

Original Article

A Novel Analytic Technique to Measure Associations Between Circulating Biomarkers and Physical Performance Across the Adult Life Span

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Abstract

Understanding associations between circulating biomarkers and physical performance across the adult life span could aid in better describing mechanistic pathways leading to disability. We hypothesized that high concentrations of circulating biomarkers would be associated with lower functioning across study populations representing the adult life span. The data were from four intervention and two observational studies with ages ranging 22–89 years. Biomarkers assayed included inflammatory, coagulation, and endothelial function markers. Physical performance was measured either by VO_{2peak} (studies of young and middle-aged adults) or usual gait speed (studies of older adults). Partialled (by age, body mass index, race, and sex) and weighted common correlations were calculated between biomarkers and physical performance. Homogeneity of the associations was also assessed. Interleukin-6 (weighted $r = -.22$), tumor necrosis factor receptor 2 (weighted $r = -.19$), D-dimer (weighted $r = -.16$), tumor necrosis factor receptor 1 (weighted $r = -.15$), granulocyte colony-stimulating factor (weighted $r = -.14$), and tumor necrosis factor alpha (weighted $r = -.10$) were all significantly inversely correlated with physical performance ($p < .05$). All significant correlations were homogeneous across studies. In summary, we observed consistent inverse associations between six circulating biomarkers and objective measures of physical performance. These results suggest that these serum biomarkers may be broadly applicable for detection, trajectory, and treatment monitoring of physical function across the life span or possibly for midlife predictors of functionally deleterious conditions.

Key Words: Physical performance—Circulating biomarkers—Aging life span

Aging is associated with dysregulation across multiple systems that may lead to reduced physical capacities and disability (1,2). The dysregulation that becomes clinically evident later in life is often the result of decades of gradual, and often unnoticed, declines in systems. Circulating biomarkers of inflammation, coagulation, and endothelial function can provide important insights related to the functioning of their representative systems (3–5). Similarly, measures of physical performance can provide insight into physical capacity as it relates to functional

decline (6,7). Understanding associations between circulating biomarkers and physical performance could aid in better describing mechanistic pathways leading to disability. For example, contrasting theories that remain unresolved to date are whether aging-related degradations occur within compartments in a stochastic nature or in a more synchronized or “cross-talk” manner across physiologic systems (8,9). Measurement of multiple markers that represent signaling in inflammation, coagulation, and endothelial function can inform these theories and aid in

the development of therapies or interventions by targeting the biologic pathways of decline (9). Further, it is important to study these associations across the adult life span to better understand the aging process; however, few studies do so because of disparate physical performance measures which lead to analytic challenges (10).

Meta-analysis as a statistical technique, independent of a systematic review that is typically performed prior to applying the statistical methodology, can be used to address the challenges that are posed when combining studies that use differing measures. This statistical method allows rich extant data sources to be combined and appropriately weighted based on sample size and associated variance in measures (11). The purpose of this report is to present new data, using meta-analytic methodology, which describe common associations between physical performance and multiple circulating biomarkers in six study cohorts including young, middle-aged, and older adults. We hypothesized that high concentrations of biomarkers of inflammation, coagulation, and endothelial dysfunction would be associated with lower functioning across different study populations representing ages across the life span.

Methods

Data Source

The data used were from six studies conducted at Duke and the Durham VA Medical Centers and coordinated by the Duke Claude Pepper Older Americans Independence Center. The participants' ages ranged across 7 decades of life (from 22 to 89 years). Of the studies, four were interventional: (i) CALERIE (Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy, conducted 2002–2004) studied of the effects of 6 months of caloric restriction or caloric restriction plus exercise in overweight, nonobese men and women on body composition and related outcomes (12); (ii) STRRIDE (Studies Targeting Risk Reduction Interventions through Defined Exercise, conducted 1998–2003) examined the effects of different amounts and intensities of aerobic exercise training on insulin sensitivity in middle-aged moderately overweight men and women (13); (iii) LIFE (Veteran's LIFE study, conducted 2004–2007) that compared the impact of a home-based physical activity counseling program versus usual primary care on physical activity and physical function in older veterans (14); and (iv) EF (Enhanced Fitness, conducted 2008–2011) that compared the impact of a home-based physical activity counseling program versus usual primary care

on selected glycemic indicators in older veterans with impaired fasting glucose (15). There were two cohort studies: (v) POP (Prediction of Osteoarthritis Progression, conducted 2003–2008) was a longitudinal cohort study to evaluate predictors of knee osteoarthritis progression in men and women (16) and (vi) CARRIAGE (CARolinas Region Interaction of Aging, Genes and Environment, conducted 2002, 2004, and 2006) study of a large extended family of primarily African and Native American ethnicity evaluated for intermediate traits of cardiovascular disease and arthritis (17). Study sizes and demographics are summarized in Table 1.

Circulating Biomarkers

Blood was collected by venipuncture, separated to yield plasma and serum, and immediately stored at -80°C . With the exception of LIFE, all samples were drawn on fasting participants. Seventeen circulating biomarkers of inflammation, coagulation, and endothelial function were assayed. These biomarkers broadly represent physiologic systems that are known to dysregulate with aging and were chosen a priori for analysis by each of the six study's research teams. Ten markers were measured as a group using a Luminex multiplex bead panel (Invitrogen, Carlsbad, CA): GCSF (granulocyte colony-stimulating factor), IL-1RA (interleukin-1 receptor antagonist), IL-2, IL-8, MCP-1 (monocyte chemoattractant protein 1), RANTES (regulated upon activation, normal T-cell expressed, and secreted), TNF α (tumor necrosis factor alpha), TNFR1 (TNF receptor 1), TNFR2, and VEGF (vascular endothelial growth factor). Five additional markers were measured by immunoassay. These included: IL-6, which was measured by the MSD Ultrasensitive Assay (Meso Scale Discovery, Gaithersburg, MD) (18); MMP-3 (matrix metalloproteinase-3; BioSource, Camarillo, CA); TRAIL (TNF-related apoptosis-inducing ligand; BioSource); leptin (Millipore, Billerica, MA); and VCAM (soluble vascular cell adhesion molecule; R&D Systems, Minneapolis, MN). The organophosphatase-specific activity of paraoxonase was assayed using a kit from Invitrogen (Eugene, OR). All of the preceding analytes were measured in serum according to the manufacturers' specifications. The coagulation marker, D-dimer, a fibrinolytic component, was measured in plasma by immunoassay, per the manufacturer's protocol (American Diagnostica, Stamford, CT). All samples were analyzed in duplicate and analyses were repeated for out of range high values and for any duplicates with coefficients of variation above 10%. In total, 17 circulating biomarkers were analyzed for this study, although not all 6 studies included all 17 biomarkers.

Table 1. Subject Demographic and Physical Characteristics for Individual Studies and Entire Group

	CALERIE	STRRIDE	CARRIAGE	POP	LIFE	Enhanced Fitness	Entire Group
N	41	68	30	137	74	253	603
% women	56.5	48.0	71.4	73.2	0	0	25
% minority	39.1	20.6	100	13.0	22.1	28.3	28
Age (years), mean (SD)	38.5 (6.4)	51.2 (7.7)	71.6 (5.5)	65.9 (11.6)	79.2 (4.8)	67.2 (6.3)	64.7 (12.8)
BMI (kg/m ²), mean (SD)	27.7 (1.7)	30.5 (2.9)	32.4 (6.9)	31.3 (6.9)	28.4 (4.1)	31.3 (3.7)	30.6 (4.8)
Usual gait speed, m/s, mean (SD)	—	—	0.85 (0.22)	0.99 (0.25)	1.10 (0.32)	1.27 (0.23)	1.13 (0.28)
VO _{2peak} , mL/kg/min, mean (SD)	30.6 (5.7)	28.3 (6.0)	—	—	—	—	29.1(6.0)

Note: BMI = body mass index; CALERIE = Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy; CARRIAGE = CARolinas Region Interaction of Aging, Genes and Environment; POP = Prediction of Osteoarthritis Progression; STRRIDE = Studies Targeting Risk Reduction Interventions through Defined Exercise.

Physical Performance Measures

The studies used one of two measures of physical performance— VO_2 peak or usual gait speed. We believe it is appropriate to combine these two measures of physical performance based on the previously reported association between the two measures. Malatesta and colleagues reported a strong correlation ($r = .70$) between VO_2 peak and usual gait speed in healthy older adults (19). Cunningham and colleagues reported that in men spanning 6 decades, the association between self-selected gait speed and VO_2 peak was independent of age (20). Further, Fiser and colleagues reported a strong association of usual gait speed and VO_2 peak after controlling for gender (partial $r = .69$, $p < .001$), which remained significant after additional control for age and muscle strength in a sample of 60–88 year olds (21). Lastly, the meta-analytic techniques used in this study enable generally related types of outcomes to be combined into a common variable of interest (11). A particular strength of using meta-analytic techniques is the ability to find common associations by combining variables with related outcomes of differing metrics. In the STRRIDE and CALERIE studies, physical performance was determined from a graded treadmill exercise tolerance test with gas exchange analysis to measure VO_2 peak, a measure of overall cardiorespiratory fitness. The standard unit of measure for this test is mL/kg/min. The four studies involving older adults (CARRIAGE, POP, LIFE, and EF) measured usual gait speed over an eight-foot course using the Short Physical Performance Battery (SPPB) protocol (22).

Statistical Analysis

Characteristics of study participants were calculated using appropriate univariate continuous or frequency statistics. All biomarker values were transformed by the natural log (with a constant of one added to the raw values to deal with values of zero) because of their skewed distribution. Partial Spearman correlations between each biomarker and physical performance (partialling age, body mass index, gender, and race via linear regression methods) were then estimated separately by study. Because correlation coefficient SEs are not normally distributed, to derive test statistics and confidence intervals, we transformed the estimates using Fisher's Z transformation which is defined as (Equation 1): Fisher's $Z = 0.5 [\ln(1 + r) - \ln(1 - r)]$ (23). We were then able to apply standard meta-analytic techniques to the data, including weighting estimates based on study

variance which is generally written as (Equation 2)
$$\bar{x} = \frac{\sum_{i=1}^n w_i x_i}{\sum_{i=1}^n w_i}$$

of the overall effect and confidence intervals. The estimate was then algebraically transformed back to produce a weighted correlation coefficient, which was the statistic of interest for this study. Tests of homogeneity were conducted using the Q statistic, which is calculated as (Equation 3): $Q = \sum w_i (Z_i - Z)^2$, where w_i is the weighting factor for the i th study assuming a fixed-effects model, and Z is the sum of effect sizes weighted by their inverse variance. The Q statistic has a chi-square distribution with $k - 1$ degrees of freedom (24). All analyses were conducted using SAS v9.4 software (SAS, Cary, NC).

Results

There was considerable variability between studies with respect to subject characteristics. The number of study subjects ranged from 33 (CARRIAGE in which only subjects 65 years and older

were evaluated for physical performance) to 253 (EF), and mean ages ranged from late thirties (CALERIE) to late seventies (LIFE). Overall, men were well represented largely due to LIFE and EF, which were studies conducted at a VA Medical Center. The mean usual gait speed in the CARRIAGE study was the slowest (0.85 ± 0.22 m/s) and indicated decreased mobility as a group; whereas, the EF group represented the highest mean gait speed of 1.27 ± 0.23 m/s. The mean VO_2 peak in the CALERIE (30.6 ± 5.7 mL/kg/min) and STRRIDE (28.3 ± 6.0 mL/kg/min) studies indicated low cardiovascular fitness, which was consistent with the target populations and inclusion criteria.

Individual Study Biomarker Concentrations

Median and interquartile ranges of biomarker concentrations for each study can be observed in Table 2. CARRIAGE and POP had data for all biomarkers; TNF α data from the LIFE study was not included due to large numbers of samples with values below the lower limit of detection. The remaining studies (CALERIE, STRRIDE, and EF) show the available biomarkers based on the assays planned a priori. There was considerable variability of concentrations within each study with a consistent pattern of right skewness. Generally, the younger cohorts (CALERIE and STRRIDE) had lower biomarker concentrations compared to the oldest cohort (CARRIAGE).

Stratified Associations Between Biomarkers and Physical Performance

Bivariate Spearman correlations, done after combining VO_2 (CALERIE and STRRIDE studies) and gait speed (CARRIAGE, POP, LIFE, and EF studies) data from the studies, generally indicated inverse associations between biomarkers and both physical performance measures (Table 3). Leptin ($r = -.78$) and D-dimer ($r = -.51$) had the strongest inverse associations with VO_2 , while TNF α ($r = -.51$) and IL-8 ($r = -.44$) were most strongly inversely associated with gait speed. Four biomarkers had correlations of differing directions with VO_2 and gait speed, including: MCP-1 ($r = .11$ and $-.25$), RANTES ($r = -.25$ and $.16$), TNFR1 ($r = .01$ and $-.41$), and TNFR2 ($r = -.17$ and $.20$). However, combining data from multiple studies can lead to misinterpretation of associations of variables within individual studies. Figure 1 provides an example, as the correlation of $r = .20$ of TNFR2 and gait speed (solid black regression line) in the combined datasets misrepresents the inverse correlations found in the individual studies, which are appropriately maintained over all studies when meta-analytic techniques are used.

Weighted Associations Between Biomarkers and Physical Performance

The weighted correlations between circulating biomarkers and physical performance can be observed in Table 4. Of the 17 biomarkers measured, 4 inflammatory markers (IL-6, TNFR2, TNFR1, and TNF α), 1 coagulation marker (D-dimer), and 1 inflammatory mediator (GCSF) were found to be inversely associated with physical performance after controlling for age, body mass index, gender, and race and adjusting for multiple comparisons. In order of strength of the association, IL-6 (weighted $r = .22$), TNFR2 (weighted $r = -.19$), D-dimer (weighted $r = -.16$), TNFR1 (weighted $r = -.15$), GCSF (weighted $r = -.14$), and TNF α (weighted $r = -.10$) were all significantly inversely correlated with physical performance (all $p < .05$).

Table 2. Median (interquartile range) Biomarker Concentrations by Study

Circulating Biomarker (group)	CALERIE	STRIDE	CARRIAGE	POP	LIFE	Enhanced Fitness
D-Dimer, ng/mL (C)	*	223.4 (168.6)	669.9 (577.8)	399.1 (389.8)	659.3 (516.7)	403.4 (380.9)
GCSF, pg/mL (I)	*	590.7 (252.6)	302.4 (144.2)	362.6 (134.7)	128.0 (53.8)	*
IL-1RA, pg/mL (I)	*	1,333 (1,124)	2,131 (3,053)	2,441 (1,651)	212.2 (455.0)	*
IL-2, pg/mL (I)	*	4.2 (17.6)	12.7 (57.9)	12.7 (52.0)	13.2 (14.9)	*
IL-6, pg/mL (I)	0.59 (0.48)	0.69 (0.63)	0.75 (0.72)	1.07 (1.08)	1.10 (1.15)	1.65 (1.70)
IL-8, pg/mL (I)	*	5.8 (10.7)	88.8 (54.2)	74.1 (45.5)	24.9 (18.0)	7.3 (3.4)
Leptin, ng/mL (I)	16.3 (19.9)	22.1 (24.0)	23.1 (33.6)	32.1 (32.1)	12.8 (8.4)	*
MCP-1, pg/mL (I)	*	1,033 (438)	2,499 (1,076)	2,418 (1,348)	572.1 (307.0)	*
MMP-3, ng/mL (I)	*	*	7.1 (4.7)	4.7 (3.3)	11.1 (6.4)	*
PXN, nmol/min/mL (E)	*	3.1 (1.1)	9.4 (4.8)	6.3 (2.5)	4.4 (1.5)	*
RANTES, pg/mL (I)	*	21,273 (15,745)	7,726 (7,831)	6,827 (6,663)	25,278 (35,852)	*
TNF α , pg/mL (I)	5.9 (7.3)	20.3 (9.2)	19.5 (9.6)	16.5 (4.7)	†	6.7 (3.1)
TNFR1, pg/mL (I)	*	2,981 (1,159)	4,654 (2,920)	3,755 (2,878)	1,700 (788)	2,064 (1,000)
TNFR2, pg/mL (I)	*	4,249 (2,214)	2,689 (2,031)	1,624 (1,851)	2,388 (1,468)	5,063 (2,288)
TRAIL, pg/mL (I)	*	*	438.0 (177.2)	501.4 (240.0)	596.8 (266.8)	*
VCAM, pg/mL (E)	*	*	992.6 (134.6)	838.7 (218.2)	890.1 (173.7)	881.4 (339.8)
VEGF, pg/mL (E)	*	†	200.4 (234.4)	254.2 (288.7)	27.0 (13.3)	*

Notes: Group refers to type of biomarker: I = inflammation related; C = coagulation related; E = endothelial function related. CALERIE = Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy; CARRIAGE = CARolinas Region Interaction of Aging, Genes and Environment; GCSF = granulocyte colony-stimulating factor; IL-1RA = interleukin-1 receptor antagonist; MCP = monocyte chemotactic protein; MMP = matrix metalloproteinase; POP = Prediction of Osteoarthritis Progression; PXN = paraoxonase; RANTES = regulated upon activation, normal T-cell expressed, and secreted; STRIDE = Studies Targeting Risk Reduction Interventions through Defined Exercise; TNF = tumor necrosis factor; TNFR = tumor necrosis factor receptor; TRAIL = tumor necrosis factor-related apoptosis-inducing ligand; VCAM = vascular cell adhesion molecule; VEGF = vascular endothelial growth factor.

*Biomarker not assayed.

†Biomarker not included due to high proportion of samples below the lower level of quantification.

This indicates that higher circulating concentrations of these particular biomarkers were associated with poorer physical performance across the study cohorts. VCAM, a marker of endothelial activation, also tended to be associated with physical performance but was not significant ($r = -.09$; Fisher's $Z = -1.87$ which equates to $p = .06$). With the exception of Leptin, the Q statistics were nonsignificant, indicating homogeneity in the associations between biomarkers and physical performance across the studies.

Discussion

We present data that demonstrate significant inverse associations between six circulating biomarkers (IL-6, TNFR2, D-dimer, TNFR1, GCSF, and TNF α) and physical performance, across a diverse group of study cohorts. The mean ages in these study cohorts ranged from late thirties to late seventies; additional demographic measures, such as gender and race, varied considerably between studies. The studies available to us provided an opportunity to test, using meta-analytic methods, if associations exist among markers of disability in dissimilar cohorts. We view this as a unique strength to this work. Despite the demographic heterogeneity, the associations of biomarkers with physical performance were remarkably homogeneous across studies, demonstrating a consistent inverse correlation of six biomarker concentrations with physical performance. The biomarkers that demonstrated no association with physical performance in our study may not represent causal pathways in the genesis of functional decline, and/or their contribution may be too small to be detected by our cross-sectional, correlative analysis. Worth noting is that the pathways that these biomarkers represent, inflammation and coagulation, have considerable "cross-talk" with respect to upregulation of systems and disease sequelae (9). Thus, their unidirectional and

consistent strength of association with physical performance as a group may represent similar activation of multiple inflammatory and coagulation pathways that would precede clinical detection in most instances.

Of the six biomarkers with associations with physical performance in our study, D-dimer and, particularly IL-6, have been most commonly discussed in previous work in this area (25). This has been work done primarily in aging cohorts (1,2,26–29) and in all the studies, higher concentrations of IL-6/D-dimer were associated with poor outcomes. Several published articles have used the Established Populations for Epidemiologic Studies of the Elderly cohorts to investigate these associations. These studies reported cross-sectional associations between IL-6 and functional disability (1), longitudinal associations between baseline IL-6/D-dimer and subsequent declines in physical functioning (2), and longitudinal associations between baseline IL-6 and incident disability (30). More recently, Adriaensen and coworkers (31) examined data from the BELFRAIL study (80+ year old cohort from Belgium) and reported cross-sectional associations between IL-6 and global functioning (physical and mental components) in men and women, and highly elevated serum IL-6 levels with poor physical performance. Additionally, while not reaching statistical significance, we observed a similar pattern of association with VCAM and physical performance, as previously described by our group (32).

We observed associations between both IL-6 and D-dimer and physical performance across all our cohorts. This both complements and extends the previous work described above. It is complementary in that it provides further confirmatory evidence of associations between IL-6/D-dimer and physical functioning when simultaneously testing associations with 15 other biomarkers. It extends previous work in that our functional assessments (gait speed and VO₂peak) are objective measures of performance that are amenable

to change and are a primary focus of interventions aimed at improving function in older adults (14,33,34).

Our finding of an association between TNFR1/TNFR2 and physical performance is a novel finding in populations whose primary diagnosis is not an inflammatory disease. Nicklas and coworkers (35) reported that after adjusting for age, race, sex, and body mass index,

Table 3. Associations (Spearman correlation coefficients) of VO₂ peak and Gait Speed With Circulating Biomarkers

Circulating Biomarker	VO ₂ peak	Gait Speed
D-Dimer	-0.51	-0.23
GCSF	-0.03	-0.26
IL-1RA	-0.08	-0.21
IL-2	-0.03	-0.10
IL-6	-0.07	-0.05
IL-8	-0.31	-0.44
Leptin	-0.78	-0.25
MCP-1	0.11	-0.25
MMP-3	NA	0.22
PXN	-0.04	-0.18
RANTES	-0.25	0.16
TNF α	-0.19	-0.51
TNFR1	0.01	-0.41
TNFR2	-0.17	0.20
TRAIL	NA	0.12
VCAM	NA	-0.15
VEGF	NA	-0.25

Note: GCSF = granulocyte colony-stimulating factor; IL-1RA = interleukin-1 receptor antagonist; MCP = monocyte chemoattractant protein; MMP = matrix metalloproteinase; PXN = paraoxonase; RANTES = regulated upon activation, normal T-cell expressed, and secreted; TNF = tumor necrosis factor; TNFR = tumor necrosis factor receptor; TRAIL = tumor necrosis factor-related apoptosis-inducing ligand; VCAM = vascular cell adhesion molecule; VEGF = vascular endothelial growth factor; NA = not available.

older individuals with osteoarthritis of the knee and homozygous for the major T allele of the TNFR2 +676 polymorphism had better physical function. In the current study, TNFR2 had the strongest association with physical performance (weighted $r = -.20$). Interestingly, the elevation of TNFR2 and its association with lower physical function may represent a counter measure as it has been shown that the TNFR2 signaling pathways appear to offer protective roles in several disorders by enhancing regeneration and repair processes. This has led to the proposal to consider using TNFR2 agonists in the treatment of such inflammatory diseases (3,36). Whether or not similar targeted therapies could improve physical performance across the life span certainly warrants consideration and future examination.

TNF α has been shown to relate to physical performance in several aged patient populations with inflammatory and cardiovascular diseases (37–39) and in well-functioning older adult cohorts such as the Health, Aging and Body Composition study (40). In our study, the CALERIE cohort (younger, overweight, and unfit) demonstrated the strongest inverse association between TNF α and physical performance ($r = -.20$). In a group of obese middle-aged women, Poelkens and coworkers found that, compared to those with metabolic syndrome, those who were metabolically healthy obese had significantly lower TNF α concentrations (41). Further study is needed to determine whether TNF α could be utilized as a preclinical marker of increased risk of developing metabolic syndrome in otherwise healthy, overweight, sedentary middle-aged adults.

The significant association of GCSF with physical performance is particularly novel. GCSF is a cytokine produced by endothelial cells, fibroblasts, and other immune cells. It is induced by, among others, TNF and ILs (42). It is a mediator of pathways found in this study to be associated with physical performance. To our knowledge, GCSF has not been comprehensively examined as a biomarker of low-level inflammation as part of the aging process. Based on its representation of multiple immune and inflammatory pathways, it may be of benefit to further examine GCSF as a global biomarker of systems dysregulation.

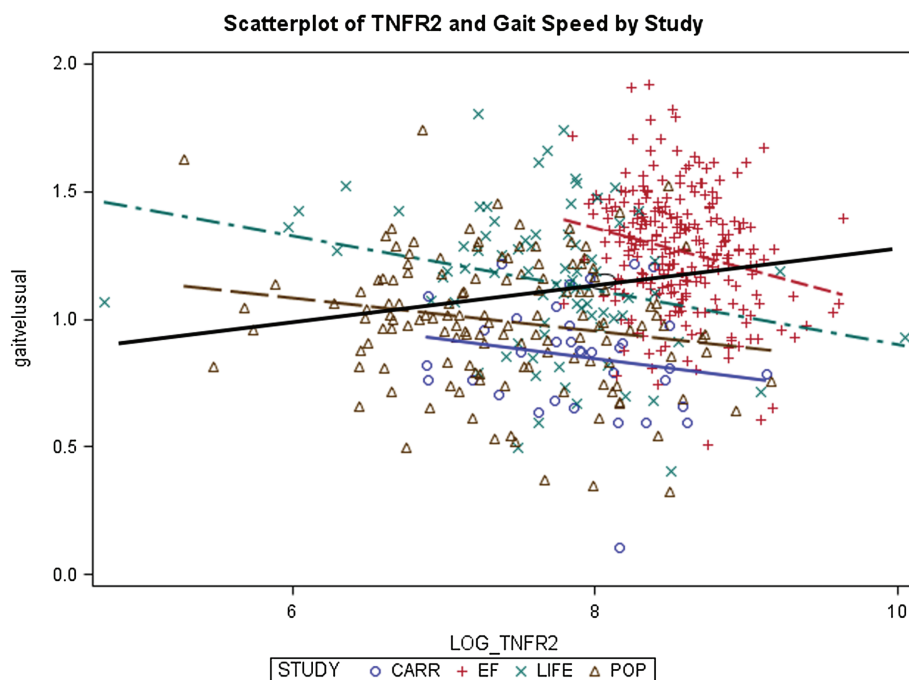


Figure 1. Example of potential misinterpretation when combining datasets for analysis.

Table 4. Weighted Correlations Between Circulating Biomarkers and Physical Performance

Circulating Biomarker	Number of Studies	N Subjects	Weighted Correlation	Fisher's Z	Q Statistic
IL-6	6	603	-0.22	-5.24*	9.27
TNFR2	5	562	-0.19	-4.40*	0.31
D-Dimer	5	562	-0.16	-3.68*	6.76
TNFR1	5	562	-0.15	-3.54*	2.85
GCSF	4	309	-0.14	-2.37*	3.61
TNF α	5	525	-0.10	-2.31*	5.78
VCAM	4	469	-0.09	-1.87	3.25
IL-8	5	562	-0.07	-1.63	1.52
RANTES	4	309	-0.09	-1.49	1.59
PXN	3	241	0.09	1.39	0.43
Leptin	5	350	-0.07	-1.26	19.68 [†]
TRAIL	3	241	0.07	1.12	1.62
VEGF	3	241	-0.05	-0.74	3.38
IL-1RA	4	309	-0.03	-0.54	0.99
IL-2	4	309	-0.03	-0.45	1.73
MMP-3	3	241	0.03	0.46	2.21
MCP-1	3	241	0.00	-0.04	0.55

Notes: GCSF = granulocyte colony-stimulating factor; IL-1RA = interleukin-1 receptor antagonist; MCP = monocyte chemotactic protein; MMP = matrix metalloproteinase; PXN = paraoxonase; RANTES = regulated upon activation, normal T-cell expressed, and secreted; TNF = tumor necrosis factor; TNFR = tumor necrosis factor receptor; TRAIL = tumor necrosis factor-related apoptosis-inducing ligand; VCAM = vascular cell adhesion molecule; VEGF = vascular endothelial growth factor.

*Adjusted $p < .05$ (adjusted for age, BMI, gender, and race).

[†]Indicates heterogeneity of correlation between studies.

Limitations to this study include the cross-sectional design, potentially uncontrolled confounders, such as medication usage and exercise history, and the incomplete measurement of all biomarkers in all the studies. Another limitation, which is a significant issue with life span research, is lack of a uniform measure of physical performance between younger and older cohorts. Finally, while we used conservative methods for significance testing, Type I error due to multiple tests of association is a potential issue that should be considered when interpreting these findings.

This study provides impetus to continue to understand if and how physiologic dysregulation occurs in a more coordinated, "cross-talk" manner, as opposed to a stochastic manner (8), in which case simultaneously measuring multiple biomarkers representing the spectrum of physiologic systems may not be necessary to quantify preclinical aging-related functional impairment. Conversely, early and midlife concentrations of particular biomarkers (eg, TNF α) may have unique predictive characteristics for increased risk of specific diseases or syndromes that impact function (41). This could be addressed in future longitudinal studies with a standardized physical performance measures and a broad spectrum of biomarkers across multiple time points throughout the adult life span.

In summary, using six cohorts that were diverse in many ways, we observed consistent inverse associations between six biomarkers and objective measures of physical performance. The generalizability of these results in cohorts spanning adulthood suggests that these serum biomarker measures may be broadly applicable for detection, trajectory, and treatment monitoring of physical function across the life span or possibly for midlife predictors of functionally deleterious conditions.

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