Do Changes in Circulating Biomarkers Track With Each Other and With Functional Changes in Older Adults?

Jason L. Sanders,1,2 Victoria Ding,3 Alice M. Arnold,3 Robert C. Kaplan,4 Anne R. Cappola,5 Jorge R. Kizer,6 Robert M. Boudreau,2 Mary Cushman,7 and Anne B. Newman2

1Medical Scientist Training Program, School of Medicine and 2Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pennsylvania. 3Department of Biostatistics, University of Washington, Seattle. 4Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Yeshiva University, New York. 5Department of Medicine, School of Medicine, University of Pennsylvania, Philadelphia. 6Department of Medicine and Pathology, University of Vermont College of Medicine, Burlington.

Address correspondence to Jason Sanders, PhD, Bellefield Professional Building 4th Floor, 130 North Bellefield Avenue, Pittsburgh, PA 15213. Email: jls196@pitt.edu

Background. It is unclear if changes in proposed circulating biomarkers of aging are strongly correlated to each other or functional change. We tested if biomarker changes track with each other and with functional measures over 9 years in older adults.

Methods. Dehydroepiandrosterone sulfate (DHEAS), adiponectin, insulin-like growth factor 1 (IGF-1), IGF binding proteins 1 (IGFBP-1) and 3 (IGFBP-3), interleukin-6 (IL-6), cholesterol, and function (gait speed, grip strength, Modified Mini Mental Status Exam [3MSE] and Digit Symbol Substitution Test [DSST] scores) were measured in 1996–1997 and 2005–2006 in the Cardiovascular Health Study All Stars study (N = 901; mean [standard deviation, SD] age 85.3 [3.6] years in 2005–2006). Adjusted Pearson correlations illustrated if biomarkers tracked together. Multivariable linear regression demonstrated if biomarker changes tracked with functional changes.

Results. Correlations among biomarker changes were mostly <0.2. In models with each biomarker entered separately, a 1-SD increase biomarker change was associated with change in function as follows: grip strength (DHEAS β = 0.61 kg, p = .001; IL-6 β = −0.46 kg, p = .012; cholesterol men β = 0.79 kg, p = .016); gait speed (DHEAS β = 0.02 meters per second, p = .039; IL-6 β = −0.018 meters per second, p = .049); and DSST score (DHEAS women β = 1.46, p = .004; IL-6 β = −0.83, p = .027). When biomarkers were entered in the same model, significant associations remaining were as follows: grip strength (DHEAS β = 0.54 kg, p = .005; IL-6 β = −0.43 kg, p = .022); 3MSE score (IGF-1 β = 0.96, p = .04; IGFBP-3 β = −1.07, p = .024); and DSST score (DHEAS women β = 1.27, p = .012; IL-6 β = −0.80, p = .04).

Conclusion. Changes in biomarkers were poorly correlated, supporting a model of stochastic, independent change across systems. DHEAS and IL-6 tracked most closely with function, illustrating that changes in inflammation and sex steroids may play dominant roles in changes of these functional outcomes.

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Thus, models based on cross-sectional designs do not accurately reflect biology.

Several questions cannot be answered using cross-sectional designs or modeling biomarkers separately. First, what is the magnitude of the correlation between biomarker changes? Determining the strength of these correlations could indicate if physiologic processes change in a coordinated or independent manner over time, shedding light on the stochastic nature of aging. Second, changes in which physiologic domains have the strongest independent associations with changes in specific functions (eg, physical vs cognitive) or functional tests (eg, walking speed vs grip strength; Digit Symbol Substitution Test [DSST] score or Modified Mini Mental Status Exam [3MSE] score)? Defining these associations may indicate which molecular or physiologic domains are most intimately linked with changes in higher order function. By extension, knowing the independent strength of these associations will help researchers target molecular pathways, which may be most beneficial for improving aging.

In this article, we use data from the Cardiovascular Health Study (CHS) All Stars study on changes in putative biomarkers of aging and physical and cognitive function to answer these questions. For biomarkers, we select dehydroepiandrosterone sulfate (DHEAS), adiponectin, interleukin-6 (IL-6), insulin-like growth factor 1 (IGF-1), and IGF binding proteins 1 (IGFBP-1) and 3 (IGFBP-3). Furthermore, we include cholesterol as a molecule often measured in clinical exams. Although hundreds of putative biomarkers of aging have been proposed, these molecules were chosen for several reasons: (a) in humans, they have been observed to change markedly with age and have been associated with disability and mortality (though some data are equivocal); and (b) they are each a member of a physiologic domain, which has been observed to change with age—reproductive endocrine capacity, body composition, immune function and inflammation, energy metabolism, and nutrition—which allows us to establish the correlation between changing systems and how multiple distinct systems may be associated with changing function.

**Methods**

**Study Population**

This is a study of 901 older adults with duplicate measures of physical and cognitive function and biomarkers all measured simultaneously and on average 9 years apart. The sample is derived from the CHS All Stars study, which included the 1,677 participants from the CHS who were alive in 2005–2006 and eligible for enrollment in a study of physical and cognitive aging into very old age. By the time of the CHS All Stars recruitment in 2005–2006, a large proportion (3,272; 58.9%) of the original 5,553 CHS participants had died, 339 (6.1%) were alive but did not give consent for the CHS All Stars study examination, and 265 (4.8%) participated in the CHS telephone follow up but not the new functional assessment. Thus, CHS All Stars includes the majority of eligible CHS participants alive at the time of All Stars recruitment. CHS All Stars examinations were conducted similarly to the original CHS examinations. Examinations were conducted at home only (429; 25.6%), in the clinic only (648; 38.6%), at home and clinic (66; 3.9%), or over the telephone (534; 31.8% including 144 telephone proxy interviews) (2). CHS itself was a four center, longitudinal, observational, community-based study of the onset, progression, and course of cardiovascular disease (CVD) in 5,888 older men and women (3,4). The CHS cohort was ≥65 years old at enrollment in 1989–1990 and was supplemented with added minority participant recruitment in 1992–1993. Participants and eligible household members were identified from a random sample of Medicare enrollees at each field center. To be eligible, participants were ≥65 years old, did not have cancer under active treatment, could not be wheelchair- or bed-bound in the home, and did not plan to move out of the area within 3 years. All procedures relating to CHS and CHS All Stars were approved by all involved institutional review boards. At recruitment, participants or their proxies gave informed consent for use of data gathered previously or future measurement of biomarkers from stored samples.

**Biomarker Measurement**

Fasting blood samples were collected and stored in 1996–1997 and 2005–2006. Samples were analyzed as pairs in 2007. Plasma DHEAS was measured with a competitive immunoassay kit (Alpco Diagnostics, Windham, NH) with inter-assay coefficient of variation of 3.8%–7.2%. Adiponectin was measured with an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN); intra- and inter-assay coefficient of variations were 2.5%–4.7% and 5.8%–6.9%, respectively. IL-6 was measured by ultrasensitive enzyme-linked immunosorbent assay (Quantikine HS Human IL-6 Immunoassay; R&D Systems); intra- and inter-assay coefficient of variations were 2.9%–8.7% and 7.3%–9.0%, respectively. IGF-1, IGFBP-1, and IGFBP-3 were measured after an extraction step using an enzyme-linked immunosorbent assay (Diagnóstics Systems Laboratory, Webster, TX) (5). Assay coefficient of variation was 4%–6% for IGF-1, 3%–14% for IGFBP-1, and 3%–5% for IGFBP-3.

**Function Measurement**

Physical function was measured using gait speed (meters per second) and average grip strength in the dominant hand (kilograms) as previously described (3). Cognitive function was evaluated with the 3MSE (6) and DSST (7). The 3MSE is an expanded 100-point version of the original Folstein Mini Mental State Examination. The DSST measures psychomotor speed and working memory (8).
Covariate Measurement

Covariates included age, sex, race, marital status, smoking, change in weight during the time period, and baseline number of chronic conditions. Disease present in 1996–1997 was tabulated for each participant. Hypertension was defined as definite if systolic blood pressure was ≥140 mmHg or diastolic blood pressure was ≥90 mmHg or if hypertension was reported as present with concomitant use of antihypertensive medication. CVD was present if either cerebrovascular disease (stroke or transient ischemic attack) or coronary heart disease (myocardial infarction, angina, history of angioplasty, or bypass surgery) was present. CVD outcomes were adjudicated by an expert panel using medical records, and medication information and prevalent CVD status were updated at each examination (9). Diabetes mellitus was classified using the American Diabetes Association criteria as not present, impaired fasting glucose, or present (10). Chronic pulmonary disease (asthma, bronchitis, or emphysema), arthritis, cancer, and kidney disease were assessed by self-report of physician diagnosis. Depression was defined as a score >10 on a modified 10-item Center for Epidemiologic Studies Short Depression Scale test (11,12).

Statistical Analysis

Prior to calculating changes in biomarker levels from 1996–1997 to 2005–2006, values exceeding 6.5 standard deviations (SD) from their respective sample means were Winsorized to the next highest number, that is, made equivalent to the nearest number, to minimize the leverage from extreme outliers without eliminating data. Three such changes were made among DHEAS and IGFBP-1 measurements taken in 2005–2006.

Pearson correlation coefficients were calculated between changes in each biomarker. Crude correlations and correlations adjusted for change in age and body mass index were determined. Multivariable linear regression was used to quantify the association of a 1-SD higher change value in biomarker level (predictor) with change in function (outcome) during the 9-year follow-up period. Covariate values were determined from the 1996–1997 visit in order to capture risk factors prior to the observed change in biomarkers and function. Modeling began by entering each biomarker change separately as a predictor of a given functional outcome. Next, adjustments were made for age in years, sex, black race (Y or N), current smoking status (Y or N), current marital status (Y or N), number of chronic conditions, change in weight during follow up in pounds, mean of 1996–1997 and 2005–2006 biomarker level in SD units, and interaction of biomarker change with sex, if the interaction term was significant in the model. Linear associations were calculated at the mean value of biomarker change. Next, all multiple biomarker changes were entered in the same model predicting functional change to allow the biomarkers to compete. Adjustment for covariates was conducted similarly to the individual biomarker models. To determine statistical significance, we used a significance level of 0.05. The R computer package was used for analysis.

RESULTS

In 1996–1997, the analytic sample was 64.9% women and 15.0% African American, and the mean (SD) age was 76.4 (3.6) years (Supplementary Table 1). Men and women had similar smoking prevalence (~6%), and men were nearly twice as likely to be currently married (83.6% vs 47.8%). In 2005–2006, the mean (SD) age was 85.3 (3.6) years. As expected, unadjusted for height or body mass index, men had faster baseline walking speed and higher grip strength, and higher levels of DHEAS.

Correlation Between Changes in Biomarkers

Levels of biomarkers and function and changes in biomarkers and function were similar to those reported from previous individual analyses from CHS (5,13–15). The largest proportional changes in biomarkers were seen for IGFBP-1, IL-6, and DHEAS, followed by adiponectin (Supplementary Table 2). On average, IL-6, adiponectin, and IGFBP-1 increased while other biomarkers decreased, though there was a wide range of change for each biomarker. Correlations among biomarker changes were relatively weak (Pearson r < 0.2). Exceptions included the following: IGF-1 and IGFBP-1 (r = −0.30, p < .001); IGF-1 and IGFBP-3 (r = 0.64, p < .001); and cholesterol and IGFBP-3 (r = 0.30, p < .001). Adjustment for change in age and body mass index throughout the time period attenuated correlations negligibly: IGF-1 and IGFBP-1 (r = −0.29, p < .001); IGF-1 and IGFBP-3 (r = 0.64, p < .001); and cholesterol and IGFBP-3 (r = 0.29, p < .001). Other notable correlations were between IL-6 and IGFBP-1 (r = 0.18, p < .001) and IL-6 and cholesterol (r = −0.15, p < .001).

Association of Biomarker Change With Functional Change: Biomarkers in Separate Models

Initial models were built including only one biomarker while adjusting for covariates. In this manner, we investigated if a change in one biomarker was associated with concurrent change in function independent of covariates but without accounting for changes in other biomarkers. This tests the possible contribution of changes in a single physiologic axis (putatively measured by changes in one biomarker) to changes in function without regard for changes in other physiologic axes. A 1-SD larger decrease in DHEAS was associated with a 0.607 kg greater decrease in grip strength, a 0.02 meters per second greater decrease in gait speed, and a 1.46 point greater decrease in DSST score (Tables 1 and 2). Changes in adiponectin, IGF-1, IGFBP-1,
or IGFBP-3 were not associated with functional changes. Greater increases in IL-6 were consistently associated with worsening decline in function. Each SD higher increase in IL-6 was associated with concurrent larger declines in grip strength (β = −0.463 kg), gait speed (β = −0.018 meters per second), and DSST score (β = −0.83). A decrease in cholesterol was only associated with decreased grip strength in men (β = −0.785 kg).

**Association of Biomarker Change With Functional Change: Biomarkers in Same Model**

Next, all biomarkers were included in the same model predicting functional change. A 1-SD larger decrease in DHEAS was associated with a 0.542 kg larger decrease in grip strength in men and women (Table 3) and a 1.27 point larger decrease in DSST score in women (Table 4). A larger increase in adiponectin was not significantly associated with changes in functional measures. Declining IGF-1 was associated with declining 3MSE score (β = −0.96). Changes in IL-6 were associated with decreased function. Each SD higher increase in IL-6 was associated with concurrent larger declines in grip strength (β = −0.428 kg) and DSST score (β = −0.80). A larger decrease in cholesterol was not associated with changing function.

**Discussion**

In this population of very old individuals, adiponectin, IL-6, and IGFBP-1 increased while DHEAS, IGF-1, IGFBP-3, and cholesterol decreased over 9 years. Concurrently, grip strength, gait speed, and DSST score decreased approximately 21% while 3MSE score declined 4%. Changes in biomarkers were weakly correlated to each other with the notable exception of changes in IGF-1 and IGFBP-3 tracking together relatively strongly. Declining DHEAS and increasing IL-6 were most consistently and strongly associated with declining function. Grip strength was more often associated with changes in biomarkers than gait speed, and DSST score was more often associated with changes in biomarkers than 3MSE score.

We were interested in determining the magnitude of the correlation between biomarker changes to test if biomarkers (and by inference the physiologic domains they may reflect)
change independently with age. These results illustrate that in older individuals these biomarkers changed independently from each other with age, with the exception of IGF-1 and IGFBP-3. There was also a wide range of change. Conceptually, disconjugate changes across systems suggest the possibility that within an individual aging is stochastic or compartmentalized rather than co-ordinated across tissues or mechanistic domains, though these data are too sparse to draw firm conclusions. Considering application of these findings to future studies, these data imply that it is necessary to measure many biomarkers in different domains to capture aging physiology accurately rather than focusing on the derangement in one or a few biomarkers. A method for quantifying the burden of dysfunction or degree of homeostatic dysregulation is constructing summary scores that represent the number of biomarkers at the extreme or with extreme changes (16). It is important to note that these conclusions are influenced by several sources of variability including the specific biomarkers selected to measure each physiologic domain, the domains themselves, the time scale between measurements, if biomarkers change in sequence rather than in parallel (ie, there is a lag between changes in one biomarker and another), when the biomarkers are measured during the life span, and measurement precision.

Previous observations support that these molecules change with age, but it is unclear how the trajectory of aging may be influenced by changes in these molecules (the reciprocal). We observed that changes in DHEAS and IL-6 were most strongly associated with functional decline, implying that, for the functional tests examined, age-associated changes in sex steroid hormones and inflammation may be most important to target for maintaining function. DHEAS has been proposed as an aging biomarker because it peaks in the third decade of life and declines steadily until after age 80 years. Over 90% of estrogens in postmenopausal women and 30% of androgens in men are derived from DHEAS (17), and the age-associated decline in DHEAS partly explains the age-associated decline in estrogen and testosterone sulfate; IGF-1 = insulin-like growth factor 1; IGFBP-1 and 3 = IGF binding proteins 1 and 3; IL-6 = interleukin-6; SD = standard deviation.
central nervous system, maintains sexual function, and alters body composition and bone integrity (21). It has been inconsistently associated with mortality and other aging-related outcomes such as CVD, higher body mass index, and worse physical and cognitive function (13,19,20,22–26). Despite these findings, the vast majority of experimental data on DHEA supplementation reveals that it has little to no benefit for reversing age-associated changes, and there is not enough evidence to recommend DHEA as a supplement to ameliorate aging (27,28).

IL-6 is a cytokine produced by immune cells, vascular endothelium, adipose tissue, and muscle that has both pro-inflammatory and anti-inflammatory properties. High levels of IL-6 have been associated with disability, age-related disease (CVD, diabetes, and cancer), loss of muscle mass (sarcopenia), frailty, and death (29,30). These associations are independent of age, sex, race, country of origin, socioeconomic status, and many other social, behavioral, and health variables. Although data are observational, the large number and consistency of studies suggest a mechanistic relationship. It is important to note that lower physical activity (31,32) and obesity, particularly visceral adiposity, are associated with higher IL-6 (33–36). Together with our data, this points toward a cycle of higher inflammation and decreased function feeding on each other. It lends more evidence for the role of physical activity in promoting healthy aging, potentially by decreasing inflammatory burden.

Why were changes in these biomarkers more strongly associated with changes in gait speed and the DSST than changes in grip strength and the 3MSE? Grip strength is a simpler phenotype than gait speed—while both require muscular and peripheral and central nervous function, gait speed also demands greater input from the vestibular system, vision, cardiorespiratory system, and central nervous system. Consequently, if a biomarker was associated particularly with the skeletal muscle component of physical function, then it might be easier to detect an association with grip strength—the signal would be drowned out using gait speed because of overlapping inputs from other systems. Similarly, the DSST is a more specific test of psychomotor processing speed (albeit with some contribution from muscle function for carrying out the test), while the 3MSE tests global executive function. It is also notable that the 3MSE has marked ceiling effect such that most individuals do not qualify as being demented. Thus, as a test with a wider range that is less skewed, the DSST might be statistically easier to detect associations with, which could mostly explain why it was more frequently associated with biomarkers changes in this analysis. Future studies employing functional measures to study biomarkers should be careful to note that nonsignificant results may be due to heterogeneity of the functional outcome obscuring an association rather than the biomarker lacking importance to aging biology.

The main strengths of this analysis include studying multiple biomarkers singly and simultaneously in change models, relating these biomarkers to clinically relevant outcomes, and adjusting for potential confounders, including baseline biomarker level. The main limitation is that this study used a retrospective survival cohort—we only measured changes in biomarkers from 1996–1997 in individuals who survived to the CHS All Stars examination in 2005–2006. Because some previous analyses have found positive associations between these biomarkers and mortality, it is possible that survival bias affects our estimates. Survival bias most likely affected estimates toward the null due to elimination of the frailest individuals with more extreme changes who would not have survived to the CHS All Stars examination. Because we measured changes in biomarkers and function over the same time period, our data do not illustrate causal relationships. We also used a subset of the CHS All Stars study with data available on all biomarkers and functional outcomes. Although this may have introduced selection bias, the population subset was relatively similar to the overall CHS All Stars population. These associations should be verified in younger, more heterogeneous populations. It is possible that stronger results would be detected in a cohort that experiences greater change in biomarkers and function—perhaps a cohort that includes a more general population or one that is followed for a long period. It is also possible that measuring biomarkers several times to calculate variability, not just twice to calculate change, models aging biology more accurately, and can result in stronger associations. For example, it was shown that DHEAS variability in older women is much more strongly associated with mortality than simple change in DHEAS or baseline DHEAS level (37). Finally, it is also possible that...
significant associations could be partially accounted for by latent variables, though additional analyses not shown here indicate that this is likely not the case.

In conclusion, we demonstrate that over 9 years in older men and women, these putative biomarkers of aging changed independently from each other, supporting the stochastic nature of aging. Changes in DHEAS and IL-6 appear to track most closely with changes in grip strength, gait speed, 3MSE score, and DSST score, suggesting that age-associated changes in the domains of sex steroid hormones and inflammation may be most targetable to preserve function. Future studies with longer follow up, a prospective design, and measurement of biomarkers at multiple time points will allow researchers to study rates of change in biomarkers (37). This trajectory analysis could help identify risk factors for large changes in biomarkers and high variability (eg, interaction with disease burden), as well as whether large changes or variability is a stronger predictor of incident events than cross-sectional biomarker level.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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Conflict of Interest

All authors have no potential conflicts of interest.

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