1. Abstract

Biomarkers are increasingly employed in empirical studies of human populations to understand physiological processes that change with age, diseases...
whose onset appears linked to age, and the aging process itself. In this chapter, we describe some of the most commonly used biomarkers in population aging research, including their collection, associations with other markers, and relationships to health outcomes. We discuss biomarkers of the cardiovascular system, metabolic processes, inflammation, activity in the hypothalamic-pituitary axis (HPA) and sympathetic nervous system (SNS), and organ functioning (including kidney, lung, and heart). In addition, we note that markers of functioning of the central nervous system and genetic markers are now becoming part of population measurement. Where possible, we detail interrelationships between these markers by providing correlations between high risk levels of each marker from three population-based surveys: the National Health and Nutrition Examination Survey (NHANES) III, NHANES 1999–2002, and the MacArthur Study of Successful Aging. NHANES III is used instead of NHANES 1999–2002 when specific markers of interest are available only in NHANES III and when we examine the relationship of biomarkers to mortality which is only known for NHANES III. We also describe summary measures combining biomarkers across systems. Finally, we examine associations between individual markers and mortality and provide information about biomarkers of growing interest for future research in population aging and health.

2. Introduction

There is no agreed upon set of biomarkers of aging; however, there is a significant body of literature discussing both what a “biomarker” is and what constitutes aging [1]. These topics are addressed briefly in the beginning of this chapter, but the majority of the chapter focuses on how biomarkers are used in empirical studies of human populations to understand physiological processes that change with age, diseases whose onset appears linked to age, and the aging process itself [2]. We limit ourselves to biomarkers related to general indicators of health and survival that are appropriate for study in human populations in vivo, and we do not include biomarkers that are specific to the diagnosis, staging, or prognosis of specific diseases. In our discussion, we indicate the health outcomes that are related to each of the markers, interrelationships between markers, the link between individual and summary biomarkers and mortality, and measures of health used in the older population that are based on multiple indicators. Finally, we indicate future challenges in studying aging populations with biomarkers.
3. **Background**

3.1. **What is a Biomarker?**

The lack of an agreed definition for the term “biomarker” was one impetus for the National Institutes of Health (NIH) to recently convene a Biomarkers Definitions working group [3]. The following definition has been offered by this group: “a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention [4].” In a recent strategic plan for the National Heart Lung and Blood Institute (NHLBI), the word “genotype” was added to the definition before normal biological processes, indicating how the focus of much research has changed since 2001 [5]. The current emphasis on biomarkers arises from an interest in understanding the molecular and physiological basis of disease as well as evaluating therapeutic interventions using surrogate end points rather than death or irreversible disease [6]. Social scientists are interested in adding biomarkers to traditional population studies of health in order to determine how social, psychological, and behavioral factors get under the skin to influence biology and subsequent health outcomes [7, 8].

In populations, biomarkers are used to monitor and predict the health of the population, to identify individuals with particular resistance or susceptibility to health problems, and to evaluate therapeutic interventions. Because of the clinical association of the word “biomarker” with risk factor, one group with a focus on aging populations has used the word “biomeasure” as a higher order term to encompass biomarkers of organic disease, physical condition or function, genetic markers, and biological indicators of aging [9]. In this chapter, we consider all of these types of measures as “biomarkers.”

3.2. **What is Aging?**

While basic scientists continue to try to separate normal aging and disease, scientists interested in population health are more empirically oriented toward defining the age-related health changes that are of interest in evaluating functional ability and survival, which typically represent some combination of aging and disease. Health change in old age has been termed the disablement process by Verbrugge and Jette [10]. In populations, health change occurs in an ordered fashion by age beginning with the development of risk factors, through the onset of diseases and conditions, to functioning loss or loss of ability to perform certain physiological functions and to the onset of disability which is often indicated by inability to work, to care for oneself, or to perform the activities necessary for independent living among older
populations. Frailty is an emerging concept in the study of health outcomes that is specific to older age [11–13]. It is a downward trajectory in health and ability to perform daily tasks resulting from the accumulation of acute and chronic diseases as well as the physiological decline and dysregulation that accompany the onset of diseases and advanced age [12]. Biomarkers can be indicators of any of these aspects of health change: risk, disease, functioning loss, disability, frailty, or imminent death.

4. Biomarkers

In human populations, the identification of biomarkers for health outcomes has resulted from large-scale community and population studies, such as the Framingham study and the NHANES. The MacArthur Study of Successful Aging was the first large-scale community-based study that provided extensive collection of biomarkers in a home-based setting. Because of increases in scientific knowledge of aging and improvements in technology for collection, a growing number of recent population studies have included biomarkers along with collection of social, economic, and psychological information [14]. We note the details of some of these studies at the end of our discussion of individual biomarkers.

In this section, we outline biomarkers that have been used in research on the health of older populations (Table 1) [15–139]. We describe the markers and why they are important in research on aging. This list represents a selection from a significantly larger number of markers that could be described. Our intent is to provide information on the currently most frequently used measures and to indicate some newer measures that are growing in use.

4.1. Cardiovascular System

We begin with indicators of cardiovascular functioning, as heart disease is the leading cause of death in the older population and one of the most important causes of disability (Table 1). The two indicators of blood pressure are probably the most commonly measured biomarkers: systolic blood pressure (SBP) is the maximum pressure in an artery at the moment when the heart is beating and pumping blood; diastolic blood pressure (DBP) is the lowest pressure in an artery in the moments between beats when the heart is resting. High levels of either measurement indicate hypertension. Current guidelines define hypertension as SBP ≥140 mm Hg or DBP ≥90 mm Hg.

SBP is thought to be more important and predictive of aging health outcomes than DBP. There are strong associations between aging, increased SBP, and cardiac and vascular diseases [140]. Studies have shown the
<table>
<thead>
<tr>
<th>Biomarkers of cardiovascular system</th>
<th>Description</th>
<th>Measure</th>
<th>Related Outcomes</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (SBP)</td>
<td>Index of cardiovascular activity: maximum pressure in an artery when the heart is pumping blood throughout the body</td>
<td>Physical exam</td>
<td>Cardiovascular death, stroke, CHD, mortality</td>
<td>[15–17]</td>
</tr>
<tr>
<td>Diastolic blood pressure (DBP)</td>
<td>Index of cardiovascular activity: lowest pressure in an artery when the heart is resting</td>
<td>Physical exam</td>
<td>Cardiovascular death, stroke, CHD, mortality</td>
<td>[15–17]</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>Indicator of increased arterial stiffness</td>
<td>Physical exam</td>
<td>Stroke, MI, heart failure, cardiovascular death, overall mortality</td>
<td>[18–20]</td>
</tr>
<tr>
<td>Resting pulse rate</td>
<td>Indicator of heart functioning and measure of overall fitness</td>
<td>Physical exam</td>
<td>CHD, mortality</td>
<td>[21]</td>
</tr>
<tr>
<td>Total homocysteine (tHcy)</td>
<td>An amino acid that plays a role in lipid metabolism; folic acid and vitamin B break down tHcy</td>
<td>Blood</td>
<td>Cardiovascular, cerebrovascular, and peripheral vascular disease, poor cognitive function</td>
<td>[22–25]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarkers of metabolic processes</th>
<th>Description</th>
<th>Measure</th>
<th>Related Outcomes</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>Aids in the synthesis of bile acids and steroid hormones</td>
<td>Blood</td>
<td>In middle-age: CHD and all-cause mortality; In older ages: U-shaped relation to death</td>
<td>[26–28]</td>
</tr>
<tr>
<td>Low-density lipoprotein (LDL)</td>
<td>Transports cholesterol from the liver to be incorporated into cell membrane tissues</td>
<td>Fasting blood</td>
<td>CHD, atherosclerosis, stroke, peripheral vascular disease</td>
<td>[29–32]</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>Description</td>
<td>Measure</td>
<td>Related Outcomes</td>
<td>Source</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Very low density lipoprotein (VLDL)</td>
<td>Transports endogenous triglycerides, phospholipids, cholesterol, and cholesteryl esters</td>
<td>Fasting blood</td>
<td>Atherosclerosis, coronary artery disease</td>
<td>[33–34]</td>
</tr>
<tr>
<td>High-density lipoprotein (HDL) cholesterol</td>
<td>Protective cholesterol</td>
<td>Blood</td>
<td>Lower atherosclerotic CVD</td>
<td>[35]</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Fat substance stored for energy use</td>
<td>Fasting blood</td>
<td>Heart attack, CHD, CAD, pancreatitis</td>
<td>[33, 36–38]</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>Measures amount of sugar in blood; indicator of diabetes</td>
<td>Fasting blood</td>
<td>Diabetes, CHD, mortality, poor cognitive function</td>
<td>[39–41]</td>
</tr>
<tr>
<td>Glycosylated hemoglobin (HbA1c)</td>
<td>Measures amount of sugar binded to hemoglobin in red blood cells</td>
<td>Blood</td>
<td>Diabetes-related complications (eye, kidney, nerve, CHD, stroke), poor cognitive function</td>
<td>[39, 42, 43]</td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
<td>Indicator of the balance between energy intake and energy expenditure</td>
<td>Physical exam</td>
<td>CVD, diabetes mellitus, stroke, mortality, some cancers, osteoarthritis</td>
<td>[44–47]</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>Indicator of abdominal obesity</td>
<td>Physical exam</td>
<td>Hypertension, CHD, noninsulin-dependent diabetes, stroke</td>
<td>[48–50]</td>
</tr>
<tr>
<td>Leptin</td>
<td>Protein hormone that regulates food intake and energy expenditure</td>
<td>Blood</td>
<td>Diabetes mellitus, metabolic syndrome (abdominal obesity, dyslipidemia, hypertension, hyperglycemia), atherosclerosis, osteoporosis</td>
<td>[51–57]</td>
</tr>
<tr>
<td>Biomarker</td>
<td>Description</td>
<td>Sample</td>
<td>Conditions/Associated Diseases</td>
<td>References</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
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</tr>
<tr>
<td><strong>Adiponectin</strong></td>
<td>Adipose-specific plasma protein that serves as a measure of insulin sensitivity</td>
<td>Fasting blood</td>
<td>Metabolic syndrome (abdominal obesity, dyslipidemia, hypertension, hyperglycemia); MI</td>
<td>[58, 59]</td>
</tr>
<tr>
<td><strong>Biomarkers of inflammation, immunity, and infection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (CRP)</td>
<td>Acute-phase response protein that indicates blood levels of inflammation</td>
<td>Blood</td>
<td>CVD, heart attack, stroke, arthritis, cancer, cognitive, physical decline</td>
<td>[60–65]</td>
</tr>
<tr>
<td>Interleukin-6 (IL-6)</td>
<td>Immune system regulator (cytokine) that responds to acute illness or injury</td>
<td>Blood, saliva</td>
<td>CVD, immune disorders, AD, diabetes mellitus, certain cancers, functional disability</td>
<td>[60, 65–67]</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Protein produced by the liver that aids in formation of blood clots to stop bleeding</td>
<td>Blood</td>
<td>CVD, mortality, AD, MCI (γ chain)</td>
<td>[40, 60, 68, 69]</td>
</tr>
<tr>
<td>Albumin</td>
<td>Protein that transports small molecules into the blood and maintains oncotic pressure</td>
<td>Blood</td>
<td>Heart attack, stroke, functioning decline, mortality, cognitive impairment</td>
<td>[60, 65, 70, 71]</td>
</tr>
<tr>
<td>Tumor necrosis factor-α (TNFα)</td>
<td>Proinflammatory cytokine that stimulates immune and vascular responses</td>
<td>Blood, CSF</td>
<td>Obesity, diabetes, arthritis, stroke</td>
<td>[72–75]</td>
</tr>
<tr>
<td>Serum amyloid A (SAA)</td>
<td>Acute-phase protein; main function involves cholesterol transport and lipid metabolism</td>
<td>Blood</td>
<td>CAD, atherosclerosis, cancer, carotid intima medial thickness, depression, obesity</td>
<td>[76–81]</td>
</tr>
<tr>
<td>Cytomegalovirus (CMV)</td>
<td>Herpesvirus infectious agent that triggers the immune system</td>
<td>Blood</td>
<td>Dementia, retinal, and gastrointestinal disease</td>
<td>[82, 83]</td>
</tr>
</tbody>
</table>

(continues)
TABLE 1 (Continued)

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Description</th>
<th>Measure</th>
<th>Related Outcomes</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epstein-Barr virus (EBV)</strong></td>
<td>B lymphotropic herpesvirus; marker of cell-mediated immune function</td>
<td>Blood, saliva</td>
<td>Cancer, infectious mononucleosis</td>
<td>[84–86]</td>
</tr>
<tr>
<td><strong>T cells</strong></td>
<td>White blood cells that protect against pathogens and tumors</td>
<td>Blood</td>
<td>Cancer, mortality, atherosclerosis, AD</td>
<td>[87]</td>
</tr>
<tr>
<td><strong>Biomarkers of the central nervous system</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Amyloid /42</strong></td>
<td>Major component of senile plaques</td>
<td>CSF</td>
<td>Inverse relation to neuropathological processes (AD); frontotemporal and vascular dementia</td>
<td>[88–91]</td>
</tr>
<tr>
<td><strong>Total (t)-Tau</strong></td>
<td>Major protein constituting neurofibrillary tangles</td>
<td>CSF</td>
<td>AD; Creutzfeldt-Jakob disease</td>
<td>[89, 92]</td>
</tr>
<tr>
<td><strong>Phosphorylated (p)-Tau</strong></td>
<td>Precedes formation of neurofibrillary tangles</td>
<td>CSF</td>
<td>AD, MI</td>
<td>[91, 93, 94]</td>
</tr>
<tr>
<td><strong>F2-isoprostanes (F2-iso)</strong></td>
<td>Isomer of prostaglandins stored in cells; stable, free radical-catalyzed products that reflect lipid peroxidation</td>
<td>CSF</td>
<td>AD, hypercholesterolemia, atherosclerotic plaque</td>
<td>[95–99]</td>
</tr>
<tr>
<td><strong>Biomarkers of the HPA and the sympathetic nervous system</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cortisol</strong></td>
<td>Steroid hormone that reflects body’s response to physiological stress</td>
<td>Blood, saliva, urine</td>
<td>CVD, poor cognitive functioning, fractures, functional disability, mortality</td>
<td>[100–104]</td>
</tr>
<tr>
<td><strong>Dehydroepiandrosterone sulfate (DHEA-S)</strong></td>
<td>Antagonist of cortisol; steadily decreases with age</td>
<td>Blood, saliva, urine</td>
<td>Inverse relation to heart disease, mortality, physical, and mental functioning, AD</td>
<td>[105–111]</td>
</tr>
<tr>
<td>Biomarker</td>
<td>Description</td>
<td>Sample</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
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<td>----------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Free insulin-like growth factor-1 (IGF-1)</td>
<td>Growth factor that regulates cell growth and development; Inhibitor of programmed cell death</td>
<td>Fasting blood</td>
<td>Fasting blood, Cancer; inverse relation to atherosclerotic plaques, CAD, osteoarthritis, mortality [112–115]</td>
<td></td>
</tr>
<tr>
<td>Norepinehrine Epinephrine (adrenaline)</td>
<td>Indicator of stress response Stress hormone important to body’s metabolism; prepares for strenuous activity of the “fight or flight” response</td>
<td>Blood, urine</td>
<td>Blood, urine, CHF, MI, mortality Cognitive decline and possibly poor survival with prior MI [116–118]</td>
<td></td>
</tr>
<tr>
<td>Biomarkers of organ function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>In clinical practice, an index of renal function</td>
<td>Blood, urine</td>
<td>Blood, urine, Cardiovascular risk, renal diseases, mortality [122–125]</td>
<td></td>
</tr>
<tr>
<td>Cystatin C (CysC)</td>
<td>Detects rapid glomerular filtration rate</td>
<td>Blood</td>
<td>Blood, Acute renal failure, diabetic nephropathy, thyroid dysfunction, mortality [126–131]</td>
<td></td>
</tr>
<tr>
<td>Peak expiratory flow (PEF)</td>
<td>Measurement of airway obstruction</td>
<td>Spirometry exam</td>
<td>Spirometry exam, Asthma, chronic obstructive pulmonary disease [132, 133]</td>
<td></td>
</tr>
<tr>
<td>Electrocardiogram (EKG)</td>
<td>Measurement of electrical impulses in the heart</td>
<td>Physical exam</td>
<td>Physical exam, Cardiovascular risk, stroke, mortality [134–136]</td>
<td></td>
</tr>
<tr>
<td>Biomarkers of oxidative stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive oxidative species (ROS)</td>
<td>Involved in programmed cell death and apoptosis, induction of host defense, mobilization of ion transport systems</td>
<td>Blood</td>
<td>Blood, Parkinson’s disease, DNA damage (cancer) [137, 138]</td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutase (SOD)</td>
<td>Important antioxidant defense in cells exposed to oxygen</td>
<td>Blood</td>
<td>Blood, Inverse relation to AD [139]</td>
<td></td>
</tr>
</tbody>
</table>

CHD = Coronary heart disease; AD = Alzheimer’s disease; MI = myocardial infarction; CAD = coronary artery disease; CVD = cardiovascular disease; MCI = mild cognitive impairment; PD = Parkinson’s disease; CHF = congestive heart failure; CSF = cerebrospinal fluid.
stronger predictive power of SBP for coronary heart disease (CHD) and life expectancy at advanced ages [27, 141, 142]. Among the Framingham Heart Study participants, SBP was directly related to CHD risk, but DBP was inversely related to the risk in older ages (60+) [143].

**Pulse pressure** (PP) is an alternative measure indicating the difference between the SBP and DBP that some researchers prefer for use in studying the aged. The rise in SBP and PP in middle-aged and elderly subjects is mainly related to increased large-artery stiffness and an associated increase in wave reflection amplitude [144]. Increasing evidence shows that PP predicts risk of CHD in middle and old ages [19, 143, 145]. During middle age, SBP and DBP change similarly; however after age 60, DBP decreases and SBP continues to rise resulting in the large increase in PP in old ages [143]. While factors such as smoking, lack of physical activity, and drinking affect PP, studies have shown the independent effect of PP on health outcomes after adjusting for such risk factors [146].

**Heart rate**, considered one of the four vital signs, is based on the number of heartbeats per minute (bpm). In most cases, the pulse is an accurate measure of heart rate, and the two terms are often used synonymously; although in individuals with certain arrhythmias, heart rate and pulse rate may not be equivalent. **Pulse rate** is commonly measured from the brachial artery (the wrist) or the carotid artery (the neck).

Since pulse rate increases with physical exercise, it is commonly measured during resting, nonphysical exertion conditions. At rest, the average adult pulse rate is 70 bpm for males and 75 bpm for females; however, these rates may vary by age, sex, race and ethnicity, and exercise status. At birth, pulse rate ranges from 100 to 180 bpm and gradually decreases to range from 60 to 110 bpm until age 16 [21, 147]. Between ages 25–74, no consistent changes in pulse rate with age have been found [148]. Gender and racial differentials indicate that women have higher resting pulse rate than men and White women have higher pulse rates than Black women [148]. Finally, athletes exhibit much lower resting pulse rates as a result of strengthened heart muscle from regular exercise [149].

A pulse rate of 90 bpm or greater is considered high [150] and is associated with increased risk of CHD, as well as cardiovascular, noncardiovascular, and all-cause mortality [21, 151]. Consequently, both medical (e.g., medication) and nonmedical modifications (e.g., life style modifications including increases in physical activity and lower fat diets) can reduce resting pulse rate, and, in turn, reduce the risk of cardiovascular disease and mortality [152–154].

All of the above markers are collected in a physical exam. There are many other biomarkers linked to cardiovascular risk that are determined in other ways. One of these is **homocysteine**, an amino acid measured from blood plasma. Homocysteine affects the development of atherosclerosis by
damaging the inner lining of arteries and promoting blood clots. For this reason, we are including it with other cardiovascular risk factors even though it differs from the others in that it is measured with blood. Homocysteine has garnered recent attention because of its importance in predicting many of the major health outcomes common in aging populations, including cardiovascular disease, peripheral vascular disease, and poorer cognitive function [22–25]. It is highly related to dietary content including folate and vitamins B12 and B6 [155, 156]. In the early 1990s, approximately one-third of those older than 65 years had elevated homocysteine levels (>14 μmol/liter) [157]; however, the prevalence has declined markedly since dietary fortification with folate began in 1996 [156, 158].

We indicate the interrelationships among the cardiovascular biomarkers for a nationally representative sample of persons aged 65 and over in the NHANES and for the MacArthur Sample of Successful Aging participants who were aged 70–79. Biomarkers are dichotomously defined using the level of each biomarker to classify sample members into those at a level defined as at clinical risk, or in the top quartile of the sample, or not. Clinical risk levels are shown in Table 2 [159–171] and the phi coefficients among cardiovascular markers in Table 3. A phi coefficient is a measure of the degree of association between two binary variables and is interpreted like a pearson correlation coefficient. The most significant coefficients are between SBP and DBP, which are moderately related with a coefficient of 0.19 (NHANES) and 0.34 (MacArthur), and between SBP and PP, which are relatively strongly related with a correlation of 0.48 (NHANES). With the exception of SBP, DBP, and PP, high risk levels of these biomarkers occur fairly independently of each other.

4.2. MARKERS OF METABOLIC PROCESSES

The next set of markers is indicators of metabolic processes, many of which are also related to cardiovascular outcomes. Cholesterol has several functions including keeping cell membranes intact and helping the synthesis of steroid hormone and bile acids. In recent years, components of total cholesterol are generally measured to determine risk for heart disease: low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low density lipoprotein (VLDL) [172]. In middle-aged populations, total cholesterol level has been shown to have a direct relation with CHD and all-cause mortality [26]. However, in older persons, the relationship between cholesterol and mortality has been found to be U- or J-shaped [27, 28]. Comorbidity may need to be considered in evaluating the risk implied by cholesterol levels among frail older persons [17, 173, 174].
<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>High risk cutpoints</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biomarkers of cardiovascular system</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>≥140 mm Hg (N)</td>
<td>[159]</td>
</tr>
<tr>
<td></td>
<td>≥148 mm Hg (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>≥90 mm Hg (N)</td>
<td>[159]</td>
</tr>
<tr>
<td></td>
<td>≥83 mm Hg (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>≥88 mm Hg (N)</td>
<td>NHANES III 1999–2002 fourth quartile$^a$</td>
</tr>
<tr>
<td>Resting pulse rate</td>
<td>≥90 bpm (N)</td>
<td>[150]</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>≥15 μmol/liter (N)</td>
<td>[161, 162]</td>
</tr>
<tr>
<td></td>
<td>≥13.38 μmol/liter (M)</td>
<td>[163]</td>
</tr>
<tr>
<td><strong>Biomarkers of metabolic processes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum total cholesterol</td>
<td>≥240 mg/dl (N)</td>
<td>[164]</td>
</tr>
<tr>
<td>Serum HDL cholesterol</td>
<td>≥40 mg/dl (N)</td>
<td>[164]</td>
</tr>
<tr>
<td></td>
<td>≥37 mg/dl (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>Total/HDL cholesterol</td>
<td>≥5.92 (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>Serum LDL cholesterol</td>
<td>≥160 mg/dl (N)</td>
<td>[164]</td>
</tr>
<tr>
<td>Serum triglycerides</td>
<td>≥200 mg/dl (N)</td>
<td>[164]</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>≥126 mg/dl (N)</td>
<td>[164]</td>
</tr>
<tr>
<td>Glycosylated hemoglobin</td>
<td>≥6.4% (N)</td>
<td>[164]</td>
</tr>
<tr>
<td></td>
<td>≥7% (M)</td>
<td>[160]</td>
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<tr>
<td>Body mass index</td>
<td>≥30 kg/m$^2$ (N)</td>
<td>[166]</td>
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<tr>
<td></td>
<td>≥28.59 kg/m$^2$ (M)</td>
<td>[163]</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>≥0.94 (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>Serum leptin</td>
<td>≥17.2 μg/liter (N)</td>
<td>NHANES III (1988–1994), fourth quartile$^a$</td>
</tr>
<tr>
<td><strong>Biomarkers of inflammation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>≥3 mg/liter (N)</td>
<td>[167]</td>
</tr>
<tr>
<td></td>
<td>≥3.19 mg/liter (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>IL-6</td>
<td>≥4.64 pg/ml (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>Plasma fibrinogen</td>
<td>≥400 mg/dl (N)</td>
<td>[168]</td>
</tr>
<tr>
<td></td>
<td>≥336 mg/dl (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>Albumin</td>
<td>&lt;3.8 g/dl (N)</td>
<td>[169]</td>
</tr>
<tr>
<td></td>
<td>≤3.9 g/dl (M)</td>
<td>[160]</td>
</tr>
<tr>
<td><strong>Biomarkers of HPA and SNS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary cortisol</td>
<td>≥25.69 μg/g creatinine (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>≤350 ng/ml (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>≥48 ug/g creatinine (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>≥4.99 ug/g creatinine (M)</td>
<td>[160]</td>
</tr>
<tr>
<td><strong>Markers of organ functioning</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>&lt;30 ml/min (N)</td>
<td>[170]</td>
</tr>
<tr>
<td></td>
<td>≤44.64 ml/min (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>Best peak flow</td>
<td>&lt;550 liter/min (males) (N)</td>
<td>NHANES III (1988–1994), fourth quartile$^a$</td>
</tr>
<tr>
<td></td>
<td>&lt;400 liter/min (females) (N)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤300 liter/min (M)</td>
<td>[160]</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>&gt;1.55 mg/liter (N)</td>
<td>[171]</td>
</tr>
</tbody>
</table>

(N) NHANES; (M) MacArthur.

HDL = high-density lipoprotein; LDL = low-density lipoprotein; IL-6 = interleukin-6; DHEA-S = dehydroepiandrosterone sulfate.

$^a$ Individual data from NHANES III (1988–1994), using the highest quartile as at risk.
LDL is sometimes referred to as “bad” cholesterol because elevated levels of LDL correlate most directly with CHD [32]. Current guidelines indicate that a desirable level of LDL cholesterol is below 130 mg/dl; borderline high is from 130 to 159 mg/dl; high is between 160 and 189 mg/dl; and very high LDL-cholesterol is ≥190 mg/dl. Recently, recommended target levels of cholesterol were adjusted to be lower for those with diabetes and other heart disease risk factors. Those who have established coronary disease and diabetes have a recommended target for an LDL cholesterol level less than 70 mg/dl [29]. Generally, a high level of LDL cholesterol has been shown to contribute to the development of coronary atherosclerosis and to increased risk of mortality and heart disease [175]; however, studies limited to older persons have shown inconsistent findings on the relationship between LDL and health outcomes [40, 173, 176–183].

Akin to LDL, levels of VLDL increase with age and are also commonly referred to as “bad” cholesterol [184]. While VLDL is not measured as frequently in population studies, it may be a better indicator of risk in older people. Among individuals aged 50 or older, VLDL was a better predictor of the development of coronary artery disease, while LDL cholesterol was more significant among people under age 50 [34].

High levels of HDL are protective for heart disease because HDL carries cholesterol away from the arteries and back to the liver, where it is passed from the body. Thus, HDL is called the “good” cholesterol and low levels are associated with higher risk. HDL cholesterol levels less than 40 mg/dl

---

**TABLE 3**

<table>
<thead>
<tr>
<th>PH COEFFICIENTS AMONG HIGH RISK LEVELS OF CARDIOVASCULAR BIOMARKERS</th>
</tr>
</thead>
</table>

(a) Ages 65+ in the NHANES 1999–2002 (N=1,884)

<table>
<thead>
<tr>
<th></th>
<th>DBP</th>
<th>SBP</th>
<th>Pulse pressure</th>
<th>Resting pulse rate</th>
<th>Homocysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP</td>
<td>0.19***</td>
<td>-0.04+</td>
<td>-0.01</td>
<td>-0.01</td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>0.48***</td>
<td>-0.02</td>
<td>-0.03</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>-0.03</td>
<td>0.05+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting pulse rate</td>
<td></td>
<td>0.07*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homocysteine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) Ages 70–79 in the MacArthur Study of Successful Aging [N=654 (N=363 for correlations to homocysteine)]

<table>
<thead>
<tr>
<th></th>
<th>DBP</th>
<th>SBP</th>
<th>Homocysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP</td>
<td>0.34***</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td></td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td>Homocysteine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DBP = diastolic blood pressure; SBP = systolic blood pressure.
*** p <0.0001, ** p <0.001, *p <0.01, +p <0.05.
(although sometimes this level is sex specific) have been related to increased risk for heart disease [185–187].

While traditional lipid measures, such as total cholesterol and HDL, are often used independently to indicate lipid profiles and their relations to health outcomes, studies have shown that total cholesterol/HDL ratio can be used as a biomarker that is associated with other cardiovascular risk factors [188, 189] and predicts ischemic heart disease risk [190] and atherosclerotic plaque rupture [191].

Triglycerides, an indicator of stored fat, are often included among the lipid indicators as part of an evaluation of coronary risk factors. Normal fasting triglyceride levels are below 150 mg/dl; 150–199 mg/dl is considered borderline high, 200–499 mg/dl high, and 500 mg/dl and above very high [33]. High triglyceride levels have been associated with heart attack [192], CHD [36], and coronary artery disease [37].

Tests for total cholesterol, LDL, HDL, and triglycerides are routinely done in lipid panels. Accurate results for the entire lipid panel assume 9–12 hours of fasting; however, total and HDL cholesterol can be measured without fasting and thus are more likely to be included in assays from large population surveys without fasting subjects. Fasting is required for valid results for LDL, VLDL, and triglycerides.

Fasting blood glucose level is indicative of diabetes and prediabetes. Higher than normal blood glucose contributes to the development of metabolic syndrome and CHD [193, 194]. About 11.9 million adults in the United States aged 45–74 had prediabetes levels of glucose in the year 2000 and this included a quarter (22.6%) of overweight adults [195]. A normal blood glucose level is between 70 and 99 mg/dl. A fasting blood glucose level between 100 and 125 mg/dl signals prediabetes and a higher level indicates diabetes [196].

Because it can be collected in a nonfasting sample, many researchers are measuring glycated hemoglobin (HbA1c) as an alternative to fasting glucose for diabetes screening [197]. The percentage of glycated cells increases with more glucose in the blood and provides an indicator of the amount of sugar that is attached to the hemoglobin in red blood cells. Because red blood cells live in the bloodstream for approximately 4 months, the HbA1c test shows the average blood sugar for the past 2–3 months and is an indicator of glucose metabolism over that time. Results of this test can indicate prediabetes and are used in managing diabetes. HbA1c levels have been related to cardiovascular disease and mortality among both diabetics and nondiabetics [198] and to CRP levels [199]. Some studies show age-related increases in HbA1c [200, 201], while others show little or no age-related increase in HbA1c [202], possibly due to its relationship to mortality.

Anthropometric measures such as weight, body mass index (BMI), waist and hip circumference, and waist-to-hip ratio (WHR) can all be used to
indicate weight and adiposity. BMI is calculated as the ratio of weight to height-squared (kg/m²). Overweight is defined as a BMI between 25 and 29.9 kg/m² and obesity as BMI ≥ 30 kg/m² [160]. However, the validity of BMI as a measure of excess fat declines in older people because of height loss and increases in fat mass occurring with age even in the absence of weight gain. Some researchers prefer WHR and waist circumference (WC) to BMI as a predictor for cardiovascular risk [102] and other adiposity-related conditions. While BMI provides an index of obesity, WHR may be more useful as an index of chronic metabolic dysregulation and adipose tissue deposition [203]. Researchers have argued that it is not obesity per se but the distribution of the adipose tissue that is related to increased risk [204, 205]. Those with an apple body shape or a central distribution of fat tend to experience higher rates of atherosclerotic heart disease, stroke, hypertension, hyperlipidemia, and diabetes than those with a pear body shape. According to the guidelines for defining metabolic syndrome [3], the use of a simple measure of WC instead of BMI is recommended to identify the body weight component of metabolic syndrome (men >40 in.; women >35 in.).

Those with higher values of BMI, waist and hip circumferences, and WHR tend to be at higher risk for hypertension, adult-onset diabetes mellitus, heart disease, stroke, various forms of cancer, atherosclerosis [44, 45, 47, 205–209], osteoarthritis [46], lower aerobic capacity and less muscle strength [210], and disability [211–215].

Leptin is a hormone that plays an important role in the long-term regulation of body weight. As a crucial regulator of food intake and energy balance, leptin is involved in the physiology of various diseases. In old age, declines in organ function and changes in hormone secretion result in the alteration of leptin secretion [216]. Although it is uncertain whether aging has an independent effect on leptin levels, it is known that some changes common in old age (e.g., declines in bone turnover and slower rates of glucose and lipid metabolism) are related to leptin levels. Studies have indicated that leptin may play an important role in several chronic diseases, including metabolic syndrome, atherosclerosis, malnutrition, diabetes mellitus, dyslipidemia, hypertension, osteoarthritis, and osteoporosis [54–57].

Examination of the interrelationships of risk levels among the metabolic markers available in the NHANES data indicates that total and LDL cholesterol are highly related (0.76) in the fasting population (Table 4a). Neither high risk levels of total or LDL cholesterol are very highly related to high risk levels of HDL cholesterol. HDL risk is moderately highly related to having high triglycerides (0.24), fasting blood glucose (0.15), and glycated hemoglobin (0.15). High-risk leptin levels are strongly related to high BMI (0.40). High BMI is moderately related to fasting blood glucose (0.12) and HbA1c (0.11), but not very closely related to any of the cholesterol indicators. The
TABLE 4
PHI COEFFICIENTS AMONG HIGH RISK LEVELS OF METABOLIC BIOMARKERS

(a) Ages 65+ in the NHANES 1999–2002 (NHANES III for Leptin) (N=1,884 for nonfasting biomarkers, N=938 for fasting biomarkers; N=2741 for nonfasting biomarkers, N=1172 for fasting biomarkers for NHANES III)

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>HDL</th>
<th>LDL(^a)</th>
<th>Triglycerides</th>
<th>Blood glucose(^a)</th>
<th>Glycosylated hemoglobin</th>
<th>BMI</th>
<th>Leptin(^{a,b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>-0.08***</td>
<td>0.76***</td>
<td></td>
<td>0.09*</td>
<td>0.02</td>
<td>0.06*</td>
<td>0.01</td>
<td>0.09*</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.05</td>
<td></td>
<td></td>
<td>0.24***</td>
<td>0.15***</td>
<td>0.15***</td>
<td>0.06</td>
<td>-0.10**</td>
</tr>
<tr>
<td>LDL(^a)</td>
<td></td>
<td></td>
<td></td>
<td>0.10*</td>
<td>-0.02</td>
<td>-0.04</td>
<td>0.00</td>
<td>0.04</td>
</tr>
<tr>
<td>Triglycerides(^a)</td>
<td></td>
<td></td>
<td>0.20***</td>
<td>0.12***</td>
<td>0.10**</td>
<td>0.04</td>
<td>0.04</td>
<td>0.10*</td>
</tr>
<tr>
<td>Blood glucose(^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.67***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycosylated hemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.12***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.11***</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Leptin(^{a,b})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.40***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) Ages 70–79 in the MacArthur Study of Successful Aging (N=654)

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol/HDL</th>
<th>HDL</th>
<th>Glycosylated hemoglobin</th>
<th>BMI</th>
<th>Waist/hip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol/HDL</td>
<td>0.55***</td>
<td>0.09(^+)</td>
<td>0.11*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>0.06</td>
<td>0.03</td>
<td>0.16***</td>
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<td></td>
</tr>
<tr>
<td>Glycosylated hemoglobin</td>
<td>0.13**</td>
<td>0.09(^+)</td>
<td>0.20***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>0.20***</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI = body mass index.
HDL = high-density lipoprotein; LDL = low-density lipoprotein.

\(^{a}\) Fasting biomarkers: LDL, triglycerides, blood glucose, leptin.

\(^{b}\) Correlations to leptin are based on biomarkers from NHANES III.
relationships among indicators of metabolic risk for the MacArthur data are shown in Table 4b. Analyses of these data have included total/HDL cholesterol ratio and the WHR among metabolic indicators [160]. Again, most of the relationships among the indicators are modest.

4.3. MARKERS OF INFLAMMATION, IMMUNITY, AND INFECTION

Markers of Inflammation are the next category of markers. Age-related changes in inflammatory markers are complex and include a wide range of potential indicators. Here we focus on the markers most commonly used in aging research. C-reactive protein (CRP) is an acute phase response protein produced in the liver that indicates general systemic levels of inflammation. CRP levels rise as part of the immune response to infection and tissue damage or injury and may be elevated due to the presence of chronic conditions, like diabetes, asthma, rheumatoid arthritis, and heart disease [61, 217–221]. In an acute response, the level of CRP can jump a thousand-fold but then drops relatively quickly when an infection passes. A blood level above 10 mg/dl is considered indicative of acute illness, although recent work has shown this level to be related to chronic conditions such as obesity and poor social conditions (e.g., living in poverty) [222]. CRP levels are also related to hormone levels in women and are elevated with the use of oral contraceptives or postmenopausal hormone replacement therapy.

Research has suggested that high levels of CRP, between 3 and 10 mg/dl [223], are related to the development of cardiovascular disease [61, 221, 224, 225] and cardiac events, including heart attack [60] and stroke [61]. This level of CRP has also been related to mortality [64, 65] and physical decline [65]. In contrast to many clinical settings, researchers use what is called a high-sensitivity CRP test (hs-CRP) to determine moderate (1–3 mg/dl) as well as higher levels of CRP. Hs-CRP can be measured with whole blood samples or blood spots [226].

Interleukin-6 (IL-6) is one of a class of immune system regulators called cytokines that serve a variety of immune functions in response to acute illness or injury and is perhaps the most commonly measured cytokine in population surveys. As a pro-inflammatory cytokine, IL-6 is involved in activating inflammatory pathways. IL-6 is always present in the body in small amounts (<1–2 µg/ml), and its concentration varies by time of day. However, in periods of immune activation, blood levels of IL-6 increase quickly, reaching as high as 40 times normal levels. IL-6 levels also rise with advancing age and are related to a variety of chronic conditions. The dysregulation of IL-6 may be a contributing factor to many of the diseases of aging.

Chronic conditions associated with high IL-6 include osteoporosis, arthritis, type-2 diabetes, certain cancers, and Alzheimer’s disease (AD) [66, 67].
High levels of IL-6 are also related to cardiovascular disease, heart attack, and stroke [60, 227–232]. In the elderly, high IL-6 levels are related to an increased risk of functional disability and functional decline [65, 70, 233], cognitive decline [234], and mortality [64, 65]. The association of IL-6 with cardiovascular disease is related to the central role this cytokine plays in promoting the production of CRP [60, 235]. Blood serum sample is required for IL-6 assays.

While less commonly included in large-scale studies, several other inflammatory cytokines have been linked to age-related outcomes. For instance, IL-10 is a pro-inflammatory cytokine also important to inflammatory and immunological responses [236]. IL-6 soluble receptor (IL-6sR) is important in the transition from acute to chronic inflammatory states [237]. IL-1β mainly stimulates T-helper cells that secrete IL-2, a cytokine that supports the proliferation of inflammatory cells [238] and influences the function of other cells by binding to IL-1 receptor antagonist (IL-1ra). IL-18, formerly called interferon (IFN)-γ inducing factor (IGIF), is closely related to IL-1. It induces IFN-γ produced in T cells, natural killer (NK) cells, gene expression, and the synthesis of tumor necrosis factor-α (TNFα) [239] (further described below). The cascade of inflammatory markers is highly interrelated and complex. Age-related increases in many of the cytokines have been noted [240], but further research on the associations of these individual markers is required before it is clear which can be included most usefully in population studies. But development of assays that can simultaneously measure a large number of inflammatory markers in dried blood spots is likely to increase markers measured in populations [241].

*Fibrinogen*, also called serum fibrinogen, plasma fibrinogen, and factor I, is a protein produced by the liver. Fibrinogen helps stop bleeding by promoting the formation of blood clots. Fibrinogen has been shown to be strongly predictive of both mortality [40] and the onset of cardiovascular disease [60, 227, 231, 242]. The relationship between socioeconomic status and fibrinogen levels has been suggested as a mechanism linking low social status and stress to cardiovascular disease [243–246]. Fibrinogen is measured using blood serum or plasma.

*Albumin* is a protein that transports small molecules in the blood and is important in maintaining oncotic pressure in the blood. Low albumin may be related to malnutrition or a low-protein diet and liver or kidney disease. Low albumin levels can also be related to inflammation. For this reason, albumin is sometimes included in indices of inflammation [247]. Low levels of albumin have been related to heart attack, stroke, functioning loss, and death among older persons [60, 65, 70, 227–233]. Data from the MacArthur study have related low levels of albumin to functional decline, death [65], and cognitive impairment [71]. Concomitant low serum cholesterol and albumin levels may
identify high-functioning older persons who are at increased risk of subsequent mortality and functional decline [248]. The test for albumin levels requires blood serum. In the MacArthur Study of Successful Aging analysis of allostatic load, low albumin has been included as a risk factor with a cutoff of 3.9 mg/dl or lower considered as high risk [160].

TNFα is a pleotropic polypeptide that plays an important role in inflammation and immune function. Expression of TNFα correlates with the expression of other cytokines, including IL-6 and IL-1. Mounting scientific evidence suggests that elevated blood plasma TNFα concentration is associated with dementia in centenarians [249] and is centrally involved in the pathogenesis of AD [250–255]. Additionally, high levels of TNFα are related to atherosclerosis [256], obesity and diabetes [72, 73], rheumatoid arthritis [74], and stroke [75].

Serum amyloid A (SAA), a grouping of acute-phase proteins, increases dramatically in response to injury and inflammation [257]. These proteins transport cholesterol to the liver for bile secretion, recruit immune cells to sites of inflammation, and induce enzymes to degrade extracellular matrix [76]. SAA is involved in chronic inflammatory diseases (e.g., atherosclerosis, coronary artery disease, and rheumatoid arthritis) [77–79, 258], and it is linked to lung cancer, depression, and obesity [78, 80, 81].

Interrelations among the inflammatory markers available in the NHANES and MacArthur studies are shown in Table 5a and b. High risk levels of fibrinogen and high risk CRP are relatively strongly related in both studies (0.33). High risk CRP and high risk IL-6 are also relatively strongly related (0.37) in the MacArthur study. There is a small relationship between high

<table>
<thead>
<tr>
<th>TABLE 5</th>
<th>PHI COEFFICIENTS AMONG HIGH RISK LEVELS OF MARKERS OF INFLAMMATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Ages 65+ in the NHANES 1999–2002 (N=1,884)</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>CRP</td>
<td>0.33***</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
</tr>
<tr>
<td>(b) Ages 70–79 in the MacArthur Study of Successful Aging (N=654)</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>IL-6</td>
</tr>
<tr>
<td>CRP</td>
<td>0.37***</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
</tr>
</tbody>
</table>

CRP = C-reactive protein; IL-6 = interleukin-6.

*** p < 0.0001, ** p < 0.001, *p < 0.01, +p < 0.05.
risk albumin and high risk CRP (0.11), between high risk albumin and high risk fibrinogen (.09) in NHANES, but not in MacArthur study.

The next set of markers is indicative of the functioning of the immune system.

*Cytomegalovirus* (CMV) is a herpesvirus that infects most people relatively early in life. The prevalence of CMV infection within the US population increases with age reaching 91% in people ages 80 and over [259–261]. It has been suggested that CMV is a “driving force” behind age-related changes in T cells [262–266]. The proposed CMV-driven pathway occurs through an increase in CMV-specific CD8+ T cells that, in turn, lead to a reduction in the immune system’s ability to respond to other infectious pathogens. CMV seropositivity and high antibody levels have been associated with inflammation, cardiovascular disease, stroke, endothelial dysfunction, frailty, and cognitive decline [267–271].

*Epstein-Barr virus* (EBV) is another common herpesvirus that affects most people during their life. The prevalence of EBV is as high as 95% among adults between ages 35 and 40 in the United States. EBV antibody level is used by some researchers as a marker of cell-mediated immunity [272–278]. The pattern of significantly higher EBV levels at older ages is suggestive of some loss of cellular immunity in older age [272].

*T-helper cells*, also known as CD4 or T4 cells, are white blood cells that are a major component of the immune system. CD4 count assesses the status of the immune system. A normal CD4 count in adults ranges from 500 to 1350 cells per cubic millimeter (mm³) of blood. A count of 250–350 CD4 cells/mm³ suggests some immune system damage and less than 200 CD4 cells/mm³ is often indicative of more serious immune system damage [279].

In addition to its value as an indicator of a compromised immune system, the CD4 count has been used in the measurement of age-related changes in the immune system [280–282]. The CD8 count has also been associated with age-related conditions. High circulating levels of CD8 T cells have been associated with chronic infections, including EBV and CMV [283]. CD8+ T cells respond to chronic systemic intracellular pathogens whereas CD4+ T cells respond to specific extracellular pathogens. A constant CD4:CD8 ratio indicates healthy aging, while a decline in this ratio can indicate increased immunological risk in the elderly [284].

### 4.4. MARKERS OF THE CENTRAL NERVOUS SYSTEM

Many potentially useful biomarkers are obtained via obtrusive or invasive measures and are not currently collected in large population studies. For instance, several potential markers for AD from cerebrospinal fluid (CSF) have been proposed but are not collected. Amyloid β42 is a major component
of senile plaques and is a suggested marker of neuropathological processes related to AD [88, 89]. Total \( (t) \)-tau is a major protein that comprises neurofibrillary tangles, and phosphorylated \( (p) \)-tau precedes formation of neurofibrillary tangles. High CSF levels of both t-tau and p-tau are associated with an increased risk of AD [89–91]. Additionally, \( F2 \)-isoprostanes (F2-iso) are prostaglandins that reflect lipid peroxidation. F2-iso are associated with AD, hypercholesterolemia, and atherosclerotic plaque [95–99]. Although several studies have used or are currently using these indicators as markers of AD, collection of CSF is not feasible for large population studies.

4.5. MARKERS OF ACTIVITY IN THE HYPOTHALAMIC PITUITARY AXIS

Cortisol is a steroid hormone produced by the adrenal cortex in response to internal or external stress [289]. Consistently high cortisol reactivity to repeated challenges is an atypical response that may reflect chronic physiological stress [285] and is associated with negative health outcomes in old age [286]. Cortisol and its antagonist, dehydroepiandrosterone sulfate (DHEA-S) (described below in more detail), are indicators of HPA activity. Cortisol has a strong diurnal variation, generally high early in the morning and falling during the day [287]. cortisol typically increases over the first few minutes of the day, reaching a peak 20–30 min after waking.

Cortisol levels have been shown to be greater among individuals experiencing chronic stress from work or emotional strain [288]. Health consequences of exposure to elevated cortisol include increased cardiovascular risk [100], poorer cognitive functioning [101, 286], and increased risks for fractures [103].

Cortisol level can be assessed using blood, saliva, and urine. Urine is collected over a 12- or 24-hour period in order to represent a daily level [286]. Researchers are often interested in the profile of cortisol change over the day; including the rise in cortisol levels after waking in the morning. For this reason, salivary cortisol may be measured four or five times in the same day—upon waking, shortly afterward, in the afternoon, evening, and night [287]. Normal levels of cortisol in the bloodstream range from 6 to 23 \( \mu g/dl \). Normal 24-hour urinary cortisol levels range from 10 to 100 \( \mu g \) per 24 hours [288]. In the MacArthur study, the level used to define risk for urinary cortisol was \( \geq 25.69 \mu g/g \) creatinine [160].

DHEA is a hormone produced by the adrenal gland. DHEA-S is synthesized from DHEA and converted into other hormones [290]. Assays measure DHEA-S instead of DHEA because DHEA-S is less rapidly cleared from the bloodstream and has less diurnal variation [290–293]. DHEA-S has been hypothesized to serve as a functional antagonist to HPA activity and thus is an important indicator of overall activity in the HPA [294–302].
The level of DHEA is age related. Production of DHEA stops at birth, then resumes around age 7 and peaks when people are in their mid-twenties. From the early thirties on, there is a steady decline (about 2% each year) until around age 75, when the level of DHEA in the body is about 5% of the peak level. Because DHEA-S is related to age and longevity [296–302], it has attracted attention for possible “antiaging” effects [303–305]. Normal values for serum DHEA-S vary with sex as well as age. Normal ranges are 800–5600 μg/liter for men, 350–4300 μg/liter for women; although there may be slight variation in these levels across laboratories. DHEA assays can be based on blood, saliva, or urine samples.

While there are mixed results by gender [306], the literature generally documents a link between low DHEA-S and poor health outcomes. Lower DHEA-S is related to a history of heart disease and mortality [105–108]. DHEA-S is hypothesized to be protective against heart disease because of its anticoagulating and antiproliferative properties [106, 307]. Low DHEA-S has also been related to worse physical and mental functioning [109, 110, 308]. Low DHEA-S has been included as one component of allostatic load [102, 309]. In addition, studies have found that DHEA-S is a marker for bone turnover predicting bone mineral density [310], and low levels have been linked to AD [111, 295].

Insulin-like growth factor-1 (IGF-1) is a polypeptide protein hormone that modulates cell growth and survival. Throughout the lifespan, IGF-1 impacts neuronal structure and function, mainly through its effects on growth hormone (GH) [311]. A meta-analysis indicated that high IGF-1 concentrations are associated with increased risk of prostate cancer and premenopausal breast cancer [112]. Conversely, low IGF-1 levels have been linked to increased mortality [114, 312, 313], coronary artery disease [113], and osteoarthritis [114]; however, a recent study on the nationally representative NHANES sample showed no relationship between low IGF-1 and all-cause mortality or mortality from heart disease or cancer [314].

4.6. MARKERS OF THE SYMPATHETIC NERVOUS SYSTEM

Norepinephrine is a neurotransmitter in the catecholamine family, which mediates chemical communication in the SNS. Norepinephrine is almost identical in structure to epinephrine, another catecholamine discussed below. Both of these are indicators of a stress response. With advancing age, there is decreased clearance of norepinephrine [118] and normal aging is associated with an increase in plasma norepinephrine levels [315–317]. High plasma norepinephrine levels have been associated with increased overall mortality in the elderly [29] as well as reduced survival in healthy older
persons, in patients with congestive heart failure [116], and in people with previous myocardial infarction (MI) [117]. Higher levels of urinary catecholamine excretion have also been shown to predict functional disability and mortality [104].

Norepinephrine is excreted in urine and 12-hour or 24-hour urine collections are used for daily levels because levels vary over the day. To adjust for body size, results for norepinephrine are reported as micrograms norepinephrine per gram creatinine of urine excretion [104, 120]. There are no normative values for urinary norepinephrine and epinephrine levels so adverse catecholamine levels have been classified as those in the top tertile or top quartile of norepinephrine for a sample. In the MacArthur study, the risk level cutoff was 48.00 µg/g creatinine. A blood plasma test is also available although used more rarely.

Epinephrine is another stress hormone, also known as adrenaline. Heightened secretion caused by fear or anger is part of the “fight or flight” response and is linked to increased heart rate and the hydrolysis of glycogen to glucose. Increases over time in urinary excretion of epinephrine predict subsequent cognitive decline in older men [120]. High plasma epinephrine has been associated with poor survival in patients with previous MI [121] but increased survival among healthy older persons [119]. Urinary epinephrine excretion is significantly lower among women and among subjects with a BMI >27 kg/m². Current smokers have higher levels of both urinary norepinephrine and epinephrine [104].

Measurement of epinephrine is similar to that of norepinephrine: usually in urine from 12-hour or 24-hour urine collections, adjusted for body size by reporting epinephrine per gram creatinine of urine excretion [104, 120]. Like norepinephrine, there are also no normative values for urinary epinephrine levels, and they are generally classified using quartiles or tertiles for

<table>
<thead>
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<th>Table 6</th>
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<tr>
<td><strong>Cortisol</strong></td>
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</tr>
<tr>
<td><strong>Cortisol</strong></td>
</tr>
<tr>
<td><strong>DHEA-S</strong></td>
</tr>
<tr>
<td><strong>Norepinephrine</strong></td>
</tr>
<tr>
<td><strong>Epinephrine</strong></td>
</tr>
</tbody>
</table>

DHEA-S = dehydroepiandrosterone sulfate.

***p <0.0001, **p <0.001, * p <0.01, +p <0.05.
individual samples. The MacArthur study used a cutoff of greater than 4.99 µg/g creatinine to denote high risk epinephrine. Epinephrine may also be determined from blood plasma assay although this is used more rarely than urinary assays. Like urinary levels, plasma levels of catecholamines may be influenced by a variety of postural, diurnal, and acute stress-related factors [318].

The MacArthur data have four biomarkers indicating SNS and HPA activity (Table 6). Epinephrine is related to norepinephrine with a coefficient of 0.27, and the correlations among other markers are weak or insignificant.

4.7. MARKERS OF ORGAN FUNCTION

Creatinine is a chemical waste molecule generated from muscle metabolism. It is transported through the bloodstream, filtered in the kidneys, and excreted in the urine. It provides information on kidney function. Normal levels of creatinine in the blood are <1.5 mg/dl in adult men and <1.4 mg/dl in adult women [319]. Although serum creatinine levels are a fairly good indicator of kidney function, multiple factors including age, sex, and ethnicity [320] affect its concentration so the use of a single set cutpoint may not be an appropriate way of defining adverse serum creatinine levels.

Creatinine can be measured via serum or urine. Serum creatinine exhibits significant individual differences [321]; while urinary creatinine and creatinine clearance show fewer individual differences and may provide a more reliable means of determining kidney function. Equations using serum creatinine to predict creatinine clearance include additional factors (e.g., age and body weight) in their prediction [322]. Reduced glomerular filtration rate (GFR), measured from serum creatinine, is associated with increased risk of cardiovascular disease and death [323]. Studies have shown that creatinine clearance predicts stroke and cardiovascular mortality [324].

Cystatin C is a cysteine protease inhibitor that is filtered out of the blood by the kidneys. As another marker of GFR, serum cystatin C is a measure of normal kidney function. Compared to serum creatinine levels (the primary clinical tool used for measuring renal function), cystatin C levels are independent of age, sex, and lean muscle mass. Hence, this is a promising biomarker for population studies. Additionally, multiple studies have indicated that cystatin C may be a more sensitive marker of kidney function than serum creatinine [131]. Cystatin C predicts all-cause and cardiovascular mortality [129, 325, 326], risk of cardiovascular disease [327], MI [328], stroke [328], and chronic kidney disease [329]. The correlation between high risk creatinine clearance and high risk cystatin C among people 65 years of age and over in NHANES III is 0.34 (Table 7a).
The peak flow rate provides an indicator of the functioning of the respiratory system. Peak expiratory flow (PEF) monitoring has been used as an objective measure of airflow obstruction. The normal range of PEF is 500–700 liter/min for men and 380–500 liter/min for women [330] but what is regarded as normal varies with differences in height and weight [331]. Studies have shown that PEF is related to mortality [332] and physical and cognitive functioning [333]. The correlation between peak flow and creatinine clearance in the MacArthur study is moderate (0.11, Table 7b).

An electrocardiogram (EKG or ECG) measures electrical impulses in the heart [134–136] and records as a graphic produced by an electrocardiograph. EKG results provide important diagnostic information on cardiac arrhythmias [334], MI [335], electrolyte disturbances [336], and ischemic heart disease [334]. A standard 12-lead resting EKG is often coded using Minnesota coding criteria [336]. The results are used to indicate probable and possible MI, and probable and possible left ventricular hypertrophy (LVH). A study based on national data showed that the age-adjusted prevalence rate of EKG-defined MI was 6.7% for those ages 40 and over which is somewhat higher than the prevalence of self-reported MI (5.8%). The prevalence was more than four times higher among those ages 65 and over compared to ages 40–64 [337].

4.8. MARKERS OF OXIDATIVE STRESS AND ANTIOXIDANTS

Oxidative stress and antioxidants are an example of a class of markers that seem to be theoretically important determinants of the aging process but are as yet not measured in such a way that they can be collected from large
populations. High levels of reactive oxidative species (ROS), enzymes important in cell signaling, have been shown to cause significant damage to cell structures. It has been suggested that ROS play an important role in the onset of age-associated loss in muscle mass (sarcopenia) [338], changes in the central nervous system, hearing loss [339], Parkinson’s disease [340, 341], and AD [342–344]. In contrast, intrinsic [e.g., superoxide dismutase (SOD) and glutathione peroxidase] and extrinsic antioxidants (e.g., vitamins A, B, C, and E) affect aging and disease by combating oxidative stress [345]. Studies suggest that SOD may function as a tumor suppressor [346–348] while carotenoids may have preventive effects against both cardiovascular disease and cancer [349–352].

4.9. Genetic Markers

Genetic markers are another category of markers that are only beginning to be employed in population studies. The growth in ability to use these markers not only as additional independent indicators of risk but also as modifiers of risk for people with other behavioral, biological, or genetic characteristics will broaden the whole approach to including biomarkers in the analysis of population health outcomes. Only a small number of genetic indicators have been used broadly in population studies to date, and the results for many of the indicators have not been as clear-cut as expected given the animal literature, which led to their selection as candidates for genes influencing human health and longevity [353]. While we cannot review all of these markers, we will highlight promising results; this is just a brief mention of the genetic markers that are likely to be commonly determined in population surveys within the next decade.

The most commonly examined genetic indicator, and the one with the most evidence of a link to health outcomes, is *apolipoprotein E* (APOE), which has been used in analysis of a variety of health outcomes in many populations. There are three alleles of the APOE gene: e2, e3, and e4. Studies have shown high risks for late-onset AD among those with the APOE4 gene [354–359]. The APOE4 gene is also known to be associated with cardiovascular diseases such as heart attack, stroke, and coronary artery disease [360, 361].

Polymorphisms for the gene coding for *angiotensin-converting enzyme* (ACE) have also been examined in a number of population surveys. Polymorphisms in ACE have been shown to be relatively strongly related to circulating ACE and may be involved in cardiovascular and renal diseases [362], AD [363, 364], and human longevity [365, 366]; but not all investigations of the role of ACE have produced positive results [367, 368].
The number of candidate genes identified and investigated in large population surveys is likely to increase exponentially in a short time. For instance, the HTR2A genotype has also been associated with memory change and is likely to be included along with APOE as risk factors for cognitive loss [369]. A set of inflammatory polymorphisms related to IL-6 and CRP has been related to circulating levels of these markers, and while there are conflicting results as to how these relate to long-term health outcomes, they are likely to be increasingly included along with blood levels of these markers in future analyses [370, 371].

*Mutations in mitochondrial DNA* (mtDNA) accumulate with age and are among the genetic factors that may eventually be shown to be associated with longevity [353, 372]. A study of Italian populations indicated that mtDNA inherited variability may be involved in longevity and healthy aging [373]. Another Italian study found a specific link between longevity and the C150T mutation in leukocytic mtDNA [374]. Additionally, a Japanese study found that three mtDNA mutations were more prevalent among centenarians compared to noncentenarian controls [375].

*Telomere length* is another genetic indicator that is currently under investigation as either an indicator of the risk of aging or as a biological marker of the aging process *per se*. Although findings have consistently related decreased telomere length to increased age [376], investigations of the link between telomere length and remaining longevity have not produced consistent results [377, 378].

Identifying biomarkers for cancer is a rapidly growing scientific undertaking partly being fueled by genomic developments. Markers of DNA damage and repair provide hope for identification of markers that are related to risk for a wide variety of cancers [379, 380]. Work in other areas shows promise that serum autoantibodies that indicate chronic inflammatory, pro-oxidant conditions can serve as bioindicators of the risk of cancer development [381, 382].

### 5. Biomarkers and Mortality

The link between high risk levels of each biomarker and mortality indicates the relative potential of each marker individually to explain the likelihood of dying in older populations and to provide evidence of how this association varies across markers. Logistic regressions were used to estimate these relationships in the MacArthur study and hazard models in the NHANES analysis. The two cohorts are persons over age 40 from the NHANES III sample and the cohort ages 70–79 from the MacArthur Study of Successful Aging. Deaths in the MacArthur sample occurred in the 7.5 years after
interview and up to 12 years after interview in the NHANES group. Odds ratios resulting from these regressions are shown in Table 8. The odds ratios indicate the relative likelihood associated with dying in years subsequent to the two surveys for each high risk biomarker. When the odds ratio is greater than 1, the likelihood of dying for those with the risk factor is higher than for those without the risk factor; when it is less than 1, the relative likelihood is lower for those with the risk factor.

A number of high risk levels of the biomarkers including SBP, pulse, HbA1c, fibrinogen, creatinine clearance, and peak flow are significantly related to mortality in the NHANES sample of middle-aged and older adults. The largest odds ratios are from biomarkers indicating organ functioning

### Table 8

**Link Between Presence of Risk Levels of Individual Biomarkers and Subsequent Mortality**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>MacArthur: Age 70–79</th>
<th>NHANES III: Age 40+</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>7.5 years mortality (N=657)</strong></td>
<td>NHANES: Age 40+ to 2000 (N=7,417)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>1.37</td>
<td>1.16*</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>1.40</td>
<td>1.01</td>
</tr>
<tr>
<td>Pulse rate at 60 s</td>
<td>–</td>
<td>1.26*</td>
</tr>
<tr>
<td>Total cholesterol (total cholesterol/HDL in MacArthur)</td>
<td>0.87</td>
<td>0.98</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.31</td>
<td>1.06</td>
</tr>
<tr>
<td>Glycosylated hemoglobin</td>
<td>1.34</td>
<td>1.31*</td>
</tr>
<tr>
<td>Body mass index (waist/hip ratio in MacArthur)</td>
<td>1.27</td>
<td>0.90</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>1.67*</td>
<td>1.00</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.41</td>
<td>–</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>1.28</td>
<td>1.29*</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.86</td>
<td>1.07</td>
</tr>
<tr>
<td>Cortisol</td>
<td>1.14</td>
<td>–</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>1.39</td>
<td>–</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>1.49</td>
<td>–</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>1.38*</td>
<td>–</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>2.22</td>
<td>1.31*</td>
</tr>
<tr>
<td>Peak flow</td>
<td>2.18*</td>
<td>1.40*</td>
</tr>
</tbody>
</table>

Source: MacArthur, Seeman et al., 2004 [163], calculated using logistic models. NHANES calculated from data using hazard models.

HDL = high-density lipoprotein; LDL = low-density lipoprotein; IL-6 = interleukin-6; DHEA-S = dehydroepiandrosterone sulfate.

*p < 0.01.

a Age, gender, and education controlled.
such as creatinine clearance (OR=1.31) and peak flow (OR=1.40). In the MacArthur sample, which includes only older people, the only indicators linked to mortality were high risk peak flow (OR=2.18), CRP (OR=1.67), and epinephrine (OR=1.38).

It is hard to compare the results of the two samples given that they differ in age, location, and different statistical models in the equations; however, the results suggest the potential of the importance of some biomarkers that are not currently used as clinical indicators such as epinephrine and markers of inflammation. This has also been true in an analysis of the links between multiple biomarkers and mortality in Taiwan [383]. In addition, the results suggest that the importance of individual biomarkers in predicting health outcomes may be related to age, with many biomarkers potentially more important in predicting mortality at younger ages.

6. Interrelationships Among Biomarkers and Summary Measures of Biological Risk

In earlier sections, we showed the interrelationships among variables in each category. We now indicate the interrelationships among the cardiovascular, metabolic, inflammatory, HPA activity and SNS activity, and organ failure indicators for both the NHANES and the MacArthur samples in Table 9. If dysregulation in one marker or system is associated with dysregulation in multiple systems, the matrix should indicate high correlations. However, there are only a few moderate relationships among the high risk levels of these biomarkers in both samples. In the NHANES sample, the highest relationships are between CRP and BMI and leptin indicating the interaction of metabolic processes and inflammatory processes; strong correlations between creatinine clearance and cystatin C and a number of markers indicate the link between kidney functioning and a number of other processes. In the MacArthur sample, some of the strongest relationships are also between the markers of inflammation and the metabolic indicators.

Development of summary measures that incorporate multiple biological risk factors has been pursued in order to more effectively combine the information from multiple markers and also because of the observation that “many individuals are exposed to several risk factors and small increases in multiple risk factors can lead to a substantial increase in overall risk, even if no single factor exceeds its clinically accepted threshold” [7] (p. 95). Some of these summary measures focus on only a few physiological systems and others include more systems; some measures are more closely linked to
### Table 9

**Phi Coefficients Indicating Relationships Among High Risk Levels of Biomarkers**

(a) Ages 65+ in the NHANES 1999–2002 (NHANES III for leptin, creatinine clearance, peak flow, and cystatin C)

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>HDL</th>
<th>LDL</th>
<th>TG</th>
<th>GL</th>
<th>HbA1c</th>
<th>BMI</th>
<th>LEP</th>
<th>CRP</th>
<th>FG</th>
<th>AL</th>
<th>CrCl</th>
<th>PF</th>
<th>CysC</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP</td>
<td>0.02</td>
<td>0.03</td>
<td>0.04</td>
<td>−0.03</td>
<td>−0.03</td>
<td>−0.05+</td>
<td>0.04</td>
<td>−0.02</td>
<td>0.02</td>
<td>0.01</td>
<td>−0.01</td>
<td>0.02</td>
<td>−0.04+</td>
<td>0.00</td>
</tr>
<tr>
<td>SBP</td>
<td>0.09***</td>
<td>−0.03</td>
<td>0.01</td>
<td>0.00</td>
<td>0.01</td>
<td>−0.02</td>
<td>−0.02</td>
<td>−0.02</td>
<td>0.06*</td>
<td>−0.00</td>
<td>−0.02</td>
<td>0.08***</td>
<td>0.07***</td>
<td>0.06*</td>
</tr>
<tr>
<td>PP</td>
<td>0.03</td>
<td>−0.01</td>
<td>−0.03</td>
<td>0.08+</td>
<td>0.09*</td>
<td>0.07*</td>
<td>−0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>0.01</td>
<td>0.00</td>
<td>0.02</td>
<td>0.10***</td>
<td>0.00</td>
</tr>
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<td>−0.03</td>
<td>0.01</td>
<td>0.07+</td>
<td>0.10***</td>
<td>0.05+</td>
<td>0.01</td>
<td>0.07*</td>
<td>0.09***</td>
<td>0.05+</td>
<td>0.03</td>
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<tr>
<td>HC</td>
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<td>−0.01</td>
<td>−0.04</td>
<td>−0.01</td>
<td>−0.05</td>
<td>−0.03</td>
<td>0.02</td>
<td>−0.04</td>
<td>0.03</td>
<td>0.12***</td>
<td>0.13***</td>
<td>0.20***</td>
<td>0.06+</td>
<td>0.25***</td>
</tr>
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<td>−0.05+</td>
<td>0.02</td>
<td>0.04+</td>
<td>0.01</td>
<td>0.04+</td>
<td>0.12***</td>
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<tr>
<td>HDL</td>
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<td>LDL</td>
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<tr>
<td>TG</td>
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<td>0.02</td>
<td>0.03</td>
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<tr>
<td>GL</td>
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<td>0.03</td>
<td>−0.04</td>
<td>−0.01</td>
<td>0.05</td>
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<tr>
<td>HbA1c</td>
<td>0.05+</td>
<td>0.08***</td>
<td>0.05</td>
<td>0.02</td>
<td>0.04+</td>
<td>0.10***</td>
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<tr>
<td>BMI</td>
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<td>0.04+</td>
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<tr>
<td>LEP</td>
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<td>0.11***</td>
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<tr>
<td>CRP</td>
<td>0.03</td>
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<tr>
<td>FG</td>
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<td>0.04+</td>
<td>0.12***</td>
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</tr>
<tr>
<td>AL</td>
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<td>0.07***</td>
<td>0.05*</td>
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</tbody>
</table>

(b) Ages 70–79 in the MacArthur Study of Successful Aging (N=654, N=363 for homocysteine)

<table>
<thead>
<tr>
<th></th>
<th>Tot/HDL</th>
<th>HDL</th>
<th>GHb</th>
<th>BMI</th>
<th>Wasit/Hip</th>
<th>CRP</th>
<th>IL6</th>
<th>FG</th>
<th>AL</th>
<th>COR</th>
<th>DHEAS</th>
<th>NE</th>
<th>EPI</th>
<th>CrCl</th>
<th>PF</th>
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<tr>
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<td>0.07</td>
<td>−0.00</td>
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<td>0.06</td>
<td>−0.01</td>
<td>0.09+</td>
<td>−0.02</td>
<td>−0.03</td>
<td>0.01</td>
<td>0.02</td>
<td>0.06</td>
<td>−0.03</td>
<td>−0.02</td>
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<tr>
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<td>0.03</td>
<td>0.09+</td>
<td>0.09</td>
<td>0.06</td>
<td>0.11*</td>
<td>0.04</td>
<td>0.00</td>
<td>−0.01</td>
<td>−0.09+</td>
<td>0.04</td>
<td>0.01</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>HC</td>
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<td>0.17*</td>
<td>−0.01</td>
<td>0.00</td>
<td>0.09</td>
<td>0.05</td>
<td>0.15*</td>
<td>0.02</td>
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<td>−0.14*</td>
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<td>0.04</td>
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<tr>
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<td>0.18***</td>
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<td>−0.07</td>
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<td>−0.00</td>
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<td>0.03</td>
<td>0.10*</td>
<td>−0.02</td>
<td>−0.07</td>
<td>−0.03</td>
<td>−0.07</td>
<td>−0.10+</td>
<td>−0.07</td>
<td>−0.15**</td>
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<tr>
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<td></td>
<td></td>
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<td>BMI</td>
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<td>0.09+</td>
<td>0.01</td>
<td>−0.03</td>
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<td>−0.11*</td>
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<tr>
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<td>−0.01</td>
<td>0.02</td>
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</tr>
</tbody>
</table>

(a) Ages 65+ in the NHANES 1999–2002 (NHANES III for leptin, creatinine clearance, peak flow, and cystatin C)

(b) Ages 70–79 in the MacArthur Study of Successful Aging (N=654, N=363 for homocysteine)
<table>
<thead>
<tr>
<th></th>
<th>IL-6</th>
<th>FG</th>
<th>AL</th>
<th>COR</th>
<th>DHEA-S</th>
<th>NE</th>
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<td>0.07</td>
<td>-0.03</td>
<td>0.00</td>
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<td>-0.00</td>
<td>-0.06</td>
<td>0.04</td>
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<tr>
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<td>0.09+</td>
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<tr>
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</tr>
<tr>
<td></td>
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<td>0.13**</td>
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</tr>
<tr>
<td></td>
<td>0.27***</td>
<td>0.10+</td>
<td></td>
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</tr>
</tbody>
</table>

Ns for analysis: NHANES 1999–2002 nonfasting biomarkers (N=1,884), fasting biomarkers (N=938); NHANES III nonfasting biomarkers (N=2,741), fasting biomarkers (N=1,172); NHANES III homocysteine nonfasting biomarkers (N=1,407), fasting biomarkers (N=571).

*** p < 0.0001, ** p < 0.001, * p < 0.01, + p < 0.05.

DBP=diastolic blood pressure; SBP=systolic blood pressure; PP=pulse pressure; PR=resting pulse rate; HC=homocysteine; TC=total cholesterol; Tot/HDL=total cholesterol/HDL; HDL=high density lipoprotein; LDL=low-density lipoprotein; TG=Triglycerides; GL=blood glucose; HbA1c=glycosylated hemoglobin; BMI=body mass index; LEP=leptin; waist/hip=waist-to-hip ratio; CRP=C-reactive protein; IL-6=interleukin-6; FG=fibrinogen; AL=albumin; COR=cortisol; DHEA-S=dehydroepiandrosterone sulfate; NE=norepinephrine; EPI=epinephrine; CrCl=creatinine clearance; PF=peak flow; CysC=cystatin C.

*a Fasting biomarkers: LDL, triglycerides, blood glucose, leptin.

*b Correlations based on biomarkers from NHANES III.
specific health outcomes like cardiovascular disease while others propose to explain a variety of health outcomes.

The Framingham risk score is a widely used index of risk for CHD [33, 384–389]. The Framingham score assigns points to different major cardiovascular risk factors including blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, and fasting blood glucose. It also includes risk related to age, gender, and smoking. For those without cardiovascular disease, the probability of CHD onset within a certain period of time is estimated. The Framingham risk score is widely used in clinical settings based on its proven ability to predict cardiovascular disease and CHD especially for women [390]. The Framingham risk score has been also shown to predict absolute risk accurately for populations other than those in North America [391–394] although some recent studies have questioned its validity in other settings [390].

Metabolic syndrome is a group of major risk factors that characterize an insulin resistance syndrome or Syndrome X [33] (p. 2488), which has been related to increased risk for cardiovascular disease and mortality [395–397]. The metabolic syndrome score is a count (0–5) of the number of the following abnormalities: hypertension, glucose dysregulation, hypertriglyceridemia, low HDL, and central obesity—based on clinical cut points [33, 398, 399]. A person with three or more of these five abnormalities is considered to have metabolic syndrome.

Allostatic load is a summary measure that is based on theories about aging and the cumulative physiological responses to stressors [400]. This summary measure involves multiple systems that are part of the body’s stress response but that may become dysregulated with chronic physical or mental stress and old age. Initially, allostatic load was measured in the MacArthur study based on 10 biological markers that represent physiological activity across the cardiovascular system, the metabolic system, the HPA and the SNS including SBP, DBP, WHR, ratio of total/HDL cholesterol, HDL cholesterol, HbA1c, cortisol, norepinephrine, epinephrine, and DHEA-S [102]. Allostatic load was measured as the number of markers out of 10 for which the subject scored in the upper 25% of the distribution. This measure has been shown to predict mortality and decline in physical and cognitive functioning [102, 309].

Subsequent analyses have included additional markers that represent renal functioning, lung capacity, inflammation and coagulation, and addition of these markers increased the explanatory power of the measure [160]. These analyses have also shown that allostatic load is a better predictor of health outcomes in the older MacArthur sample than the set of individual markers or the indices of the cardiovascular and metabolic markers [309].

Seeman and colleagues have explored several alternative approaches to measurement including allowing differential contribution of individual
indicators through their entire range of values and different weights for different outcomes [160, 401, 402]. While these refined approaches indicate that differential weighting of the individual components of biological risk by the outcome of interest might be optimal, the original count index and the more complex approach do not differ significantly in their predictive ability [7].

7. Surveys with Biomarkers

Biomarkers are available in many large samples representative of national populations and communities, which allow examination of the diversity of biomarkers within the population and the large numbers needed for examination of longitudinal change. They are designed to examine the relationship of not only the risk associated with biological factors but also social and economic factors and the interaction among these risks. They do not expect to provide evidence of new biological relationships or risks for health outcomes but they could be used to identify important interactions between biological factors and health outcomes. These surveys generally include measurement of risk factors and physiological states known to be related to highly prevalent major health outcomes. We describe a selection of these studies below. In each case, we give some idea of the biomarkers available but in many cases we are not exhaustive in our listing. Also, because many of the studies have stored samples, biomarkers are added regularly from new assays.

Our analyses have used biomarker information from the NHANES, which include interviews, clinical exams, and extensive laboratory analysis which results in the most extensive set of biomarkers for a large population. These studies are undertaken by the National Center for Health Statistics, and exams and biological specimens are collected by medical staff working in mobile exam units in trucks that move across the country. NHANES, with the exception of the first study, is cross-sectional except for passive follow-up of administrative death records and Medicare records. The available biomarkers are too extensive to be mentioned individually but in addition to those mentioned above other indicators include hematology antibody tests, hormones, toxicology, and assessments of anemia and sexually transmitted diseases (STDs). Exams include vision, audiometry, periodontal assessments, cardiovascular fitness, physical functioning, balance, cognition, and reaction.

The MacArthur Study of Successful Aging was the first large-scale study to collect information on a significant number of biomarkers in the home rather than in a medical setting [403]. This survey was of people aged 70–79 in three
communities and biomarkers were collected at multiple time points. A phlebotomist collected blood samples and interviewers collected overnight urine collections. Many of the measures available from this study have been indicated above. Some were from assays done at the time of collection and others from stored samples (e.g., antioxidants, homocysteine, folic acid, CRP, fibrinogen, IL-6, and extraction of DNA). There are additional performance tests for balance, walking ability, strength, and cognitive functioning.

The number of large population and community studies including the collection of biomarker data has multiplied in recent years partly in response to the technological changes that have allowed interviewers or respondents rather than medical professionals to collect samples. These developments include the use of dried blood spots [404], and buccal swabs and salivary assays for DNA. The Health and Retirement Survey is a nationally representative longitudinal study of the US population over age 50. It has been ongoing since 1992 and added the collection of biomarkers and performance measures in 2006 [405]. This study collects blood samples using the dried blood spot, which have been assayed for HbA1c, total cholesterol, HDL cholesterol, and CRP. DNA has been extracted from saliva. Participants also completed several performance tests for strength, balance, and lung function.

National samples from other countries have also introduced these approaches to collecting information on biomarkers. The Taiwan Biomarker Project has collected a set of biomarkers [406], as has the English Longitudinal Study of Aging [407]. The Mexican Family Life Survey and the Indonesian Family Life Survey are both collecting blood using dried blood spots [408]. Additionally, the Mexican Family Life Survey is collecting information on anemia at the time of the survey using a hemocue meter.

### 8. Future of Biomarkers in Studying Aging Populations

The increase in the number of population and community studies including the collection of biomarker data has resulted from theoretical imperatives, scientific advances, and improvements in collection opportunities. The theoretical demands require a fuller explanation of how the aging process proceeds. The scientific advances have dramatically increased our knowledge of the multiple biological pathways affecting the aging process. The collection opportunities have increased with the development of less invasive measurement offered by salivary and dried blood spot assays. The future is likely to see further expansion of biomarker collection using saliva not only for DNA but also for RNA and certainly an increase in markers based on scanning. Many samples for well-characterized populations are available now for further genetic analysis; development of inexpensive genotyping techniques.
will result in an expansion of genetic biomarkers. The developments in metabonomics, analysis of metabolic profiles, and proteomics will lead to the inclusion of many new classes of biomarkers.

Multiple biomarker measurements that are more indicative of the physiological response to challenge are likely to be included in population surveys in the future. Monitoring through telephone or small electronic device (e.g., paging devices, palmtop computers, and programmable wristwatches) will be increasingly used to collect and stimulate responses.

Finally, further methodological developments will be required to analytically integrate the increasingly complex indicators that will be collected. The number of biomarker indicators and the interrelationships among them demand new analytic approaches.

**Acknowledgement**

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**REFERENCES**


BIOMARKERS RELATED TO AGING


