Supplementary materials for "Physiological dysregulation and aging in evolutionary perspective"

Thomas S. Kraft, Jonathan Stieglitz, Benjamin C. Trumble, Angela R. Garcia, Hillard Kaplan, Michael Gurven

1. Health and aging in non-industrial societies

Absolute increases in mortality rates with age are higher in small-scale subsistence societies than contemporary industrialized countries [1,2], consistent with a faster pace of actuarial senescence. Transitioning subsistence populations often experience improvements in life expectancy associated with increased access to healthcare and other modern amenities [3]. As conditions improve, developed countries show a greater "rectangularization" of survival curves consistent with temporal declines in rates of actuarial aging [4]. Yet how actuarial aging relates to physiological condition is not straightforward.

Numerous studies suggest that human subsistence populations with distinct lifestyles and genetic backgrounds experience radically different age trajectories in health-related biomarkers compared to industrial populations [5–7]. For example, risk of hypertension increases with age in almost all industrialized populations, but not among some subsistence populations of hunter-gatherers and forager-horticulturalists [8,9]. Such differences in physiological aging are likely responsible for the very low prevalence of hypertension and atherosclerosis in subsistence populations [10]. In examples from our own studies of Tsimane horticulturalists of Bolivia, we have documented high mortality risk and major infectious sources of mortality and morbidity [3], unique age profiles of immune cells [11], slow age-related loss of cardiorespiratory fitness and muscular strength [12], age-related cognitive decline [13], and minimal cardiovascular disease [10,14,15]. Based on these findings, one might expect large population differences in physiological aging profiles.

2. Additional details of data analysis

2.1 Sampling design

Sample sizes vary by biomarker and across the duration of the study period for several reasons. First, sampling strategy varies by data type such that some variables were targeted towards individuals of a particular sex or age demographic (e.g. vertebral bone mineral density not measured in young individuals). Second, absent or sick THLHP personnel precluded the collection of specific data types at certain times. Finally, the number of study villages and thus enrolled participants has increased over time, and the data types collected have changed. As a result, our dataset contains missing values that require special procedures for the calculation of the Mahalanobis distance, described below.

2.2 Biomarker analysis

Following a fasting morning blood draw, serum was separated and frozen in liquid nitrogen. Samples then were either measured at our clinic laboratory located in San Borja, Bolivia, or sent on dry ice to the Human Biodemography Laboratory at UC Santa Barbara.

In Bolivia, at the time of collection, glucose was measured using a point of care device (Prodigy Diabetes Care, Charlotte NC). A manual complete blood count with a five-part differential was conducted using a hemocytometer, and erythrocyte sedimentation rate (ESR) was measured using the conventional (Westergren) method. Hemoglobin was assessed on a QBC Autoread Plus Dry Hematology System (Drucker Diagnostics, PA). Serum lipids (total cholesterol, HDL, LDL, triglycerides) were measured on a Stat Fax 1908 (Awareness Technology, Palm City, FL).

At UC Santa Barbara, commercial immunoassays were used to measure oxidized LDL (oxLDL) (Mercodia, Winston Salem, NC), Apolipoprotein A (ApoA) (Abcam, Cambridge, MA), Apolipoprotein B (ApoB) (R&D Systems, Minneapolis, MN) [10]. Cytokines were measured via multiplex assay on a Luminex MagPix (EMD Millipore, Darmstadt, Germany) [10,16]. High sensitivity C-Reactive Protein was assessed from serum via immunoassay, and was cross-validated by the University of Washington laboratory, using the protocols utilized for the National Health and Nutrition Evaluation Survey (NHANES) [17]. Cortisol was measured via an in-house enzyme immunoassay [18]. Serum IgE and IgG were assessed via commercial enzyme immunoassay with consistent lot numbers (per assay) used across all samples (Bethyl, Montgomery, TX) [11]. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) was measured with a commercial ELISA (Highly Sensitive 8-OHdG Check ELISA, Genox Corp., Baltimore, MD), as was urinary 15-F2t-isoprostane (Oxford Biomedical Research, Rochester Hills, MI). Urinary creatinine was measured with the colorimetric Jaffe reaction [19].

Several other biomarkers are included here that have not been previously described in other publications. These include body temperature, respiratory rate, forced expiratory volume, and peak expiratory flow. Body temperature was measured using an oral thermometer up through mid-2006 and a Braun digital tympanic thermometer (Thermoscan 5) thereafter. Respiratory rate was assessed by counting the number of breaths at rest over a set time period. Forced expiratory volume and peak expiratory volume (two different standardized measures of lung function) were measured in one of two ways using spirometry tests: 1) using a Cosmed Fitmate PRO metabolic device, or 2) using a Piko peak flow meter device. In either case, subjects were asked to blow a single breath as full and long as possible through a measurement device, which then calculated the desired parameters.

2.3 *Checking reference values*

Baseline or reference means and covariance matrix are required to calculate D_m. As noted in the main text, we set reference values as the median of all observations for individuals between the ages of 20 and 45 years old and generated separate baselines for males and females to account for potential sex differences in traits. Similar approaches have been employed by Cohen and colleagues [20]. Nonetheless, we corroborated this reference by calculating mean values on all biomarkers from a subset of our data that included individuals between 20 and 45 years old who received a “healthy” diagnosis from medical doctors during examinations at the time of data collection. Values from the two calculations were highly correlated ($r_{female} = 0.79$, $p < 0.001$; $r_{male} = 0.88$, $p < 0.001$), suggesting that our reference values reflect biomarker values that are indicative of a healthy condition.

2.4 Further details on the calculation of Mahalanobis distance

Milot et al. [21] note that D_m cannot be calculated if cases contain missing values. It would have been impractical to remove all missing values from our dataset. To address this constraint, we used *MDmiss* in the *R* package *modi* to calculate the "marginal" D_m over all available data [22]. This function handles missingness by looping over observations and omitting missing dimensions before calculating D_m, and then incorporates a correction factor based on the ratio of total to observed dimensions [22]. Distances are therefore calculated based solely on existing values.

The magnitude of D_m is influenced by the number of biomarkers analyzed. To ensure that the number of biomarkers did not influence our results, we included the number of biomarkers as a covariate in all models and weighted observations by the number of biomarkers. To test whether missing data and methodological procedures affected results, we re-ran models of D_m while restricting the dataset to observations with at least 5, 10, 15, 20, and 25 biomarkers. Despite reductions in sample size, this procedure produced qualitatively similar results at all levels (Fig. S2).

We also could not directly estimate a sample covariance matrix from our reference population due to the presence of missing values. We thus calculated the covariance matrix using pairwise complete cases of individuals 20-45 years old. Because some combinations of biomarkers were rarely measured in tandem in young individuals, we then identified pairwise combinations of variables that had less than 50 observations in the reference population, and replaced such cases with a covariance calculation from either the 50 observations of lowest age with pairwise information, or all cases in the population (people of all ages) if there were less than 40 observations overall. Because this procedure can lead to covariance matrices which are not positive-definite (problematic for calculation of Mahalanobis distance), we then used the algorithm of Higham [23] via the *nearPD* function in *R* to compute the nearest positive-definite approximation when necessary. An alternative approach to calculate a positive definite covariance matrix with missingness is to use maximum likelihood estimation or an expectation maximization algorithm. We applied this approach using a penalized expectation maximization algorithm [24] and found similar results.

2.5 Non-human primate data and methods

D_m estimates from non-human primates were taken from a recent study by Dansereau et al. [25]. In brief, this study analyzed longitudinal biomarker data from a recently available database (Internet Primate Aging Database (iPAD); http://ipad.primate.wisc.edu/) on 10 species of non-human primates. Observations in this database come from nonexperimental adult animals housed in captive settings.

This dataset contained variable numbers of biomarkers measured across the different study species. As such, the authors conducted two types of analyses of D_m: 1) using all available biomarkers for each species (what they call "Set 1"), and 2) using a fixed set of 12 biomarkers that were available for all species and industrialized humans ("Set 2"). Because our analysis of Tsimane D_m used a different array of biomarkers (all that were available) and thus corresponds closely with their "Set 1," we use focus on this set for comparison. Age slopes from [25] derive from linear mixed effects models without polynomial effects, allowing comparison with our models including only linear age terms (see [25] for more details). Data were taken from their

Table S3, and 95% confidence intervals that were not reported were taken directly from figure 1 in [25] using ImageJ.

Dansereau et al. [25] present only standardized coefficients that correspond to changes in D_m per standard deviation of age. To calculate unstandardized coefficients, we obtained the appropriate sample SDs from the original authors (T. Wey and A. Cohen, personal communication), and then transformed estimates by dividing reported age slopes by species-specific sample SDs (Fig. 3).

2.6 Industrialized human population data

D_m from industrialized humans also come from [25]. Average "industrialized human" values were generated from two longitudinal datasets, the Baltimore Longitudinal Study of Aging [26] and the Invecchiare in Chianti study [27]. See [25] and references within for more details.

3. Additional advantages and disadvantages of Mahalanobis distance

There are several additional advantages to the use of Mahalanobis distance as a measure of systemic physiological aging beyond those described in the main text. First, by comparing observations to a baseline reference population, D_m can be interpreted explicitly as a relative deviation from a presumed healthy state. This contrasts with alternative approaches that have been applied to measure physiological aging, such as PCA [28]. Second, previous studies of allostatic load sometimes utilize *a priori* designations of “healthy” versus “unhealthy” levels of individual biomarkers, and then sum across biomarkers to create an overall score. D_m preserves biomarkers as continuous variables and avoids arbitrary cutoffs identifying directional values as indicative of poor health, and so averts the circularity of tallying up indicators of poor health (i.e. deficits) to create a measure of poor health and aging [29]. This makes D_m useful as a measure of an emergent process of dysregulation. Third, by simultaneously taking into account both means and covariances of biomarkers, D_m more directly captures a breakdown in the capacity of complex regulatory networks that maintain homeostasis [30], as embodied by the theoretical concepts of allostatic load [31], “homeostenosis” [32], and physiological dysregulation [33]. Finally, in principle D_m can be compared across physiological systems when appropriately standardized. Such utility is bolstered by the fact that D_m is robust to choice of biomarkers, with little marginal change beyond 10-15 biomarkers, and with D_ms calculated from mutually exclusive sets of biomarkers showing correlations of 0.4-0.5 [34].

A recent study comparing D_m with other measures of biological aging in New Zealanders showed low correlations with telomeres and epigenetic measures, and moderate correlations with other longitudinal, biomarker-based measures [35]. In that study, D_m was the best predictor of most health outcomes examined, including physical and cognitive condition, and self-reported health, yet associations were moderate.

There are also several disadvantages to our usage of D_m. First, the calculation of D_m includes the assumption of multivariate normality, which is often unfeasible for high-dimension data sets. Second, D_m cannot traditionally be calculated with missing values, and thus requires multiple imputation or methodological procedures such as those employed here to enable estimation. Finally, unlike individual biomarker values, absolute values of D_m measure relative

distance from a reference centroid and lack straightforward interpretation that would allow inference about how much dysregulation is "too high." It therefore implicitly assumes that the average healthy reference biomarker profile is "ideal" for everyone. Individual studies can, however, evaluate determinants of D_m, and the extent to which different levels of D_m are associated with morbidity or mortality, to better interpret the meaning of D_m levels.

Supplementary References

1. Gurven MD, Kaplan HS. 2007 Longevity among hunter-gatherers: a cross-cultural comparison. *Popul. Dev. Rev.* **33**, 321–365.
2. Gurven MD, Fenelon A. 2009 Has the rate of actuarial aging changed over the past 250 years? A comparison of small-scale subsistence populations, and Swedish and English cohorts. *Evolution* **63**, 1017–1035.
3. Gurven MD, Kaplan HS, Zelada Supa A. 2007 Mortality experience of Tsimane Amerindians: regional variation and temporal trends. *Am. J. Hum. Biol.* **19**, 376–398.
4. Yashin AI, Ukraintseva S V, Boiko SI, Arbeev KG. 2002 Individual aging and mortality rate: how are they related? *Soc. Biol.* **49**, 206–217.
5. Pontzer H, Wood BM, Raichlen DA. 2018 Hunter-gatherers as models in public health. *Obes. Rev.* **19**, 24–35. (doi:10.1111/obr.12785)
6. Liebert MA, Snodgrass JJ, Madimenos FC, Cepon TJ, Blackwell AD, Sugiyama LS. 2013 Implications of market integration for cardiovascular and metabolic health among an indigenous Amazonian Ecuadorian population. *Ann. Hum. Biol.* **40**, 228–242. (doi:10.3109/03014460.2012.759621)
7. Eaton SB, Konner MJ, Shostak M. 1988 Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. *Am. J. Med.* **84**, 739–749.
8. Gurven MD, Blackwell AD, Rodríguez DE, Stieglitz J, Kaplan HS. 2012 Does blood pressure inevitably rise with age? Longitudinal evidence among forager-horticulturalists. *Hypertension* **60**, 25–33.
9. Koopman JJE, van Bodegom D, Jukema JW, Westendorp RGJ. 2012 Risk of cardiovascular disease in a traditional African population with a high infectious load: a population-based study. *PLoS One* **7**, e46855.
10. Kaplan HS *et al.* 2017 Coronary atherosclerosis in indigenous South American Tsimane: a cross-sectional cohort study. *Lancet* **389**, 1730–1739. (doi:10.1016/S0140-6736(17)30752-3)
11. Blackwell AD, Trumble BC, Maldonado Suarez I, Stieglitz J, Beheim BA, Snodgrass JJ, Kaplan HS, Gurven MD. 2016 Immune function in Amazonian horticulturalists. *Ann. Hum. Biol.* **43**, 382–396. (doi:10.1080/03014460.2016.1189963)
12. Pisor AC, Gurven MD, Blackwell AD, Kaplan HS, Yetish G. 2013 Patterns of senescence in human cardiovascular fitness: VO2max in subsistence and industrialized populations. *Am. J. Hum. Biol.* **25**, 756–769.
13. Gurven MD, Fuerstenberg E, Trumble BC, Stieglitz J, Beheim B, Davis H, Kaplan HS. 2017 Cognitive performance across the life course of Bolivian forager-farmers with limited schooling. *Dev. Psychol.* **53**, 160–176. (doi:http://dx.doi.org/10.1037/dev0000175)
14. Gurven MD, Kaplan HS, Winking J, Eid D, Vasunilashorn S, Kim J, Finch C, Crimmins E. 2009 Inflammation and infection do not promote arterial aging and cardiovascular

disease among lean Tsimane forager-horticulturalists. *PLoS One* **4**, e6590. (doi:doi:10.1371/journal.pone.0006590)
15. Gurven MD, Trumble BC, Stieglitz J, Blackwell AD, Michalik DE, Finch CE, Kaplan HS. 2016 Cardiovascular disease and type 2 diabetes in evolutionary perspective: A critical role for helminths? *Evol. Med. Public Heal.* , 338–357. (doi:10.1093/emph/eow028)
16. Trumble BC, Blackwell AD, Stieglitz J, Thompson ME, Suarez IM, Kaplan HS, Gurven MD. 2016 Associations between male testosterone and immune function in a pathogenically stressed forager-horticultural population. *Am. J. Phys. Anthropol.*
17. Brindle E, Fujita M, Shofer J, O'Connor KA. 2010 Serum, plasma, and dried blood spot high-sensitivity C-reactive protein enzyme immunoassay for population research. *J. Immunol. Methods* **362**, 112–120.
18. Trumble BC, Brindle E, Kupsik M, O'Connor KA. 2010 Responsiveness of the reproductive axis to a single missed evening meal in young adult males. *Am. J. Hum. Biol.* **22**, 775–781. (doi:10.1002/ajhb.21079)
19. Taussky HH. 1954 A microcolorimetric determination of creatinine in urine by the Jaffe reaction. *J. Biol. Chem.* **208**, 853–861.
20. Cohen AA, Milot E, Yong J, Seplaki CL, Fülöp T, Bandeen-Roche K, Fried LP. 2013 A novel statistical approach shows evidence for multi-system physiological dysregulation during aging. *Mech. Ageing Dev.* **134**, 110–117. (doi:10.1016/j.mad.2013.01.004)
21. Milot E, Cohen AA, Vézina F, Buehler DM, Matson KD, Piersma T. 2014 A novel integrative method for measuring body condition in ecological studies based on physiological dysregulation. *Methods Ecol. Evol.* **5**, 146–155. (doi:10.1111/2041-210X.12145)
22. Bill M, Hulliger B. 2016 Treatment of multivariate outliers in incomplete business survey data. *Austrian J. Stat.* **45**, 3–23. (doi:10.17713/ajs.v45i1.86)
23. Higham NJ. 2002 Computing the nearest correlation matrix-a problem from finance. *IMA J. Numer. Anal.* **22**, 329–343.
24. Chen LS, Prentice RL, Wang P. 2014 A penalized EM algorithm incorporating missing data mechanism for Gaussian parameter estimation. *Biometrics* **70**, 312–322. (doi:10.1111/biom.12149)
25. Dansereau G, Wey TW, Legault V, Brunet MA, Kemnitz JW, Ferrucci L, Cohen AA. 2019 Conservation of physiological dysregulation signatures of aging across primates. *Aging Cell* **18**, e12925. (doi:10.1111/acel.12925)
26. Ferrucci L. 2008 The Baltimore Longitudinal Study of Aging (BLSA): a 50-year-long journey and plans for the future. *Journals Gerontol. Ser. A Biol. Sci. Med. Sci.* **63**, 1416–1419. (doi:10.1093/gerona/63.12.1416)
27. Ferrucci L, Bandinelli S, Benvenuti E, Di Iorio A, Macchi C, Harris TB, Guralnik JM. 2000 Subsystems contributing to the decline in ability to walk: bridging the gap between epidemiology and geriatric practice in the InCHIANTI study. *J. Am. Geriatr. Soc.* **48**, 1618–1625. (doi:10.1111/j.1532-5415.2000.tb03873.x)
28. Cohen AA *et al.* 2015 Detection of a novel, integrative aging process suggests complex physiological integration. *PLoS One* **10**, e0116489. (doi:10.1371/journal.pone.0116489)
29. Cohen AA, Milot E, Li Q, Legault V, Fried LP, Ferrucci L. 2014 Cross-population validation of statistical distance as a measure of physiological dysregulation during aging. *Exp.*

Gerontol. **57**, 203–210. (doi:10.1016/j.exger.2014.04.016)
30. Cohen AA. 2016 Complex systems dynamics in aging: new evidence, continuing questions. *Biogerontology* **17**, 205–220. (doi:10.1007/s10522-015-9584-x)
31. McEwen BS. 1998 Stress, adaptation, and disease: allostasis and allostatic load. *Ann. N. Y. Acad. Sci.* **840**, 33–44.
32. Taffett GE. 2003 Physiology of aging. In *Geriatric Medicine*, pp. 27–35. Springer.
33. Seplaki CL, Goldman N, Glei D, Weinstein M. 2005 A comparative analysis of measurement approaches for physiological dysregulation in an older population. *Exp. Gerontol.* **40**, 438–449. (doi:10.1016/j.exger.2005.03.002)
34. Cohen AA, Li Q, Milot E, Leroux M, Faucher S, Morissette-Thomas V, Legault V, Fried LP, Ferrucci L. 2015 Statistical distance as a measure of physiological dysregulation Is largely robust to variation in its biomarker composition. *PLoS One* **10**, e0122541. (doi:10.1371/journal.pone.0122541)
35. Belsky DW *et al.* 2017 Eleven telomere, epigenetic clock, and biomarker-composite quantifications of biological aging: do they measure the same thing? *Am. J. Epidemiol.* **187**, 1220–1230. (doi:10.1093/aje/kwx346)
36. Vasunilashorn S, Crimmins EM, Kim JK, Winking J, Gurven MD, Kaplan HS, Finch CE. 2010 Blood lipids, infection, and inflammatory markers in the Tsimane of Bolivia. *Am. J. Hum. Biol.* **22**, 731–740. (doi:10.1002/ajhb.21074)
37. Anderson A, Trumble BC, Hove C, Kraft TS, Kaplan HS, Gurven MD, Blackwell AD. 2019 Old friends and friendly fire: potential costs of fetal tolerance among tropical horticulturalists in a hookworm-endemic environment. *Am. J. Hum. Biol.*
38. Blackwell AD, Urlacher SS, Beheim BA, von Rueden C, Jaeggi A V, Stieglitz J, Trumble B, Gurven MD, Kaplan HS. 2017 Growth references for Tsimane forager-horticulturalists of the Bolivian Amazon. *Am. J. Phys. Anthropol.* **162**, 441–461.
39. Gurven MD, Trumble BC, Stieglitz J, Cummings D, Blackwell AD, Beheim BA, Kaplan HS, Pontzer H. 2016 High resting metabolic rate among Amazonian forager-horticulturalists experiencing high pathogen burden. *Am. J. Phys. Anthropol.* **161**, 414–425.
40. Blackwell AD, Gurven MD, Sugiyama LS, Madimenos FC, Liebert MA, Martin MA, Kaplan HS, Snodgrass JJ. 2011 Evidence for a peak shift in a humoral response to helminths: age profiles of IgE in the Shuar of Ecuador, the Tsimane of Bolivia, and the U.S. NHANES. *PLoS Negl Trop Dis* **5**, e1218. (doi:10.1371/journal.pntd.0001218)
41. von Rueden CR, Trumble BC, Thompson ME, Stieglitz J, Hooper PL, Blackwell AD, Kaplan HS, Gurven MD. 2014 Political influence associates with cortisol and health among egalitarian forager-farmers. *Evol. Med. Public Heal.* **2014**, 122–133.
42. Gurven MD, Kaplan HS, Gutierrez M. 2006 How long does it take to become a proficient hunter? Implications for the evolution of extended development and long life span. *J. Hum. Evol.* **51**, 454–470. (doi:10.1016/j.jhevol.2006.05.003)
43. Stieglitz J, Trumble BC, Kaplan HS, Gurven MD. 2017 Horticultural activity predicts later localized limb status in a contemporary pre-industrial population. *Am. J. Phys. Anthropol.* **163**, 425–436. (doi:10.1002/ajpa.23214)
44. Stieglitz J, Trumble BC, Finch CE, Dong L, Budoff MJ, Kaplan H, Gurven MD. 2019 Computed tomography shows high fracture prevalence among physically active forager-horticulturalists with high fertility. *Elife* **8**. (doi:10.7554/eLife.48607)

45. Stieglitz J, Madimenos F, Kaplan HS, Gurven MD. 2016 Calcaneal quantitative ultrasound indicates reduced bone mineral status among physically active adult forager-horticulturalists. *J. Bone Miner. Res.* **31**, 663–671.

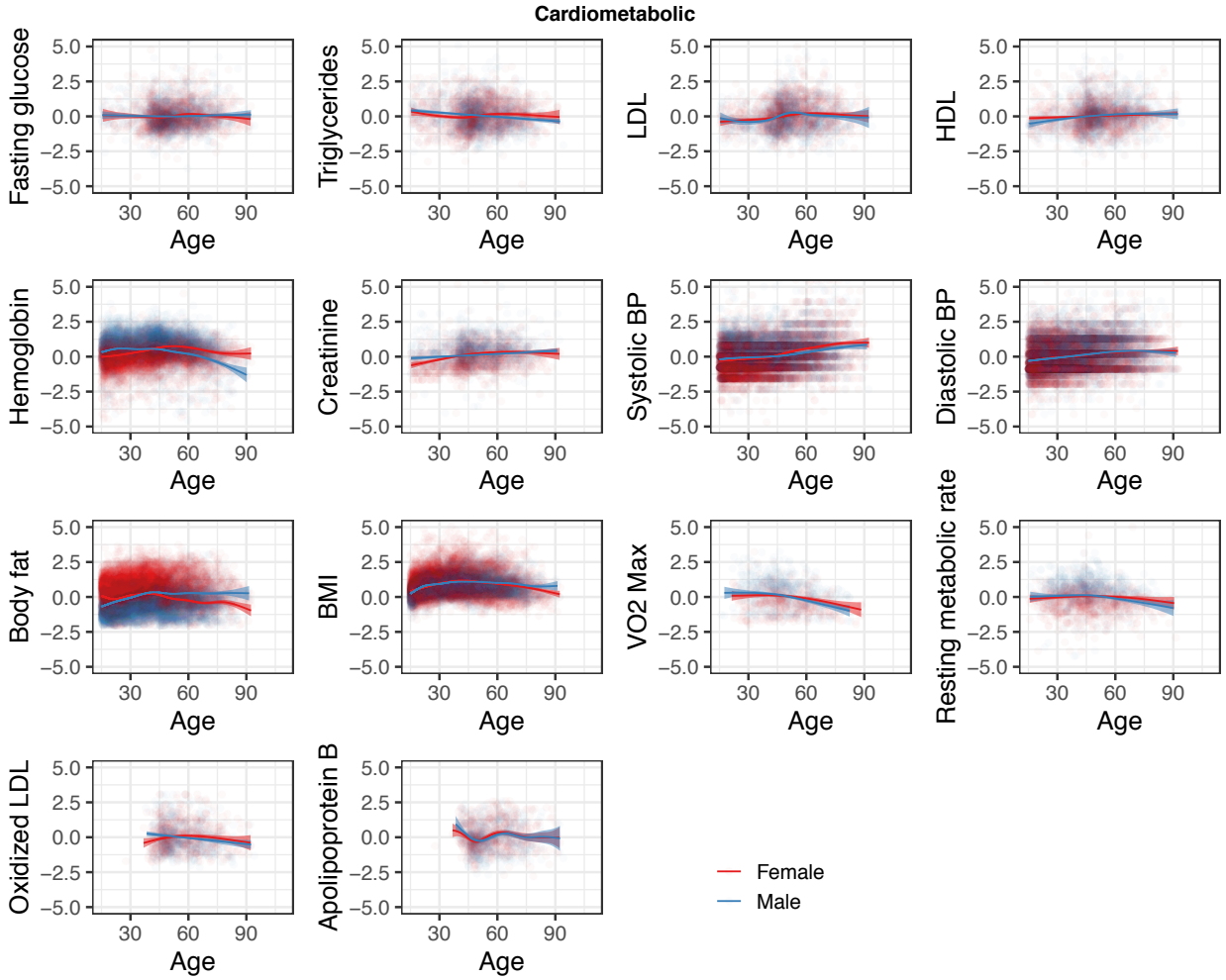
Supplemental tables and figures

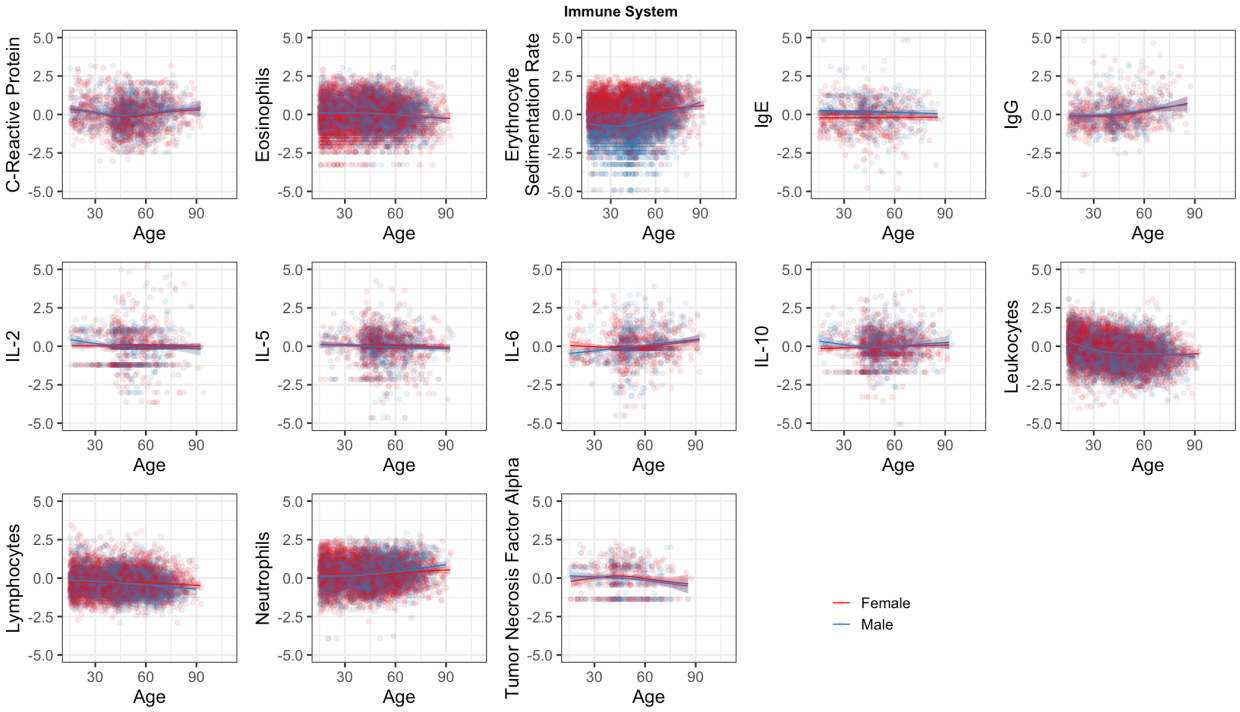
Table S1: List of biomarkers with published references containing more details about data collection. Reported means and SDs are untransformed.

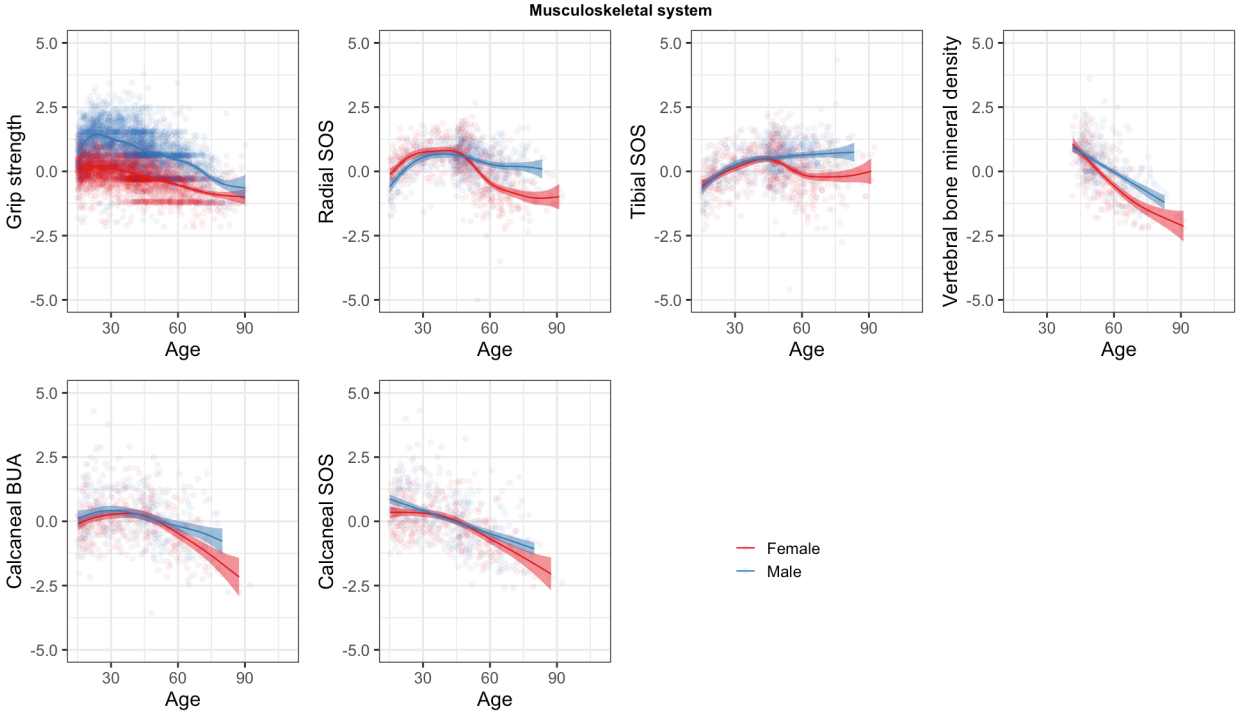
Biomarker	Original units	Transformation applied	References	Mean	SD
Cardiometabolic					
Fasting glucose	mg/dL	none	[10]	80.6	16.1
Triglycerides	mg/dL	natural log	[10,36]	108.7	57.1
LDL	mg/dL	none	[10,36]	85.6	32.5
HDL	mg/dL	none	[10,36]	37.1	9.9
Creatinine	mg/dL	none	Unpublished	1.0	2.7
Hemoglobin	g/dL	none	[37]	13.1	1.6
Systolic BP	mmHg	none	[8]	109.9	12.8
Diastolic BP	mmHg	none	[8]	68.3	9.3
Body fat	%	none	[10]	21.2	7.8
BMI	kg/m^2	none	[38]	23.6	3.0
VO2 Max	$ml*kg^{-1}*min^{-1}$	none	[12]	43.8	17.1
RMR	kcal/day	none	[39]	1794.6	423.5
Oxidized LDL	units/L	none	[10]	76.4	23.9
Apolipoprotein B-100	mg/dL	natural log	[10]	97.8	40.8
Immune					
C-reactive protein	mg/dL	natural log	[10,14,36]	34.9	30.8
Eosinophils	%	square root	[11]	17.0	9.0
Erythrocyte sedimentation rate	mm/hr	natural log	[10]	30.0	20.1
IgE	IU/mL	none	[11,36,40]	10,175	6526
IgG	mg/dL	none	[11,36]	11.0	3.4
Interleukin 2	pg/mL	natural log	[10]	2.0	5.6
Interleukin 5	pg/mL	natural log	[10]	2.4	6.7
Interleukin 6	pg/mL	natural log	[10]	3.1	6.7
Interleukin 10	pg/mL	natural log	[10]	4.8	7.4
Leukocytes	$cells/ml^3$	natural log	[11]	9866	3235
Lymphocytes	%	none	[11]	29.0	7.9
Neutrophils	%	none	[11]	53.3	10.6
Tumor necrosis factor alpha	pg/mL	natural log	[10]	9.2	22.5
Other (Oxidative stress)					
8-hydroxy-2'-deoxyguanosine (8-OH-dG)	ng/specific gravity	natural log	Unpublished	9.8	9.1

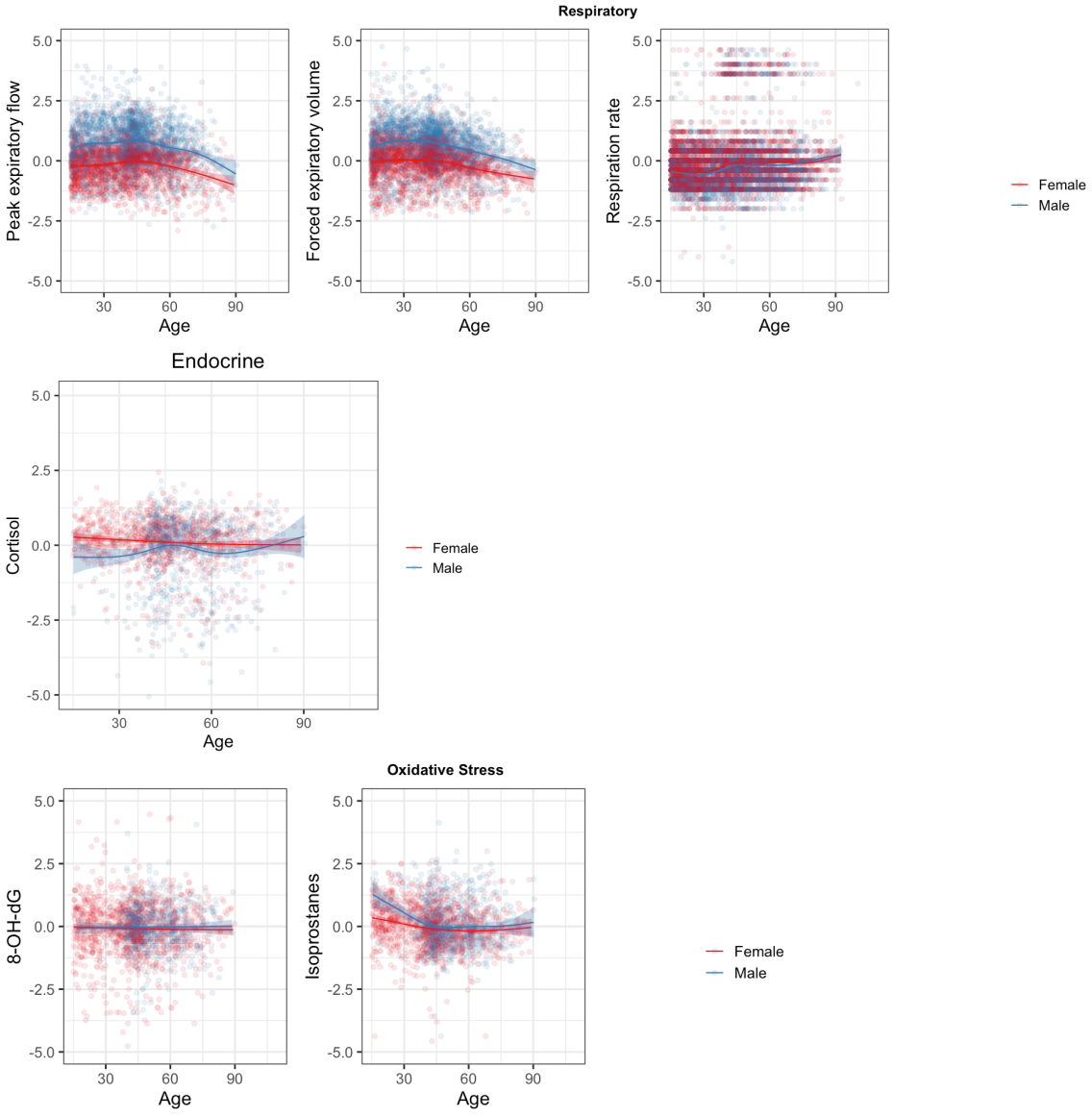
Isoprostanes	ng/specific gravity	natural log	Unpublished	2.1	1.7
Other (Endocrine)					
Cortisol	pg/mL (specific gravity corrected)	natural log	[41]	244,651	191,399
Other (Respiratory)					
Respiratory rate	breaths/min	none	Unpublished	20.2	4.4
Forced expiratory volume	L	square root	Unpublished	2.1	0.9
Peak expiratory flow	L/min	square root	Unpublished	318.9	131.4
Musculoskeletal					
Hand strength	kg	none	[42]	27.6	9.3
Radial speed of sound	m/s	none	[43]	3873	130
Tibial speed of sound	m/s	none	[43]	3798	130
Vertebral bone mineral density	g/cm^2	none	[44]	165.3	41.5
Calcaneal broadband ultrasound attenuation	dB/MHz	none	[45]	64.7	14.0
Calcaneal speed of sound	m/s	none	[45]	1527.1	24.6
Other					
Body temperature	°C	none	Unpublished	36.7	0.5

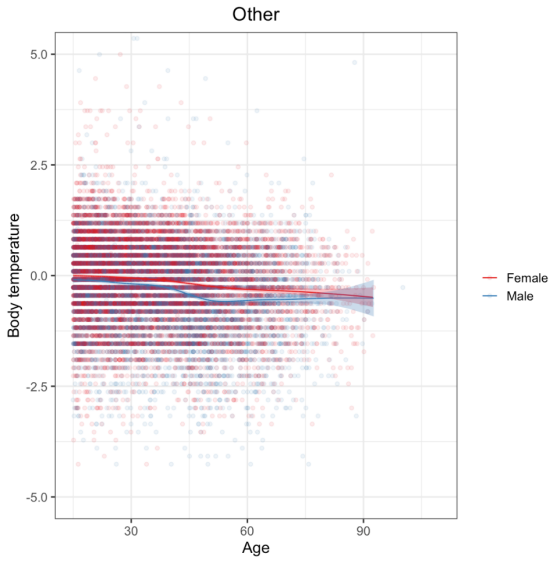
Figure S1. Age profiles of study biomarkers. Lines and shaded 95% confidence intervals represent splines for age from generalized additive mixed models with individual-level random intercepts. All variables are standardized to units of standard deviations.




Immune System
C-Reactive Protein
Eosinophils
Erythrocyte Sedimentation Rate
IgE
IgG
IL-2
IL-5
IL-6
IL-10
Leukocytes
Lymphocytes
Neutrophils
Tumor Necrosis Factor Alpha
Age
Female
Male


Musculoskeletal system
Grip strength
Radial SOS
Tibial SOS
Vertebral bone mineral density
Calcaneal BUA
Calcaneal SOS
Age
Female
Male


Respiratory
Peak expiratory flow
Forced expiratory volume
Respiration rate
Age
Female
Male
Endocrine
Cortisol
Oxidative Stress
8-OH-dG
Isoprostanes


Other
Body temperature
5.0
2.5
0.0
-2.5
-5.0
30
60
90
Age
Female
Male

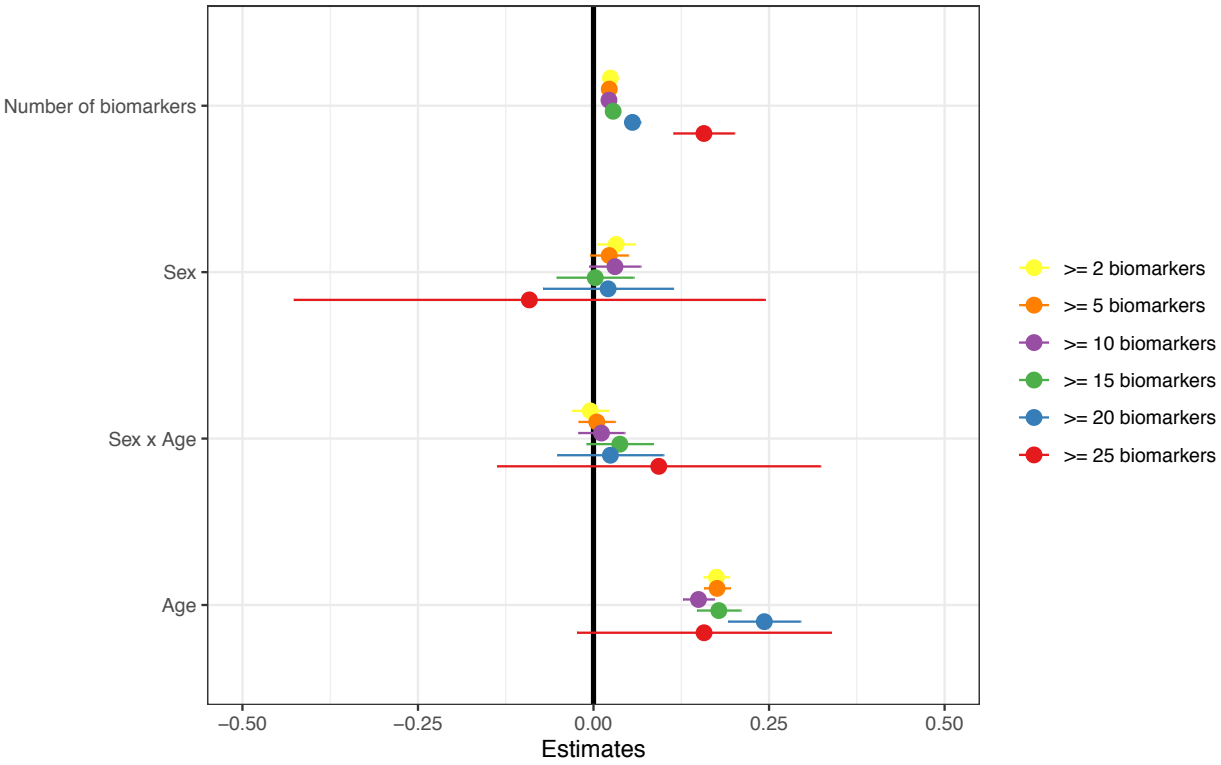


Figure S2: Forest plots (means ± 95% CIs) of models testing for robustness. Models were run on restricted data sets with different minimum thresholds for the number of biomarkers an observation required to be included in the analysis. Note that the >= 2 biomarker condition represents the model included in the main analysis.

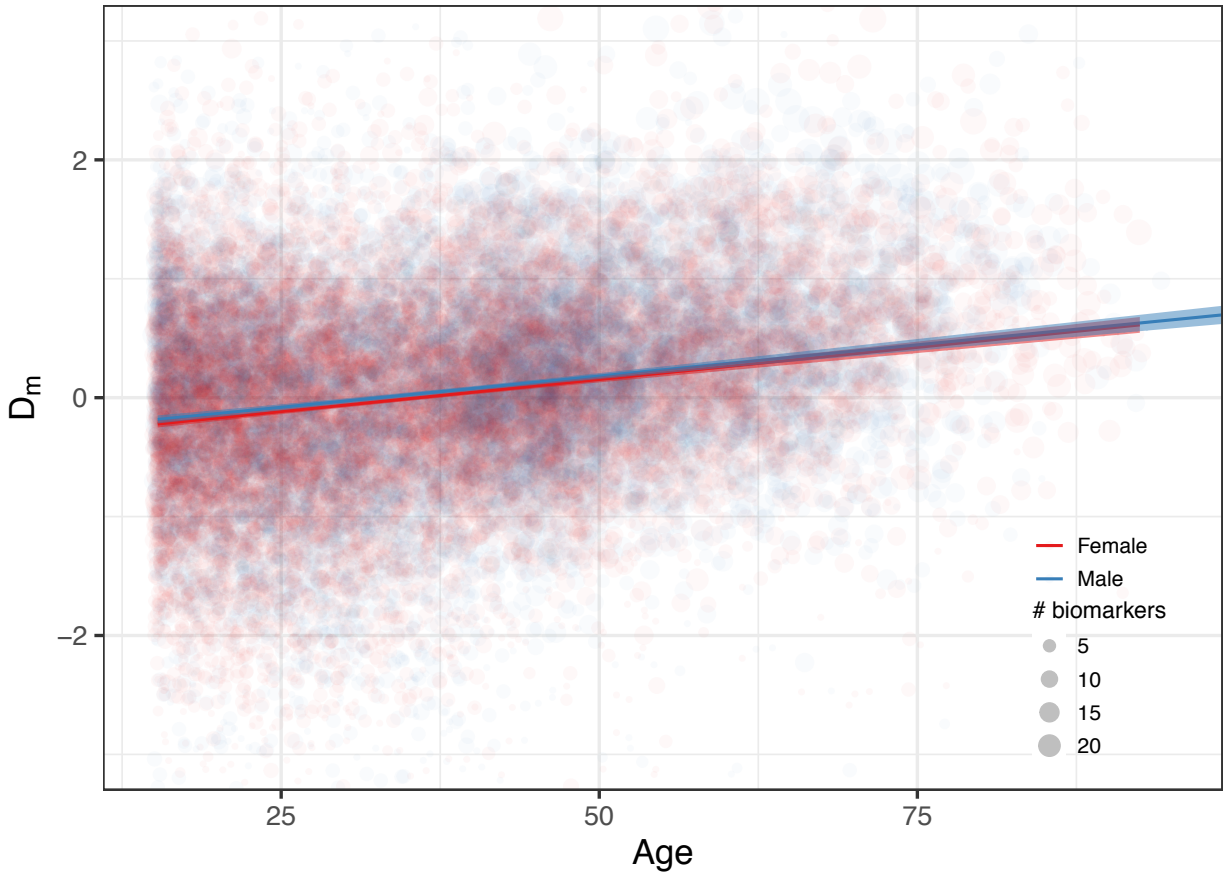


Figure S3: Physiological dysregulation (D_m) as a function of age (linear) and sex. Trend lines and confidence intervals are calculated from non-quadratic models in Table 2. Mahalanobis distance (D_m) on the y-axis was natural log-transformed and standardized (subtracted from the mean and divided by the standard deviation). Each point represents a "person-observation", and point size denotes the number of biomarkers measured. Points with only a single biomarker were excluded from the analysis. The range of the y-axis omits some points to improve view of the main trends.

Figures S4-S6: Correlation matrices of biomarkers and D_m by physiological systems. Numbers represent Pearson correlation coefficients. Correlations involving D_m are weighted by the total number of biomarkers measured at each observation. Question marks represent combinations of variables that were not available in tandem.

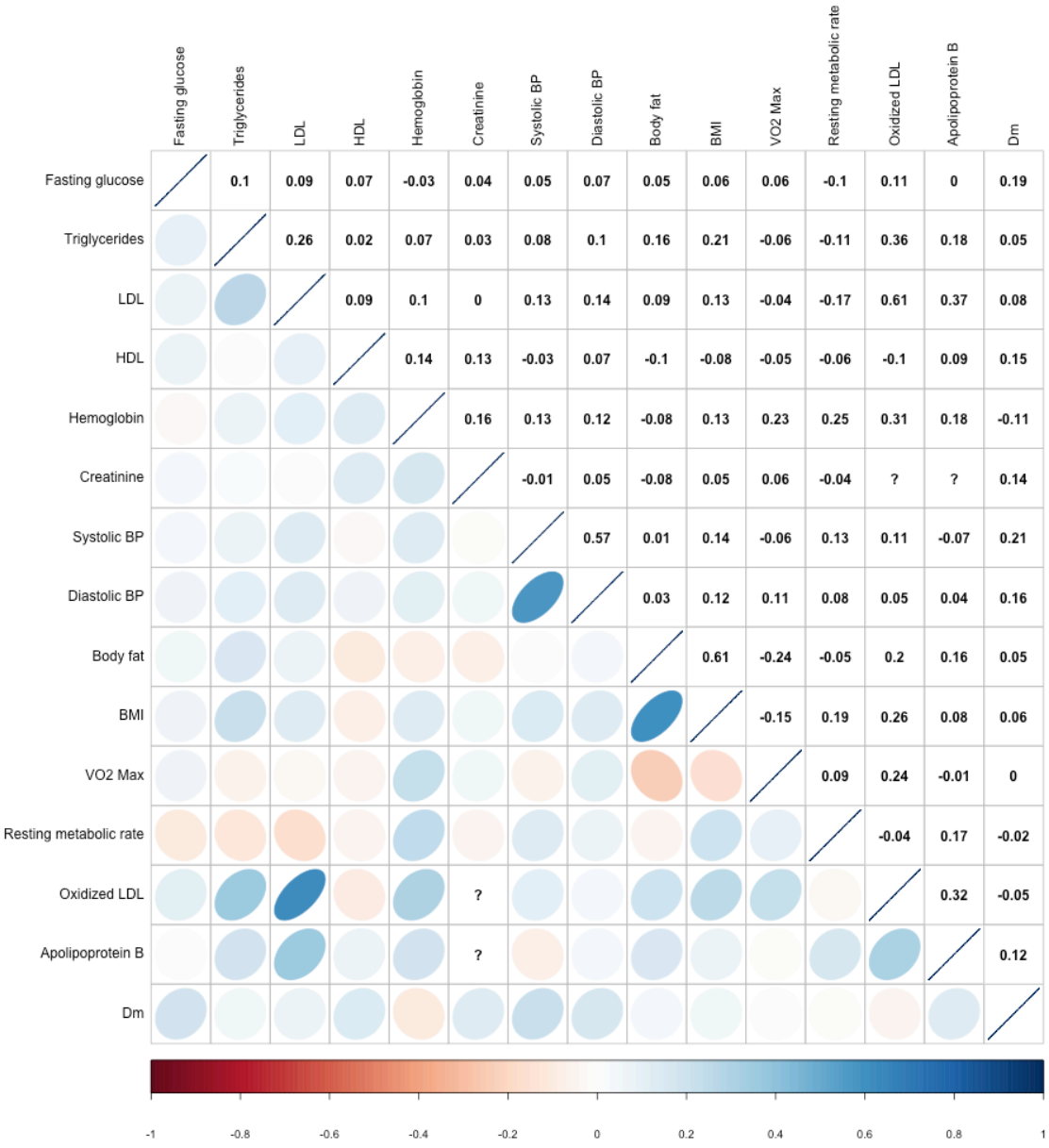


Figure S4: Cardiometabolic correlation plot, including D_m (last row/column).

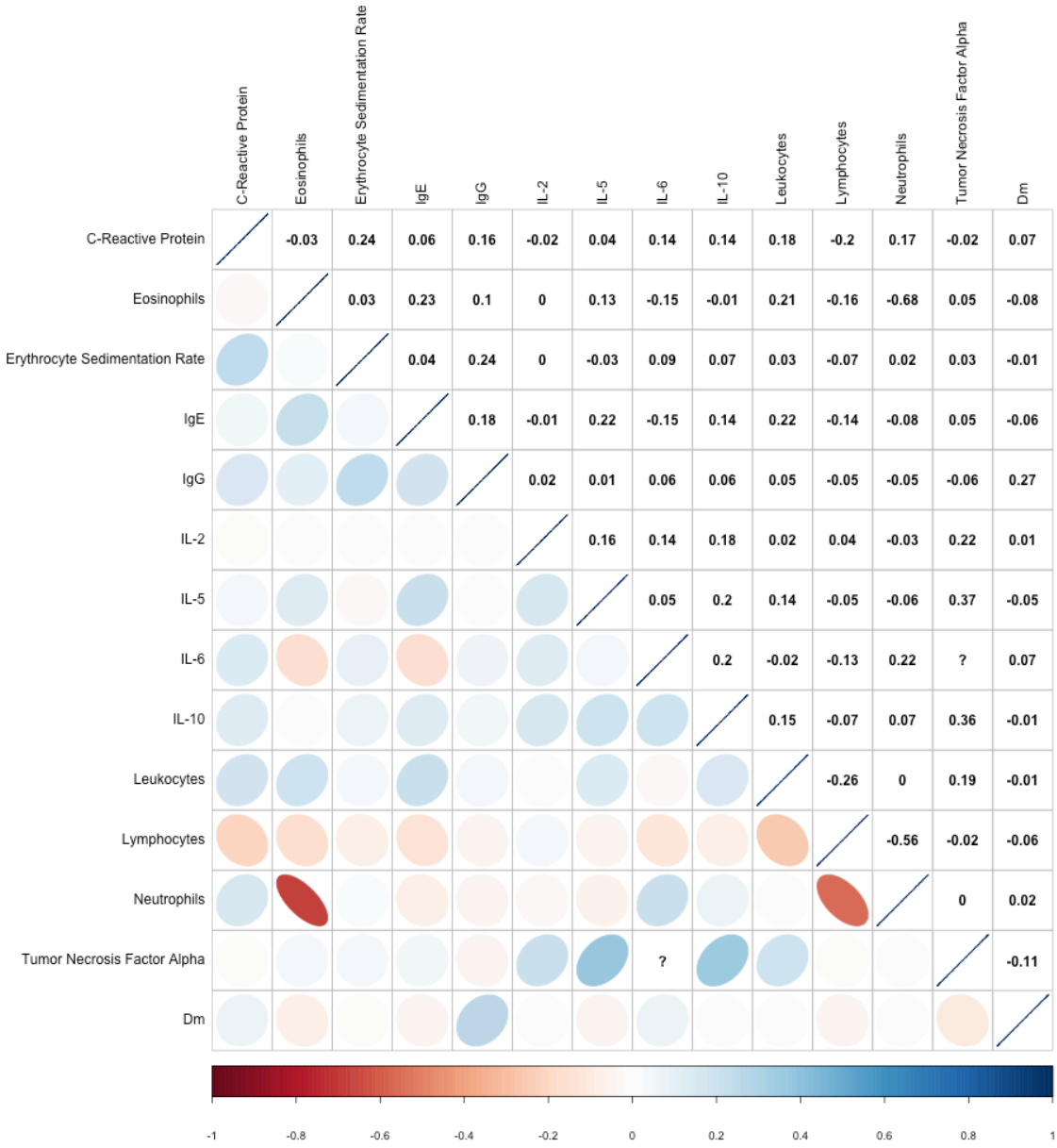


Figure S5: Immune correlation plot, including D_m (last row/column).

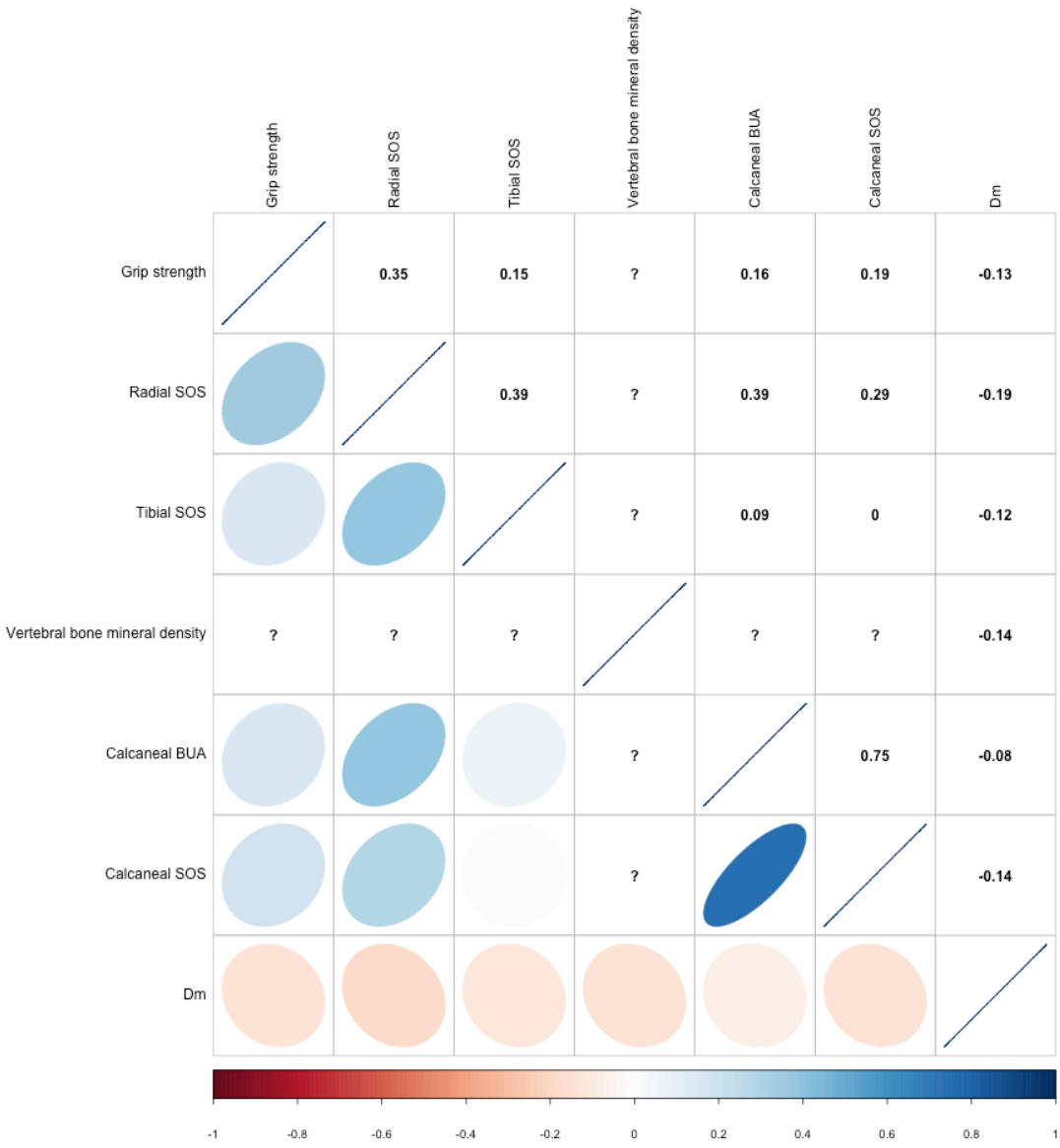


Figure S6: Musculoskeletal correlation plot, including D_m (last row/column).

Figure S7: Individual age trajectories of D_m as a function of age at first measurement. There was a very minor relationship between age at first measurement and D_m trajectory ($\beta_{\text{age of first measurement}} = 1\text{x}10^{-4}$, $p = 0.0004$, $\beta_{\text{sex x age of first measurement}} = 1\text{x}10^{-5}$, $p = 0.81$). Variability in age slopes is notably greater at later ages.