predictors of mortality and potential confounders. We evaluated for multicollinearity by examining intercorrelations between independent variables using variance inflation factors (VIF) or tolerance values[14]. The proportional hazards assumption was confirmed by inspection of log (-log [survival]) curves as well as examination of time-dependent covariates[15].
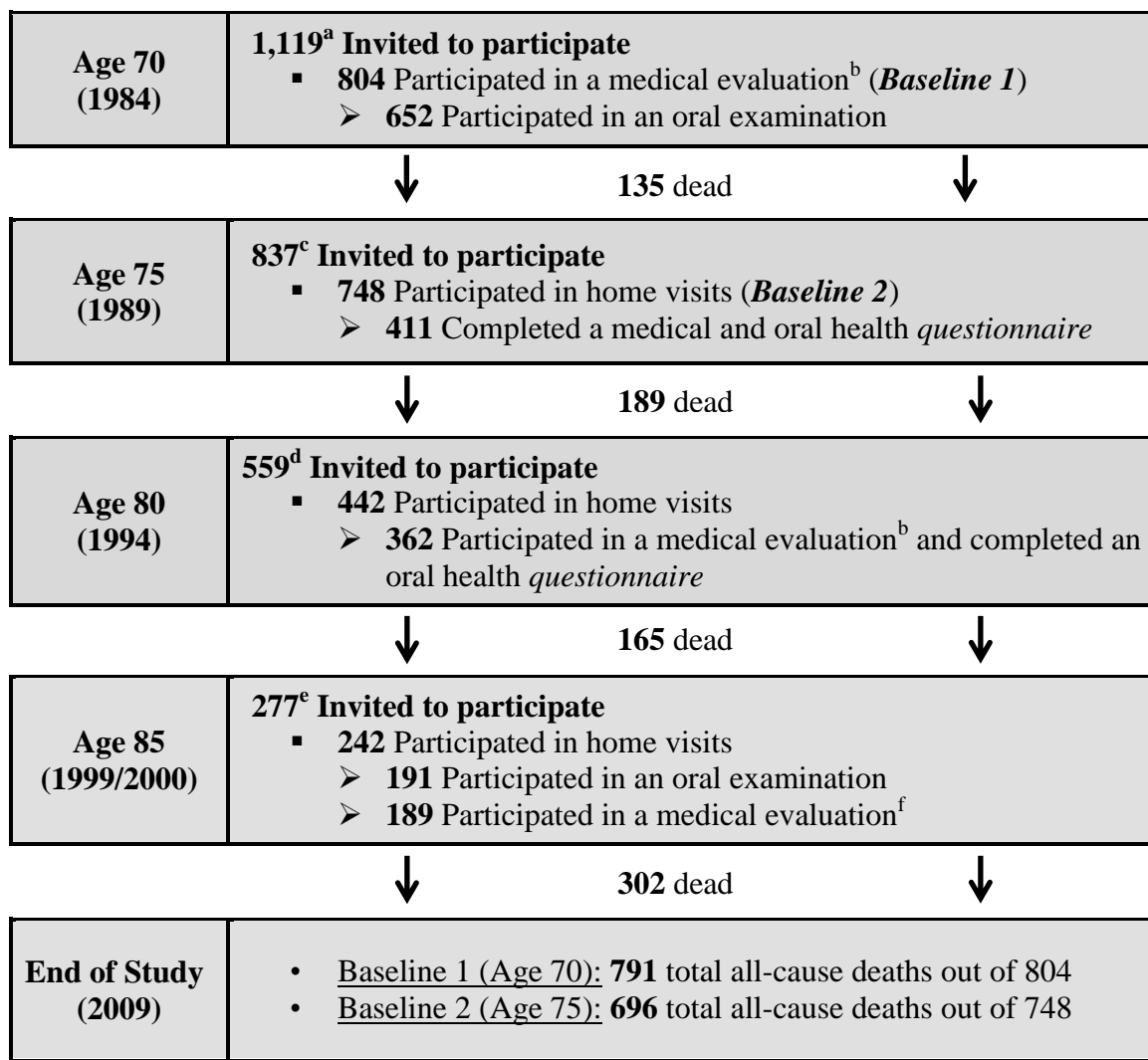
To evaluate whether the AL-mortality relationship differed by periodontal inflammation, we stratified the models by edentulous status and by severity of periodontal inflammation, with

cut-points chosen at the median. I explored an interaction between edentulism and AL using a cross-product term entered into a multivariable Cox model and used a likelihood ratio test to assess statistical significance, comparing models with and without the interaction term. Hazard-risks were additionally illustrated by adjusted survival curves using an inverse probability weighting approach to control for covariates[16]. Inverse probability is the best approach to graphically represent survival curves when the adjusted covariates are continuous variables. The main advantage for using this approach is that the curves are not forced to be proportional.

6.2. References

1. Schroll M, Jorgensen T, Ingerslev J. The Glostrup Population Studies, 1964-1992. Dan Med Bull 1992;39:204-7.

2. Rose G, Blackburn H. Cardiovascular survey methods. In. Geneva: WHO monograph series No. 56; 1968.

3. Schroll M. A ten-year prospective study, 1964-1974, of cardiovascular risk factors in men and women from the Glostrup population born in 1914. Dan Med Bull 1982;29:213-52.

4. Avlund K. Disability in old age. Longitudinal population-based studies of the disablement process. Danish medical bulletin 2004;51:315-49.

5. Bruunsgaard H, Ladelund S, Pedersen AN, Schroll M, Jorgensen T, Pedersen BK. Predicting death from tumour necrosis factor-alpha and interleukin-6 in 80-year-old people. Clin Exp Immunol 2003;132:24-31.

6. Seeman TE, Singer BH, Rowe JW, Horwitz RI, McEwen BS. Price of adaptation--allostatic load and its health consequences. MacArthur studies of successful aging. Arch Intern Med 1997;157:2259-68.

7. Seplaki CL, Goldman N, Glei D, Weinstein M. A comparative analysis of measurement approaches for physiological dysregulation in an older population. Exp Gerontol 2005;40:438-49.

8. Joliffe IT, Morgan BJ. Principal component analysis and exploratory factor analysis. Stat Methods Med Res 1992;1:69-95.

9. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. Journal of periodontology 2007;78:1387-99.

10. Papapanou PN, Teanpaisan R, Obiechina NS, et al. Periodontal microbiota and clinical periodontal status in a rural sample in southern Thailand. Eur J Oral Sci 2002;110:345-52.

11. Demmer RT, Papapanou PN, Jacobs DR, Jr., Desvarieux M. Bleeding on probing differentially relates to bacterial profiles: the Oral Infections and Vascular Disease Epidemiology Study. J Clin Periodontol 2008;35:479-86.

12. Papapanou PN, Baelum V, Luan WM, et al. Subgingival microbiota in adult Chinese: prevalence and relation to periodontal disease progression. J Periodontol 1997;68:651-66.

13. World Health Organization. International statistical classification of diseases and related health problems. 10th revision, 2nd edition. ed. Geneva: World Health Organization; 2004.

14. Fox J, Monette G. Generalized Collinearity Diagnostics. Journal of the American Statistical Association 1992:178-83.

15. Hosmer D, Lemeshow S, May S, eds. Applied Survival Analysis. Regression Modeling of Time to Event Data. New York: John Wiley & Sons Inc; 2008.

16. Cole SR, Hernan MA. Adjusted survival curves with inverse probability weights. Comput Methods Programs Biomed 2004;75:45-9.

Figure 5.1. Overview of the 1914 Glostrup study population

Age 70 (1984)	**1,119[a] Invited to participate** ▪ **804** Participated in a medical evaluation[b] (***Baseline 1***) ➢ **652** Participated in an oral examination

↓ **135** dead ↓

Age 75 (1989)	**837[c] Invited to participate** ▪ **748** Participated in home visits (***Baseline 2***) ➢ **411** Completed a medical and oral health *questionnaire*

↓ **189** dead ↓

Age 80 (1994)	**559[d] Invited to participate** ▪ **442** Participated in home visits ➢ **362** Participated in a medical evaluation[b] and completed an oral health *questionnaire*

↓ **165** dead ↓

Age 85 (1999/2000)	**277[e] Invited to participate** ▪ **242** Participated in home visits ➢ **191** Participated in an oral examination ➢ **189** Participated in a medical evaluation[f]

↓ **302** dead ↓

End of Study (2009)	• Baseline 1 (Age 70): **791** total all-cause deaths out of 804 • Baseline 2 (Age 75): **696** total all-cause deaths out of 748

[a] 1,119 sample was based on 736 survivors of the 50-year-old cohort and an additional 383 random sample born in Glostrup in 1914.

[b] The medical evaluations involved a physical examination, blood work, and completion of a health questionnaire at the Copenhagen County Hospital in Glostrup.

[c] 837 sample was based on 599 survivors of the 70-year-old cohort and an additional 238 random sample born in Glostrup in 1914.

[d] 559 sample included all surviving participants from the previous follow-up assessment (748) less the deceased (189).

[e] 277 sample included all surviving participants from the previous follow-up assessment (442) less the deceased (165).

[f] The medical evaluation at age 85 took place at the subject's home.

Figure 5.2. Overview of dissertation aims
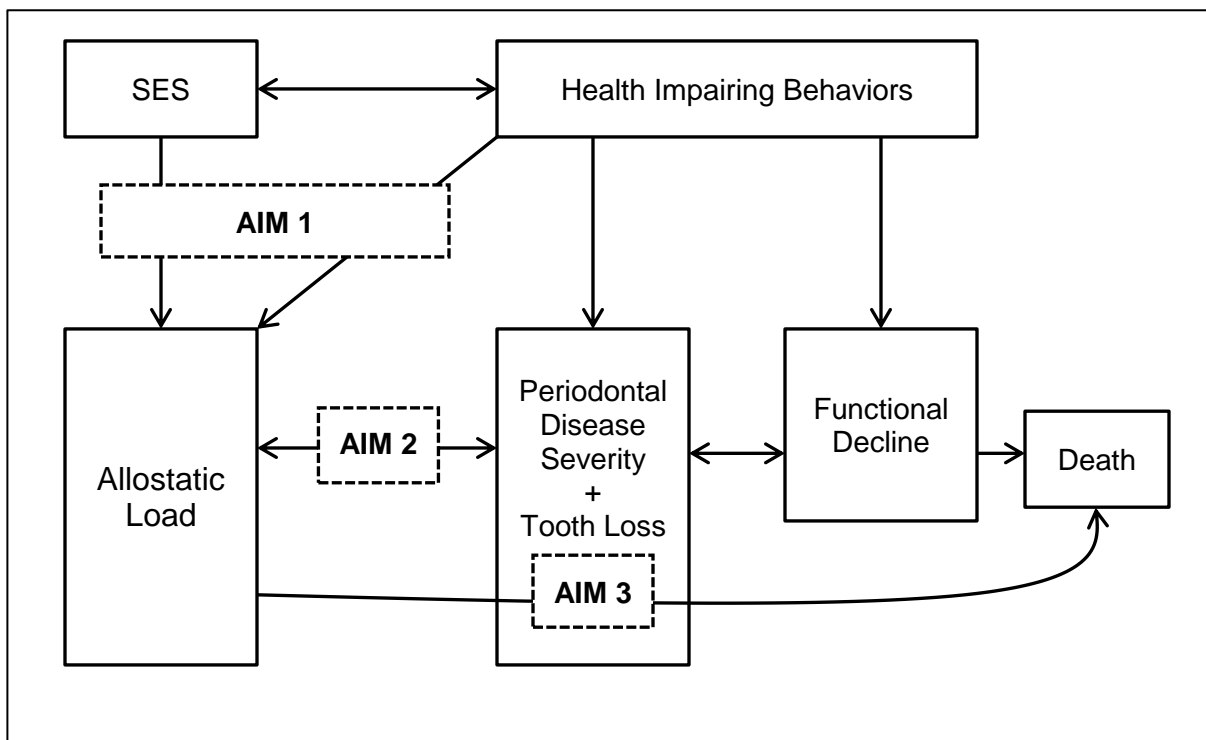
Appendix 2:
Supplemental tables and figures for chapter 2

Supplemental Tables

Supplemental Table 2.1. Distribution of missing behavioral characteristics at age 75 by vocational training, income, and housing

	Smoking status			Alcohol consumption			Physical activity		
	Complete (n=409)	Missing (n=339)	P-value	Complete (n=406)	Missing (n=342)	P-value	Complete (n=410)	Missing (n=338)	P-value
Vocational Training, %									
No training	41.1%	49.4%	0.03	40.6%	49.9%	0.02	41.2%	49.3%	0.04
<3 years training	17.4%	11.9%		17.5%	11.8%		17.3%	11.9%	
≥3 years training	41.6%	38.7%		41.9%	38.4%		41.5%	38.8%	
Last occupation, %									
Managerial	15.0%	17.8%	0.21	15.1%	17.6%	0.24	14.9%	17.8%	0.23
Skilled	28.9%	22.8%		28.9%	22.9%		28.9%	22.9%	
Unskilled	34.6%	38.8%		34.6%	38.7%		34.7%	38.6%	
Other	21.6%	20.7%		21.5%	20.8%		21.5%	20.8%	
Income, %									
High	67.7%	69.9%	0.52	68.5%	69.0%	0.88	67.8%	69.8%	0.55
Low	32.3%	30.1%		31.5%	31.0%		32.2%	30.2%	
Housing Status, %									
Owner	42.8%	37.8%	0.02	42.8%	37.8%	0.03	42.7%	37.9%	0.02
Renter	52.1%	51.9%		52.0%	52.1%		52.2%	51.8%	
Other	5.1%	10.3%		5.2%	10.2%		5.1%	10.4%	

Supplemental Table 2.2: Summary statistics for physiological markers used in the allostatic load summary score at age 80 stratified by sex

Physiological Marker	Men (N=160)		Women (N=170)		
	Range	Mean (SD)	Range	Mean (SD)	P-value
BMI (kg/m^2)	16.6 – 35.0	24.9 (3.2)	11.6 – 37.0	24.8 (4.4)	0.76
Ratio of total cholesterol/HDL	1.9 – 9.0	4.7 (1.5)	2.1 – 14.6	4.4 (1.5)	0.07
HDL cholesterol (mg/dL)	17.8 – 104.8	50.4 (14.9)	26.7 – 122.6	59.9 (16.8)	<0.0001
Resting DBP (mmHg)	59.0 – 120.0	83.4 (12.1)	60.0 – 116.0	85.0 (10.6)	0.23
Resting SBP (mmHg)	103.0 – 210.0	148.1 (20.7)	111.0 – 224.0	152.9 (21.5)	0.04
IL-6 (pg/ml)	0.5 – 137.5	7.0 (15.8)	0.7 – 412.0	8.1 (33.2)	0.70
TNF-α (pg/ml)	1.6 – 24.8	4.2 (2.2)	1.9 – 32.0	4.5 (2.7)	0.31
Albumin (g/dL)	2.6 – 5.0	4.1 (0.3)	3.5 – 4.8	4.1 (0.2)	0.09
Resting heart rate (bpm)	48.0 – 124.0	73.4 (12.1)	48.0 – 108.0	72.1 (11.5)	0.35
Blood glucose (mg/dL)	61.2 – 392.4	107.0 (47.6)	54.0 – 237.6	97.1 (30.1)	0.03

Supplemental Table 2.3. Mean allostatic load and odds ratios for having a higher level of allostatic load in relation to smoking behavior at ages 70 - 80

Smoking behavior	Analysis of variance			ORs for having higher allostatic load	
	n	Mean (SE)	p-value[a]	Crude ORs (95% CI)	Adjusted[b] ORs (95% CI)
Smoking status (ages 75 – 80)					
Current smoker (75 and 80)	57	2.52 (0.27)	0.19	1.00	1.00
Ex-smoker (75 and 80)	72	2.61 (0.23)		1.04 (0.55 – 1.96)	1.09 (0.57 – 2.07)
Never smoker (75 and 80)	53	2.90 (0.27)		1.30 (0.65 – 2.58)	1.55 (0.76 – 3.14)
Current smoker (75), ex-smoker (80)	16	1.67 (0.50)		0.39 (0.14 – 1.10)	0.35 (0.12 – 0.98)
Smoking status (ages 70 – 80)					
Current smoker (70, 75, and 80)	46	2.61 (0.28)	0.21	1.00	1.00
Ex smoker (70, 75, and 80)	37	2.17 (0.31)		0.64 (0.30 – 1.41)	0.67 (0.30 – 1.46)
Never smoker (70, 75, and 80)	35	2.94 (0.33)		1.36 (0.61 – 3.07)	1.58 (0.69 – 3.59)
Current smoker (70 and 75), ex-smoker (80)	12	1.82 (0.56)		0.43 (0.13 – 1.38)	0.36 (0.11 – 1.19)

[a] P-value for ANOVA
[b] Adjusted for sex and physical activity

Supplemental Table 2.4: Odds ratios for having a higher level of allostatic load among men and women by level of physical activity, N= 194

Physical activity	ORs for higher levels of allostatic load			
	n	Women	n	Men
Crude Model				
None/light exercise	70	1.00 (referent)	48	1.38 (0.72 – 2.65)
Moderate/rigorous exercise	32	0.86 (0.41 – 1.81)	44	0.55 (0.28 – 1.08)
		P for interaction[b] = 0.15		
Adjusted Model[a]				
None/light exercise	70	1.00 (referent)	48	1.85 (0.89 – 3.83)
Moderate/rigorous exercise	32	0.91 (0.42 – 1.98)	44	0.61 (0.29 – 1.31)
		P for interaction[b] = 0.07		

[a] Adjusted for vocational training, occupation, housing status, smoking and alcohol consumption
[b] P-value for Wald chi-square of interaction term (sex*physical activity)

Supplemental Table 2.5. Bivariate associations between social/behavioral factors and factor scores in men, n=160

Social and Behavioral Characteristics	N	ORs for high factor scores in relation to social and behavioral characteristics			
		Lipid pattern	Blood pressure pattern	Inflammation pattern	Glucose Met. pattern
Vocational Training					
Training	109	1.00	1.00	1.00	1.00
No training	35	1.48 (0.75 – 2.92)	1.33 (0.68 – 2.62)	1.49 (0.75 – 2.92)	1.57 (0.80 – 3.10)
Last occupation					
Managerial	41	1.00	1.00	1.00	1.00
Skilled/Other	66	2.05 (1.02 – 4.13)	1.17 (0.58 – 2.38)	0.99 (0.50 – 1.98)	1.40 (0.70 – 2.79)
Unskilled	35	2.33 (1.04 – 5.23)	1.38 (0.62 – 3.08)	1.47 (0.66 – 3.28)	2.05 (0.91 – 4.58)
Income					
High	125	1.00	1.00	1.00	1.00
Low	35	1.19 (0.61 – 2.32)	0.81 (0.42 – 1.57)	1.48 (0.76 – 2.88)	1.74 (0.89 – 3.40)
Housing Status					
Owner	80	1.00	1.00	1.00	1.00
Renter	60	1.67 (0.92 – 3.04)	1.76 (0.97 – 3.21)	1.48 (0.82 – 2.68)	0.79 (0.44 – 1.43)
Smoking					
Never	13	1.00	1.00	1.00	1.00
Ex-smoker	43	1.24 (0.41 – 3.71)	0.43 (0.14 – 1.29)	0.59 (0.20 – 1.78)	0.71 (0.23 – 2.12)
Current smoker	36	1.12 (0.39 – 3.20)	0.43 (0.14 – 1.34)	0.76 (0.25 – 2.34)	0.66 (0.21 – 2.02)
Alcohol consumption					
Rarely/never	15	1.00	1.00	1.00	1.00
1-2 times per month	11	0.30 (0.42 – 1.23)	2.35 (0.58 – 9.49)	0.73 (0.18 – 2.90)	1.58 (0.40 – 6.30)
Once per week	13	1.04 (0.27 – 3.93)	1.75 (0.46 – 6.63)	0.45 (0.12 – 1.71)	0.78 (0.21 – 2.93)
Daily/3x per week	52	0.48 (0.17 – 1.34)	2.25 (0.80 – 6.37)	1.30 (0.47 – 3.60)	1.55 (0.56 – 4.
Physical activity					
Moderate/rigorous exercise	44	1.00	1.00	1.00	1.00
Light exercise	38	2.02 (0.93 – 4.41)	2.45 (1.11 – 5.38)	1.44 (0.42 – 4.87)	0.91 (0.42 – 1.96)
Mostly sedentary	10	2.89 (0.83 – 10.0)	0.97 (0.28 – 3.29)	1.05 (0.49 – 2.26)	4.01 (1.11 – 14.5)

Supplemental Table 2.6. Bivariate associations between social/behavioral factors and factor scores in women, n=170

Social and Behavioral Characteristics	N	ORs for high factor scores in relation to social and behavioral characteristics			
		Lipid pattern	Cardiovascular pattern	Inflammation pattern	Glucose met. pattern
Vocational Training					
Training	75	1.00	1.00	1.00	1.00
No training	86	1.15 (0.66 – 1.98)	0.73 (0.42 – 1.26)	1.51 (0.87 – 2.62)	1.08 (0.63 – 1.88)
Last occupation					
Managerial	24	1.00	1.00	1.00	1.00
Skilled	35	0.99 (0.39 – 2.49)	0.80 (0.32 – 2.02)	0.92 (0.37 – 2.31)	1.02 (0.41 – 2.58)
Unskilled/Other	102	1.50 (0.68 – 3.30)	0.77 (0.35 – 1.69)	1.01 (0.46 – 2.26)	1.44 (0.66 – 3.18)
Income					
High	131	1.00	1.00	1.00	1.00
Low	39	2.16 (1.14 – 4.10)	1.01 (0.54 – 1.90)	0.83 (0.44 – 1.57)	1.67 (0.88 – 3.15)
Housing Status					
Owner	67	1.00	1.00	1.00	1.00
Renter	83	0.83 (0.47 – 1.47)	0.70 (0.39 – 1.23)	1.00 (0.57 – 1.77)	1.36 (0.77 – 2.41)
Smoking					
Never	39	1.00	1.00	1.00	1.00
Ex-smoker	31	0.78 (0.34 – 1.80)	0.87 (0.37 – 2.02)	0.83 (0.36 – 1.91)	0.95 (0.41 – 2.19)
Current smoker	31	0.50 (0.21 – 1.15)	0.51 (0.22 – 1.18)	0.66 (0.28 – 1.52)	0.53 (0.23 – 1.24)
Alcohol consumption					
Rarely/never	44	1.00	1.00	1.00	1.00
1-2 times per month	17	0.99 (0.37 – 2.68)	0.98 (0.37 – 2.64)	1.66 (0.61 – 4.56)	1.02 (0.38 – 2.75)
Once per week	10	0.82 (0.24 – 2.77)	1.69 (0.50 – 5.78)	0.46 (0.13 – 1.56)	0.80 (0.24 – 2.70)
Daily/3x per week	31	0.47 (0.21 – 1.08)	2.22 (0.96 – 5.10)	0.32 (0.14 – 0.74)	1.05 (0.47 – 2.36)
Physical activity					
Moderate/rigorous exercise	32	1.00	1.00	1.00	1.00
Light exercise	54	1.74 (0.79 – 3.80)	0.54 (0.24 – 1.18)	1.38 (0.64 – 3.00)	0.52 (0.24 – 1.13)
Mostly sedentary	16	2.10 (0.72 – 6.15)	0.39 (0.13 – 1.14)	0.89 (0.31 – 2.58)	0.53 (0.18 – 1.53)

Appendix 3:
Supplemental tables and figures for chapter 3

Supplemental Tables

Supplemental Table 3.1. Modified version of the Community Periodontal Index Need (MCPI) at age 70

MCPI Scores[a]	Criteria
0	no sign of inflammation or periodontal pockets around the tooth
1	signs of inflammation partially circumscribing the tooth, no periodontal pockets
2	signs of inflammation circumscribing the tooth, no periodontal pockets
3	signs of inflammation circumscribing the tooth, periodontal pockets 4 – 6 mm
6	signs of inflammation circumscribing the tooth, periodontal pockets > 6mm
8	signs of inflammation circumscribing the tooth, advanced periodontal destruction

[a] Each tooth was examined and assigned a score

Supplemental Table 3.2. Measures of periodontal disease at ages 70 and 85

Age group	Measure	Analytic approach	Categories
70-year old	MCPI scores (0 – 8)	Mean MCPI scores	**(a)** 0.40 – 1.54, **(b)** 1.56 – 2.10, **(c)** 2.11 – 2.90, **(d)** 2.94 – 6.00, **(e)** edentulous
		% of mean MCPI ≥ 3	**(a)** 0 – 5.0%, **(b)** 6.3 – 25.0%, **(c)** 26.1 – 62.5%, **(d)** 63.6 – 100%, **(e)** edentulous
		% of mean MCPI ≥ 6	**(a)** 0%, **(b)** 3.6 – 18.2%, **(c)** 19.1 – 33.3%, **(d)** 34.8 – 80%, **(e)** edentulous
85 year-old	Periodontal pocket depth (PD)	% sites with PD ≥ 3 mm	Continuous measure
		% sites with PD ≥ 4 mm	Continuous measure
		% sites with PD ≥ 5 mm	Continuous measure
		≥ 2 sites with PD ≥ 5 mm (on at least 2 teeth present)	Case definition
	Clinical attachment loss (CAL)	% sites with CAL ≥ 3 mm	Continuous measure
		% sites with CAL ≥ 4 mm	Continuous measure
		% sites with CAL ≥ 5 mm	Continuous measure

Supplemental Table 3.3: Summary statistics and high-risk cut-points for allostatic load markers at ages 70 and 80

Domain	Physiological Marker	Range	Median	Mean (SD)	High risk cut-point[a]	Number at high risk/ total
Anthropometric	BMI at 70 (kg/m^2)	16.4 – 46.7	24.6	25.2 (4.2)	≥ 27.2	113 / 453
	BMI at 80 (kg/m^2)	11.6 – 37.0	24.5	24.9 (3.8)	≥ 27.2	82 / 330
Metabolic	Total cholesterol/HDL at 70	1.6 – 10.1	4.6	4.8 (1.5)	≥ 5.6	113 / 453
	Total cholesterol/HDL at 80	1.9 – 14.6	4.3	4.6 (1.5)	≥ 5.3	82 / 330
	HDL cholesterol at 70 (mg/dL)	24.0 – 155.8	54.9	57.9 (17.5)	≤ 45.3	114 / 453
	HDL cholesterol at 80 (mg/dL)	17.8 – 122.6	52.0	55.3 (16.6)	≤ 44.5	82 / 330
	Triglycerides at 70 (mg/dL)	44.0 – 915.0	125.0	145.5 (79.4)	≥ 176.0	113 / 453
	Blood glucose at 80 (mg/dL)	54.0 – 392.4	90.0	101.9 (39.8)	≥ 106.2	82 / 330
Cardiovascular	Resting DBP at 70 (mmHg)	52.0 – 128.0	80.0	80.7 (10.6)	≥ 88.0	117 / 453
	Resting DBP at 80 (mmHg)	59.0 – 120.0	83.0	84.2 (11.4)	≥ 93.0	78 / 330
	Resting SBP at 70 (mmHg)	98.0 – 218.0	138.0	139.8 (19.8)	≥ 152.0	116 / 453
	Resting SBP at 80 (mmHg)	103.0 – 224.0	150.0	150.6 (21.2)	≥ 164.0	83 / 330
	Resting heart rate at 70 (bpm)	32.0 – 116.0	72.0	73.5 (11.0)	≥ 84.0	107 / 453
	Resting heart rate at 80 (bpm)	48.0 – 124.0	72.0	72.7 (11.8)	≥ 84.0	69 / 330
Pulmonary	FEV$_1$ at 70 (ml)	33.0 – 358.0	174.0	176.8 (60.3)	≤ 135.0	116 / 453
Inflammation	IL-6 at 80 (pg/ml)	0.5 – 412.0	3.2	7.5 (26.2)	≥ 5.4	82 / 330
	TNF-α at 80 (pg/ml)	1.6 – 32.0	4.1	4.4 (2.5)	≥ 5.0	82 / 330
	Albumin at 80 (g/dL)	2.6 – 5.0	4.1	4.1 (0.3)	≤ 3.9	80 / 330

BMI= Body Mass Index, HDL= High Density Lipoprotein, SD= Standard Deviation, DBP= Diastolic blood pressure, SBP= Systolic blood pressure

[a] High risk cut-points are based on the upper or lower 25th percentile of the marker's distribution in the study population

Supplemental Table 3.4a. Longitudinal associations of allostatic load at ages 70 and 80 with periodontal disease at age 85 using a case definition determined apriori[a]

Allostatic load (AL)	Cases/ non-cases	Adjusted means[b] (SE)			ORs[c] for periodontal disease (95% CI)	
		Cases	Non-cases	*P*-value	Model 1	Model 2
AL at age 70 (AL 70)	25 / 28	2.18 (0.30)	0.80 (0.32)	0.003	1.95 (1.20 – 3.18)	1.99 (1.13 – 3.52)
AL at age 80 (AL 80)	49 / 38	2.17 (0.22)	2.18 (0.25)	0.97	0.98 (0.88 – 2.41)	1.03 (0.76 – 1.39)

[a] ≥ 2 sites with PD ≥ 5 mm on at least 2 teeth
[b] Adjusted for number of teeth at age 85
[c] ORs represent a one-score difference in allostatic load. Model 1 is adjusted for number of teeth at age 85. Model 2 additionally adjusted for sex and income (high, low)

Supplemental Table 3.4b. Longitudinal associations of allostatic load at ages 70 and 80 with periodontal disease at age 85 using a case definition determined apriori[a]

Allostatic load (AL) at ages 70 and 80	Cases/ non-cases	ORs[b] for periodontal disease at age 85 (95% CI)
Low AL 70 / Low AL 80	10 / 15	1.00
Low AL 70 / High AL 80	2 / 4	1.01 (0.14 – 7.17)
High AL 70 / Low AL 80	4 / 4	3.10 (0.47 – 20.6)
High AL 70 / High AL 80	9 / 2	6.80 (1.20 – 38.4)

[a] $\geq$ 2 sites with PD $\geq$ 5 mm on at least 2 teeth
[b] Adjusted for number of teeth at age 85

Supplemental Table 3.5. Cross-sectional and longitudinal associations of edentulism at age 70 with allostatic load at ages 70 and 80

Edentulism	Allostatic load scores			ORs for having higher allostatic load	
	n	Mean (SE)	*P*-value	Crude ORs (95% CI)	Adjusted[a] ORs (95% CI)
Model 1[b]					
Dentulous	199	1.80 (0.12)	0.04	1.00	1.00
Edentulous	140	2.18 (0.14)		1.50 (1.01 – 2.21)	1.49 (1.01 – 2.20)
Model 2[c]					
Dentulous	161	2.36 (0.14)	0.65	1.00	1.00
Edentulous	67	2.48 (0.21)		1.19 (0.71 – 1.99)	1.15 (0.68 – 1.94)

[a] OR for model 1 was adjusted for physical activity and OR for model 2 additionally adjusted for sex and physical activity

[b] Model 1 evaluates the association of edentulism with allostatic load cross-sectionally at age 70

[c] Model 2 evaluates the association of edentulism at age 70 with allostatic load at age 80

Supplemental Table 3.6. Cross-sectional and longitudinal associations of allostatic load at age 70 with edentulism at ages 70 and 85

Allostatic load (AL)	n	ORs for edentulism	
		Crude ORs (95% CI)	Adjusted[a] ORs (95% CI)
Model 1[b]			
AL 70 quartile 1	75	1.00	1.00
AL 70 quartile 2	85	1.27 (0.66 – 2.43)	0.91 (0.44 – 1.89)
AL 70 quartile 3	122	1.59 (0.87 – 2.89)	1.30 (0.67 – 2.55)
AL 70 quartile 4	57	1.93 (0.95 – 3.92)	1.53 (0.68 – 3.43)
Model 2[c]			
AL 70 quartile 1	33	1.00	1.00
AL 70 quartile 2	29	0.59 (0.20 – 1.71)	0.56 (0.17 – 1.87)
AL 70 quartile 3	25	1.42 (0.50 – 4.06)	1.31 (0.40 – 4.27)
AL 70 quartile 4	17	1.08 (0.33 – 3.55)	1.01 (0.26 – 3.90)
Model 3[d]			
AL 80 quartile 1	58	1.00	1.00
AL 80 quartile 2	39	0.95 (0.40 – 2.24)	1.83 (0.63 – 5.31)
AL 80 quartile 3	30	1.66 (0.68 – 4.08)	1.55 (0.51 – 4.72)
AL 80 quartile 4	28	1.06 (0.41 – 2.71)	1.00 (0.31 – 3.26)
Model 4[e]			
low AL 70 / low AL 80	44	1.00	1.00
low AL 70 / high AL 80	9	0.97 (0.21 – 4.42)	0.91 (0.16 – 5.08)
high AL 70 / low AL 80	13	1.21 (0.34 – 4.34)	1.27 (0.31 – 5.27)
high AL 70 / high AL 80	18	1.23 (0.40 – 3.83)	0.88 (0.22 – 3.46)

[a] ORs were adjusted for sex, income, education, housing status, smoking, and physical activity
[b] Model 1 examines the cross-sectional association of allostatic load at age 70 with edentulism at age 70
[c] Model 2 examines the longitudinal association of allostatic load at age 70 with edentulism at age 85 (15 – year follow-up)
[d] Model 3 examines the longitudinal association of allostatic load at age 80 with edentulism at age 85 (5 – year follow-up)
[d] Model 4 examines the longitudinal association of allostatic load at ages 70/80 with edentulism at age 85

Supplemental Table 3.7. Rotated factor pattern and final communality estimates from principal component analysis at age 70, n=453

Physiological Markers	Rotated Factor Pattern		
	Lipid pattern	Blood pressure pattern	Cardio-pulmonary pattern
BMI	0.46	0.38	0.07
Total cholesterol/HDL	0.93	0.01	0.05
HDL cholesterol	-0.87	0.02	-0.18
Triglycerides	0.83	0.11	-0.16
Resting SBP	0.03	0.87	-0.11
Resting DBP	0.09	0.91	-0.03
Heart rate	-0.08	0.35	-0.62
FEV_1	-0.01	0.12	0.87
Proportion of variance explained	33%	23%	14%

Supplemental Table 3.8. Spearman correlations between factor scores and physiological markers at age 70

Physiological Markers	Factor scores		
	Lipid pattern	Blood pressure pattern	Cardio-pulmonary pattern
BMI	0.48	0.35	-0.09
Total cholesterol/HDL	0.93	0.02	-0.06
Triglycerides	0.81	0.11	0.14
HDL cholesterol	-0.87	0.01	0.17
Resting SBP	0.04	0.86	0.09
Resting DBP	0.08	0.90	0.01
Heart rate	-0.07	0.32	0.62
FEV_1	0.01	0.13	-0.86

Supplemental Table 3.9: Cross-sectional associations of factor scores with periodontal disease and edentulism at age 70

| Factor scores	ORs[a] for having higher periodontal disease and edentulism				
	n	Mean MCPI scores	Mean % of MCPI scores ≥ 3	n	Edentulism[b]
Lipid					
Quartile 1	58	1.00	1.00	84	1.00
Quartile 2	52	0.86 (0.42 – 1.74)	1.10 (0.54 – 2.21)	85	1.44 (0.70 – 2.96)
Quartile 3	42	0.60 (0.28 – 1.29)	0.77 (0.36 – 1.64)	85	2.28 (1.10 – 4.75)
Quartile 4	47	1.21 (0.57 – 2.60)	1.56 (0.73 – 3.32)	85	1.62 (0.77 – 3.37)
Blood pressure					
Quartile 1	50	1.00	1.00	84	1.00
Quartile 2	45	0.60 (0.28 – 1.29)	0.82 (0.39 – 1.75)	85	1.63 (0.81 – 3.30)
Quartile 3	48	0.83 (0.38 – 1.82)	0.96 (0.44 – 2.09)	85	1.09 (0.53 – 2.25)
Quartile 4	56	0.75 (0.36 – 1.55)	0.81 (0.39 – 1.67)	85	1.02 (0.50 – 2.07)
Cardio-pulmonary					
Quartile 1	55	1.00	1.00	84	1.00
Quartile 2	50	1.83 (0.84 – 3.96)	1.15 (0.48 – 2.26)	85	1.49 (0.71 – 3.13)
Quartile 3	58	1.69 (0.72 – 3.98)	1.01 (0.43 – 2.37)	85	1.36 (0.60 – 3.09)
Quartile 4	36	2.24 (0.87 – 5.76)	1.70 (0.67 – 4.32)	85	2.59 (1.16 – 5.77)

[a] ORs for periodontal disease were adjusted for the same covariates as in table 3.3b, final model: number of retained teeth, sex, smoking and physical activity.

[b] ORs for edentulism were additionally adjusted for income, housing status, and education

Supplemental Table 3.10: Longitudinal associations of factor scores at ages 70 and 80 with periodontal pocket depths at age 85

| Factor Scores (quartile 4 vs. 1-3)	Periodontal pocket depths (PD) at varying thresholds					
	% PD ≥ 3 mm		% PD ≥ 4 mm		% PD ≥ 5 mm	
	ß-coefficient[a] (SE)	*P*-value	ß-coefficient[a] (SE)	*P*-value	ß-coefficient[a] (SE)	*P*-value
Age 70, N=53						
Lipid	4.15 (7.11)	0.56	5.86 (0.30)	0.30	2.06 (4.48)	0.65
Blood pressure	10.0 (6.59)	0.14	11.17 (5.23)	0.04	6.01 (4.16)	0.16
Cardio-pulmonary	-2.88 (7.97)	0.72	1.47 (6.33)	0.82	1.65 (5.02)	0.74
Age 80, N=87						
Lipid	6.42 (5.65)	0.26	7.81 (4.67)	0.10	6.13 (3.20)	0.06
Blood pressure	1.07 (5.38)	0.84	-1.40 (4.46)	0.75	-0.59 (3.06)	0.85
Inflammation	-2.47 (6.47)	0.70	-4.96 (5.35)	0.36	-1.83 (3.67)	0.62
Glucose metabolism	-0.76 (8.09)	0.93	-1.79 (6.70)	0.79	-0.08 (4.59)	0.99

[a] Adjusted for number of teeth, income, and sex.
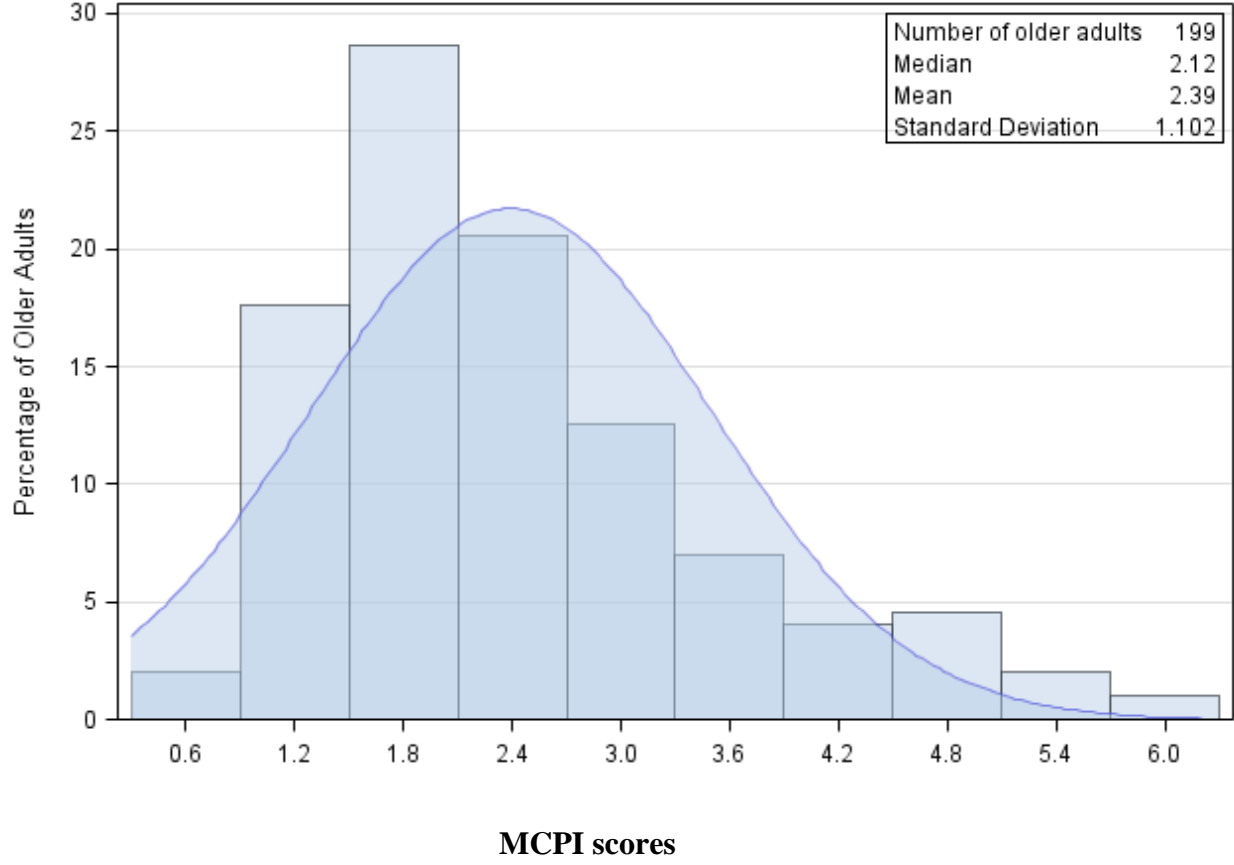
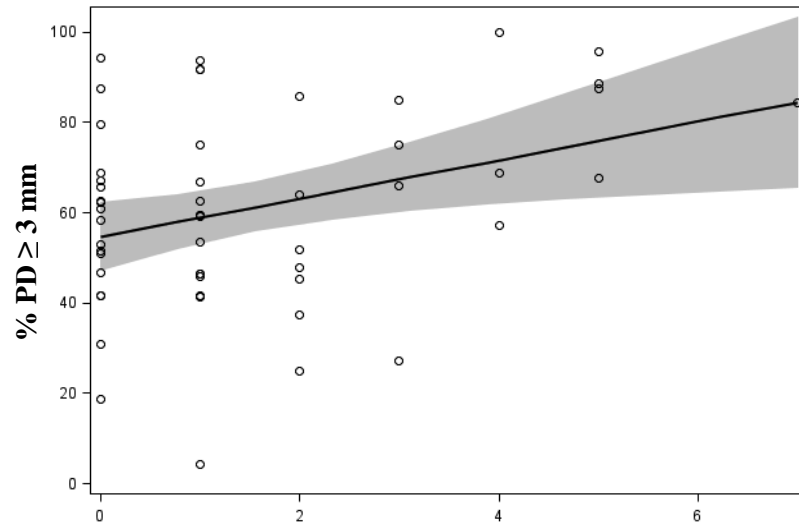**Supplemental Figure 3.1**. Distribution of MCPI score in the study population (dentate individuals)
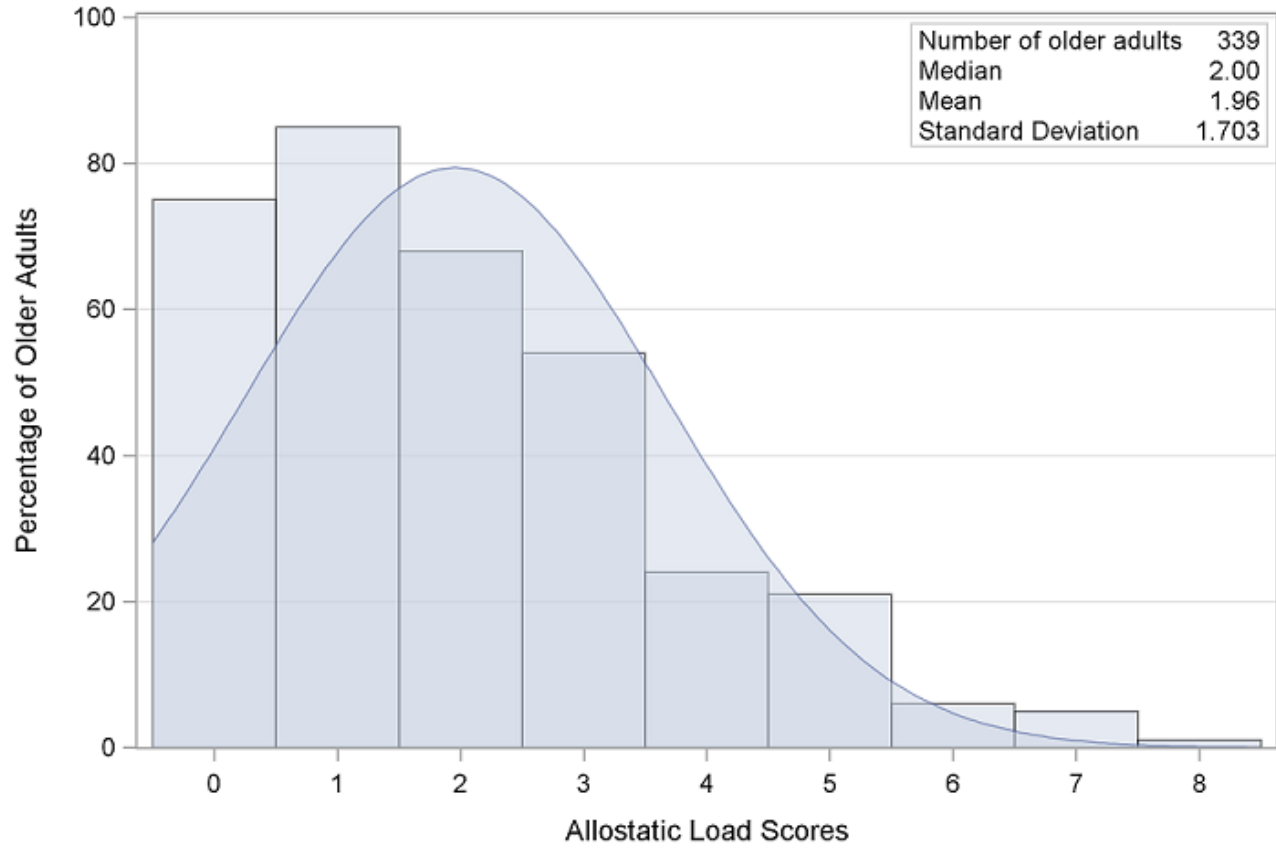
Supplemental Figure 3.2. Distribution of allostatic load scores in the study population at age 70
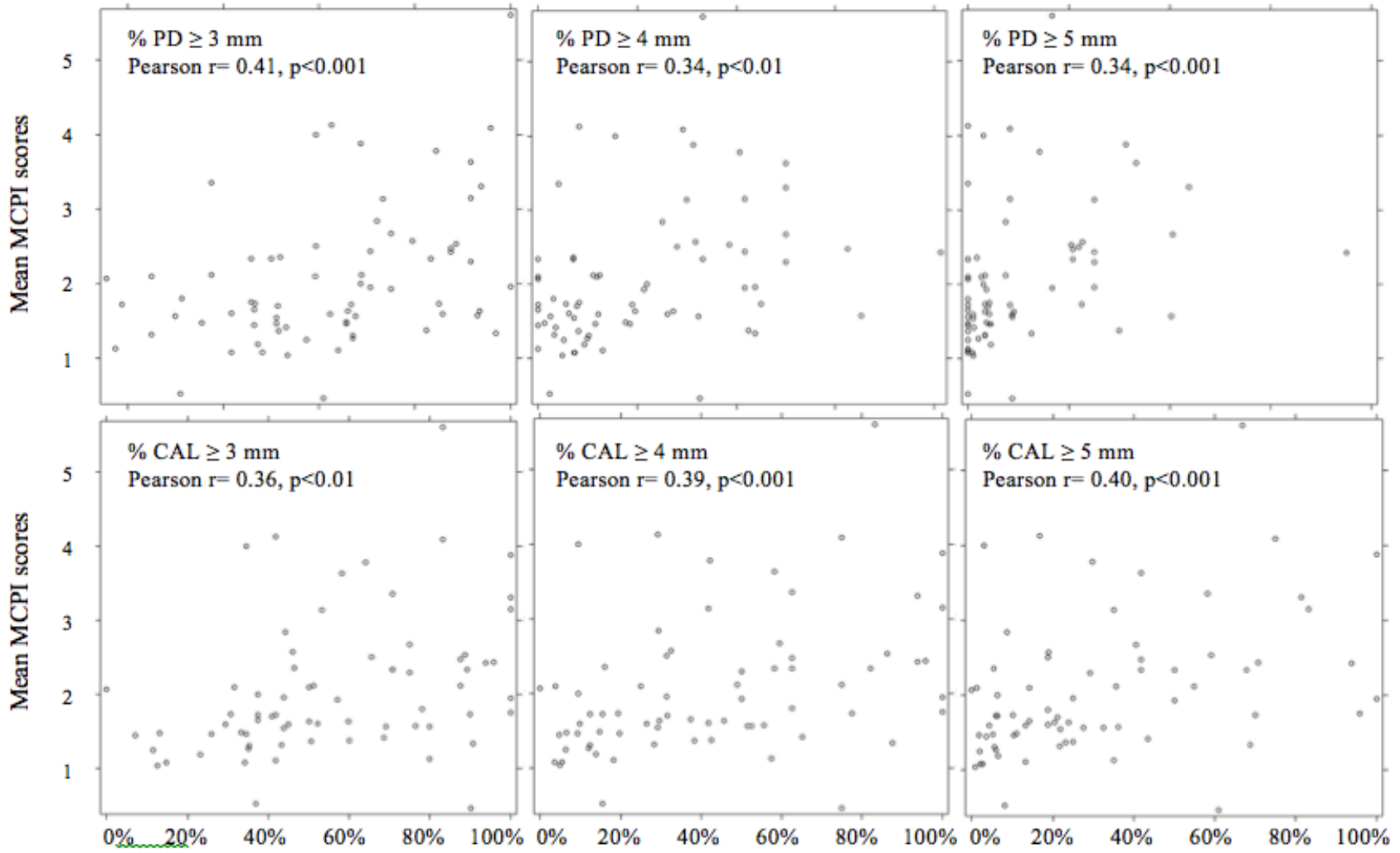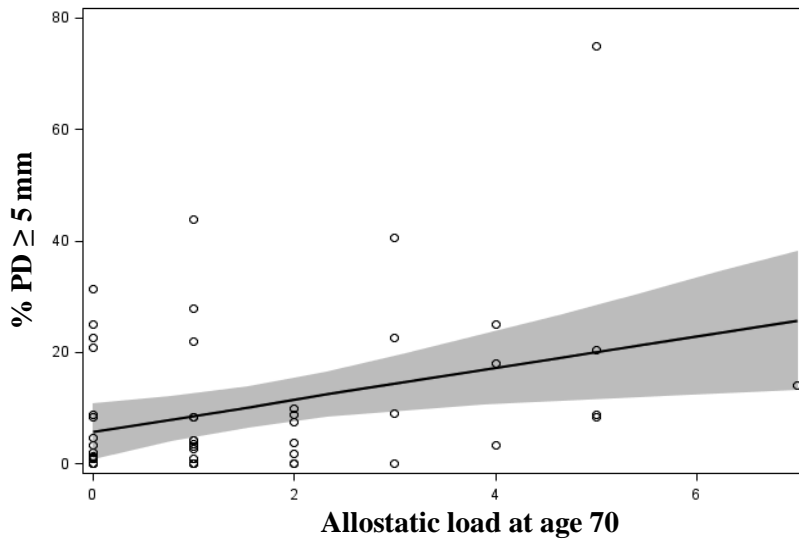
Supplemental Figure 3.3 Scatter plots of mean MCPI scores at age 70 with periodontal indices at varying thresholds at age 85
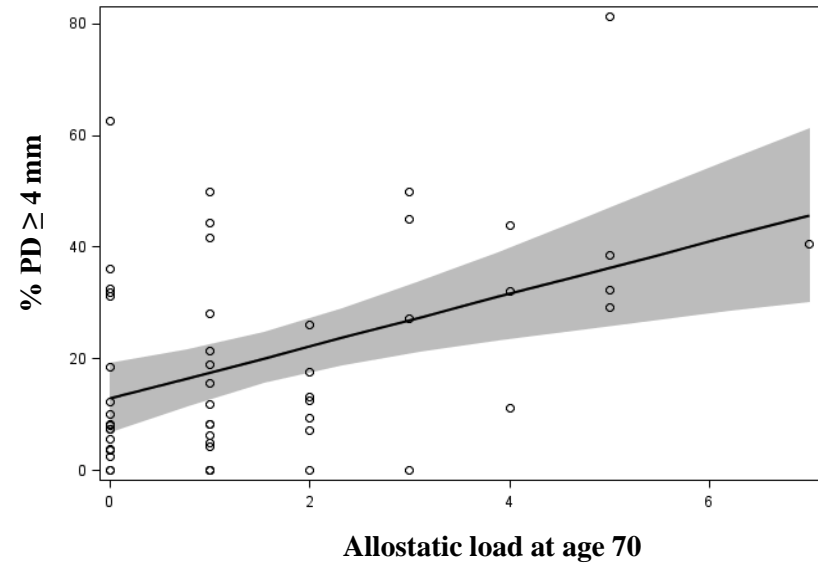
A.

B.

C.

Supplemental Figure 3.4.
Scatterplots of allostatic load scores measured at age 70 against percentage (%) of tooth sites with periodontal pocket depths (PD) at various thresholds at age 85. Top left panel **(A)** is a scatterplot of % of sites with PD ≥ 3mm. Top right panel **(B)** is a scatterplot of % of sites with PD ≥ 4mm. Bottom left panel **(C)** is a scatterplot of % of sites with PD ≥ 5mm. Included in the figures are unadjusted regression lines. The shaded area depicts 95% confidence intervals.
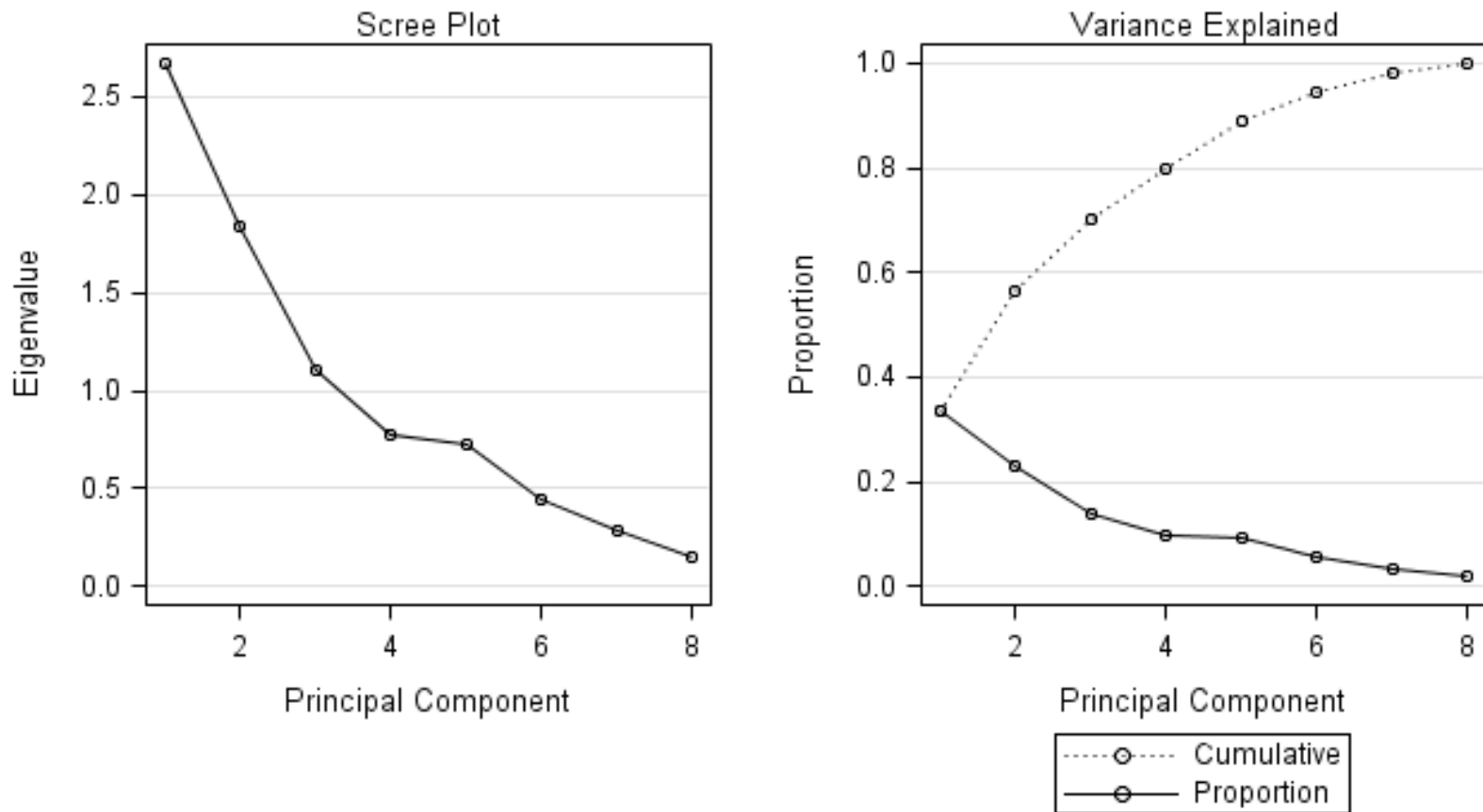
Supplemental Figure 3.5: Scree plot and proportion of variance explained. The left panel is a scree plot of principal components against eigenvalues derived from PCA. The right panel depicts the percent of total variance explained by each principal component. Each component was derived from a maximally weighted linear combination of 8 physiological markers assessed at age 70, n=453.