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Human plasma proteomic profiles indicative of cardiorespiratory fitness

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Maximal oxygen uptake (VO_2max) is a direct measure of human cardiorespiratory fitness and is associated with health. However, the molecular determinants of interindividual differences in baseline (intrinsic) VO_2max, and of increases of VO_2max in response to exercise training (ΔVO_2max), are largely unknown. Here, we measure ~5,000 plasma proteins using an affinity-based platform in over 650 sedentary adults before and after a 20-week endurance-exercise intervention and identify 147 proteins and 102 proteins whose plasma levels are associated with baseline VO_2max and ΔVO_2max, respectively. Addition of a protein biomarker score derived from these proteins to a score based on clinical traits improves the prediction of an individual's ΔVO_2max. We validate findings in a separate exercise cohort, further link 21 proteins to incident all-cause mortality in a community-based cohort and reproduce the specificity of ~75% of our key findings using antibody-based assays. Taken together, our data shed light on biological pathways relevant to cardiorespiratory fitness and highlight the potential additive value of protein biomarkers in identifying exercise responsiveness in humans.

Oxygen uptake (VO_2) represents a measure of the body's capacity to supply oxygen to skeletal muscle to perform physical work. VO_2 reflects the integration of multiple organ systems and cellular processes, including pulmonary ventilation, oxygen carrying capacity and transport through the circulatory system, cardiac output, central nervous system recruitment of motor units, oxygen diffusion and extraction at the capillary–skeletal muscle level, as well as mitochondrial respiration. VO_2max defines the limits of these processes and is thus widely considered the gold-standard measure of cardiorespiratory fitness (CRF)[1,2].

It is thus not surprising that VO_2max (as a direct measure of CRF) has been firmly established as a powerful prognostic marker of cardiovascular disease (CVD) and all-cause mortality[3]. VO_2max's inverse relationship with CVD and mortality risk applies to both its baseline measure (intrinsic VO_2max[4,5]) and capacity to improve VO_2max through regular physical activity (acquired or adaptive VO_2max; ΔVO_2max)[6,7]. Consequently, there has been significant interest in characterizing the relative contributions of different organ systems to VO_2max. Several lines of evidence point to cardiac output and oxygen delivery as being the principal determinants of VO_2max[8,9]; however, even the precise contributions of these processes, including oxygen diffusion, convection and mitochondrial oxidative capacity, are not fully resolved[10,11].

Furthermore, both baseline measures of VO_2max and ΔVO_2max appear to vary greatly in the general population. In the HERITAGE Family Study, a subgroup of 429 apparently healthy but sedentary members of family units, who were of European descent, underwent direct measurements of baseline VO_2max through cardiopulmonary exercise testing (CPET) on 2 separate days, and the s.d. (9 ml O_2 kg^{-1} min^{-1}) was ~29% of the mean (31 ml O_2 kg^{-1} min^{-1}) after adjustment for age, sex, body mass and body composition[12]. Similarly, among 720 HERITAGE participants who completed the supervised 20-week endurance-exercise training programme, the s.d. was 53% of the mean change in VO_2max. Interestingly, there was no relationship between baseline and ΔVO_2max in this group ($r^2 = 0.011$). This suggests that these traits may have different biologic underpinnings and underscores our inability to predict VO_2max 'trainability' using existing clinical factors[13].

Given our incomplete understanding of the biologic basis of CRF and its close relationship to long-term health outcomes, uncovering the molecular determinants of VO_2max may provide insights into the mechanistic links between physical fitness and well-being. Indeed, this has become an important goal of the medical community. Prior efforts to characterize both baseline and acquired CRF at the molecular level have included genetic analyses, transcriptomic profiling of skeletal muscle and plasma metabolomics[14–16]. Although biochemical profiling of plasma proteins has yielded insights into differences in substrate metabolism among different fitness states in animal models[17] and has provided biologic 'snapshots' of human metabolism[18], few data exist regarding plasma

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Table 1 | HERITAGE cohort clinical characteristics

Clinical characteristics	Participants with baseline V O_2max (n= **745**)	Participants with baseline and post-training V O_2max (n= **654**)
Age, mean (s.d.), years	34.3 (13.4)	34.8 (13.6)
Female, n (%)	409 (54.9)	361 (55.2)
European descent, n (%)	457 (61.3)	424 (64.8)
BMI, median (interquartile range), kg/m^2	25.5 (22.4–29.7)	25.5 (22.5–29.7)
Maximal oxygen uptake, mean (s.d.), ml min^{-1}		
Baseline	2,345 (726)	2,348 (732.5)
Change after exercise training	–	383 (202.8)
SBP, mean (s.d.), mmHg	119 (12.0)	119 (11.8)
DBP, mean (s.d.), mmHg	69 (8.9)	68 (8.8)
Resting heart rate, mean (s.d.)	65 (8.9)	65 (8.9)

Mean (s.d.) and median (25–75%) values are shown.

proteomics profiling of CRF in humans, particularly in the context of exercise training. These limitations are in part due to the technical challenges involved in capturing the highly dynamic range of circulating proteins. Advancements in aptamer-based profiling methods now allow for the high-throughput measurement of over 5,000 proteins[19]. This technology spans a dynamic range of at least 7 orders of magnitude (~100 fM–1 μM) with demonstrated high assay reproducibility across both hospital- and population-based cohorts[20,21], and was recently applied in the HERITAGE study[22].

Here, we sought to compare the circulating proteomic profiles of baseline VO_2max as well as its adaptation to an exercise programme by applying a large-scale, affinity-based platform in more than 650 healthy but sedentary participants before and after a 20-week supervised endurance-exercise training intervention. We hypothesized that plasma protein signatures associated with VO_2max would reflect its integrative biology and highlight proteins related to skeletal muscle, hematopoiesis and the vascular system, among other determinants of CRF. Further, given that clinical traits are weakly correlated with VO_2max changes following exercise training, we anticipated that the addition of plasma proteins would improve the capacity to predict VO_2max responsiveness. Finally, given that both baseline VO_2max as well its capacity to change in response to exercise training are associated with future risk of death, we tested whether plasma proteins related to these measures would be associated with incident all-cause mortality in a separate population-based study.

Results

HERITAGE participant characteristics. The HERITAGE cohort was composed of adult parents and their biologic offspring. The mean (s.d.) age of the full cohort (n = 745) used for baseline VO_2max analyses was 34.3 (13.4) years; 288 were African American (39%), 409 were women (55%) and 503 were offspring (68%). Mean (s.d.) baseline VO_2max was 2,345 (726) ml min^{-1}. Among the participants with VO2max measurements before and after exercise training (n = 654), the mean Δ VO_2max was 383 (203) ml O_2 min^{-1} (Table 1).

Plasma proteins associated with baseline levels of VO_2max. We measured ~5,000 proteins using a multiplexed, single-stranded DNA aptamer (SOMAmers) assay (Supplementary Table 1). We first tested for age- and sex-adjusted protein associations with baseline VO_2max in the offspring generation (n = 503) and then sought to replicate our findings in the parent generation (n = 242). We identified 94 proteins that were associated with VO_2max in the offspring by using a false-discovery rate (FDR) threshold of <1%. Fifty of 94 proteins were associated with VO_2max in the parents at nominal significance (P < 0.05) and 90/94 were directionally consistent (Fig. 1). We subsequently collapsed these subgroups for all further analyses.

In the full cohort, we identified 147 circulating proteins that were associated with baseline VO_2max (Fig. 2), including 85 proteins that were positively associated and 62 proteins negatively associated in analyses that were adjusted for age, sex, body mass index (BMI) and race (Supplementary Table 2). Proteins positively associated with baseline VO_2max spanned organ systems and biologic processes relevant to CRF including angiogenesis (for example extracellular matrix protein 1 (ECM1) and anthrax toxin receptor 2 (ANTXR2)), coagulation and hematopoiesis (for example, complement decay-accelerating factor (DAF) and tetranectin (TN)) and lipid metabolism (for example apolipoprotein F (APOF) and lipase member K (LIPK)). Interestingly, we found a large number of circulating proteins related to striated muscle structure and function (Fig. 3 and Supplementary Table 3). These included actin and myosin stabilizing molecules (for example, alpha-actinin 2 (ACTN2) and myomesin-2 (MYOM2)); proteins involved in muscle contraction (for example, troponin-I (TNNI2) and myosin-binding protein C (MYBPC1)); and two essential myosin light-chain elements (MYL3 and MYL6B) that regulate force production during muscular cross-bridge cycles. We also identified several muscle-isoform-specific enzymes involved in glycolysis in plasma, including beta-enolase (ENOB), ALDOA, phosphoglycerate mutase 1 (PGAM1) and 2 (PGAM2) and lactate dehydrogenase alpha (LDHA) and beta (LDHB).

These baseline cross-sectional analyses also identified several well-known markers of metabolic dysregulation known to be positively associated with adiposity, including leptin, CRP and insulin, which were inversely associated with baseline VO_2max. Thus, we adjusted for additional measures of body composition—body fat percentage and fat-free mass—to further examine the role of adiposity in our results. We found that the relationships between these proteins and VO_2max were no longer significant after adjustment for body fat percentage but remained significant after adjustment for fat-free mass (Supplementary Table 4). In contrast to these markers of metabolic dysregulation, the striated muscle proteins described above (and in Supplementary Table 3) maintained their correlation with baseline VO_2max after adjustment for body fat percentage but not fat-free mass, suggesting that their association with CRF may proceed through their relationship to lean body mass.

Among the 85 proteins positively associated with baseline VO_2max following multivariate adjustment, 25 were known to be secreted based on UniProt Consortium data (Supplementary Table 2). The group of secreted proteins included multiple proteins related to bone homeostasis, including members of osteoblast differentiation (SPARC-related modular calcium binding protein 1 (SMOC1)), bone metabolism via TGF-ß signalling (NOG, bone morphogenic protein 8B (BMP8B)) and structural components of hyaline cartilage (COL9A1, COMP, EPYC; Extended Data Fig. 1).

Test results for the interaction of generation, sex and race on protein–VO_2max relationships are shown in Supplementary Table 5. Although we identified 23 protein–generation interactions at nominal significance (P value < 0.05; highlighted in Supplementary Table 2), all were directionally consistent among parents and offspring. Similarly, all 20 protein X sex interactions were directionally consistent among males and females. Only Tartrate-resistant

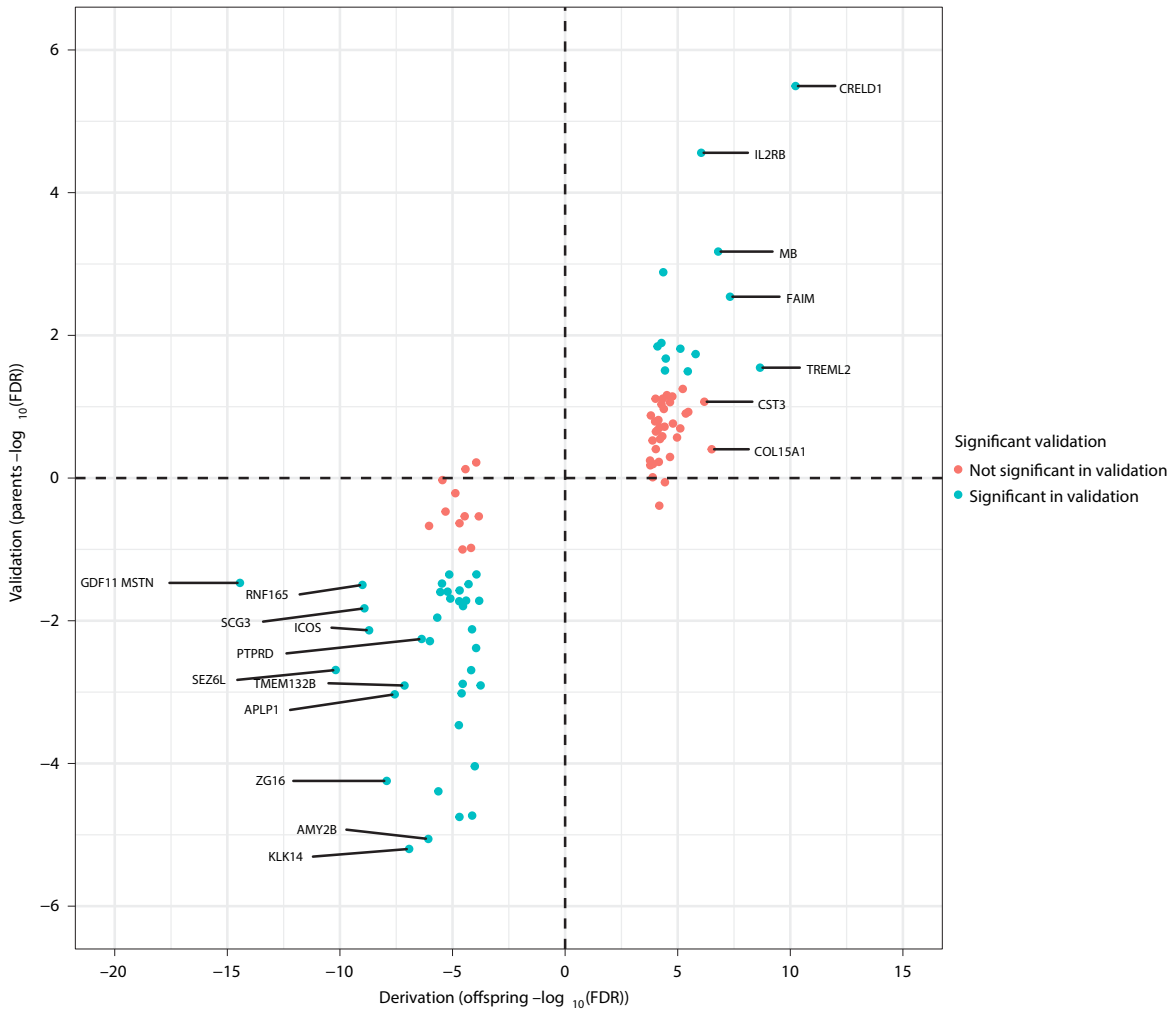


Fig. 1 | Proteins associated with baseline V O_2max among offspring and parents. Protein associations (FDR < 1%) using linear regression were determined first in the offspring cohort (n = 433). We subsequently validated 50/94 proteins in the cohort of parents (n = 221; P < 0.05). Ninety of 94 proteins were directionally consistent, indicated by quadrant (increase in parents and offspring, upper right; decrease in parents and offspring, lower left).

acid phosphatase type 5 (ACP5) and Neural cell adhesion molecule L1-like protein (NRCAM) were directionally different among the 29 protein-VO_2max associations that were different between racial groups, with both ACP5 and NRCAM having a positive association with VO_2max among African Americans and negative association among Caucasians (ACP5, β = 2.5 and −93.6, respectively; *P* for interaction = 0.003; NRCAM, β = 21.1 and −10.6, respectively; *P* for interaction = 0.02). All data have been made available and are available through the NIH Common Fund Molecular Transducers of Physical Activity Consortium (MoTrPAC; https://motrpac-data.org/related-studies/heritage-proteomics).

Validation of baseline VO_2max findings in an external cohort. To further assess the generalizability of our findings, we performed a similar proteomics screen in a separate cohort of abdominally obese individuals who were enroled in a dose–response trial of endurance exercise[23]. Participants in the validation study subgroup were older (mean age = 47) and had larger body mass (median BMI = 32.8) than HERITAGE participants. A higher percentage of the validation study subgroup was female (71%), and all participants were of European descent (Supplementary Table 6). Of the top 147 proteins associated with baseline VO_2max in HERITAGE, 107 were available in the validation dataset. Seventy-nine proteins were directionally consistent, and 24 met statistical significance in the validation cohort in a linear regression model adjusted for age, sex and BMI (*P* < 0.05; Supplementary Table 7).

Proteins associated with VO_2max changes to exercise training. We found 102 baseline proteins that were associated with ΔVO_2max in a linear regression model adjusted for age, sex, BMI, race and the baseline level of VO_2max (Supplementary Table 8). The proteins with the strongest associations with ΔVO_2max included: 5 nucleotidase (NT5E), a cell-surface protein that hydrolyses extracellular nucleotides into membrane permeable nucleosides and in which cognate gene variants have been associated with premature arterial calcification[24]; IL-22 binding protein (IL22RA2), a soluble receptor whose ligand is involved in insulin and glucose homeostasis[25]; and fibromodulin (FMOD), a secreted protein that has been implicated in tissue repair and myogenic regulation through its interaction with myostatin[26].

A generation–protein interaction on ΔVO_2max was found for four proteins, with hepcidin (LEAP1) having directionally different associations among parents and offspring (Supplementary Table 9). Eleven proteins demonstrated a sex–protein interaction, with β-1,3-galactosyltransferase (B3GALT1) and triggering receptor expressed on myeloid cells 1 (TREM1) having directionally different associations among males and females. Among the 18 proteins that demonstrated a race–protein interaction on ΔVO_2max,

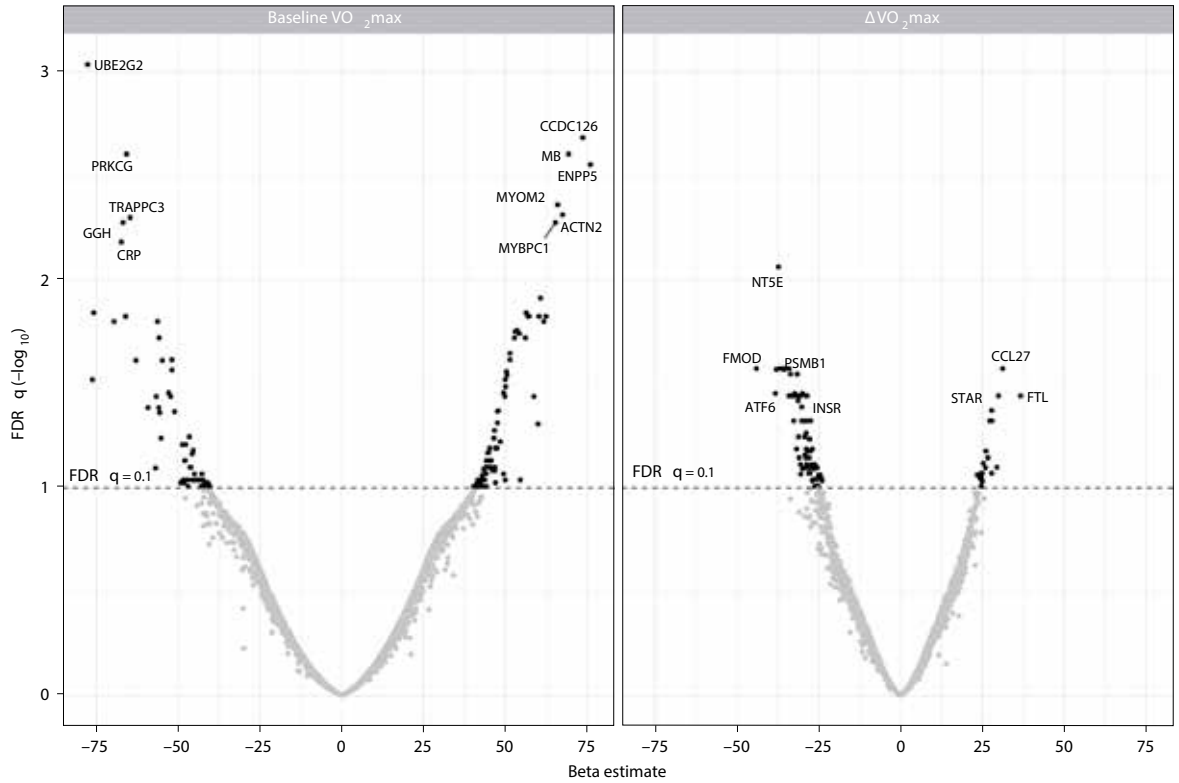
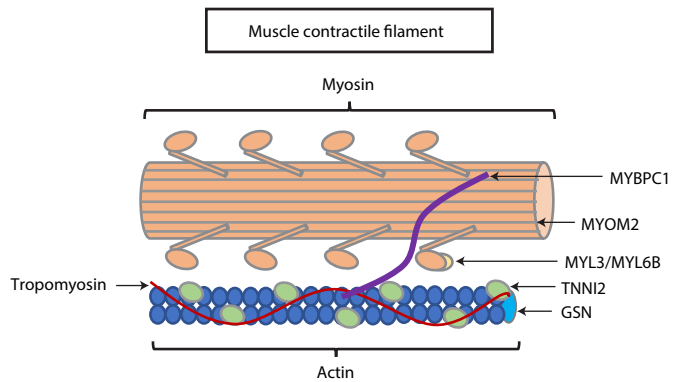


Fig. 2 | Plasma proteins associated with baseline and ΔVO$_2$max. Protein relationships to baseline VO$_2$max (ml O$_2$ min^{-1}) in a linear regression model adjusted for age, sex, BMI and race. The value of leptin's relationship with baseline VO$_2$max extends beyond the scale.



Gene symbol	Protein name
TNNI2	Troponin I, skeletal, fast twitch
MYOM2	Myomesin-2
MYBPC1	Myosin-binding protein C, slow type
MYL3	Myosin light chain 3
MYL6B	Myosin light chain 6B
ACTN2	Alpha-actinin 2
GSN	Gelsolin

Fig. 3 | Muscle proteins positively associated with baseline V O$_2$max. Left, muscle filament depiction highlighting the proteins positively associated with baseline VO$_2$max that participate in striated muscle structure and/or function. Myosin-binding protein slow-skeletal isoform (MYBPC1) regulates myosin–actin cross-bridge formation. Troponin I (TNNI2) inhibits actin-activated myosin ATPase activity. Gelsolin (GSN) is an actin severing protein. Myosin light-chain elements (MYL3 and MYL6B) regulate mechano-enzymatic function of myosin. Alpha-actinin 2 (ACTN2; not shown) and myomesin-2 (MYOM2) are actin and myosin stabilizing proteins. Right, table of names of proteins depicted at left and their associated gene symbols.

6 demonstrated directionally different associations among African Americans and those of European descent: C–C motif chemokine 27 precursor (CCL27), retinal rod rhodopsin-sensitive cGMP 3 ,5 -cyclic phosphodiesterase subunit delta (PDE6D), phosphatidylinositol polyphosphate 5-phosphatase type IV (INP5E), plexin-A1 (PLXA1), pleiotropin (PTN), and EGF-like repeat and discoidin I-like domain-containing protein 3 (EDIL3).

We next performed gene set enrichment analysis (GSEA) to further elucidate biochemical pathways among this set of proteins, as well as those previously identified in the baseline VO$_2$max analyses (Supplementary Tables 10 and 11, respectively). Proteins negatively associated with ΔVO$_2$max were most enriched for ECM-related proteins (the 'matrisome')[27] (Fig. 4a,b). Positively associated proteins, however, were enriched for core signalling pathways that include platelet-derived growth factor receptor, neurotrophin and hepatocyte growth factor pathway signalling, among others (Fig. 4a,c,d). These biochemical pathways contrast with those enriched after GSEA was applied to proteins ranked by their association with baseline VO$_2$max (Fig. 4e).

We also compared the group of proteins associated with baseline VO$_2$max with those associated with adaptive VO$_2$max changes to exercise training and found minimal overlap between the two

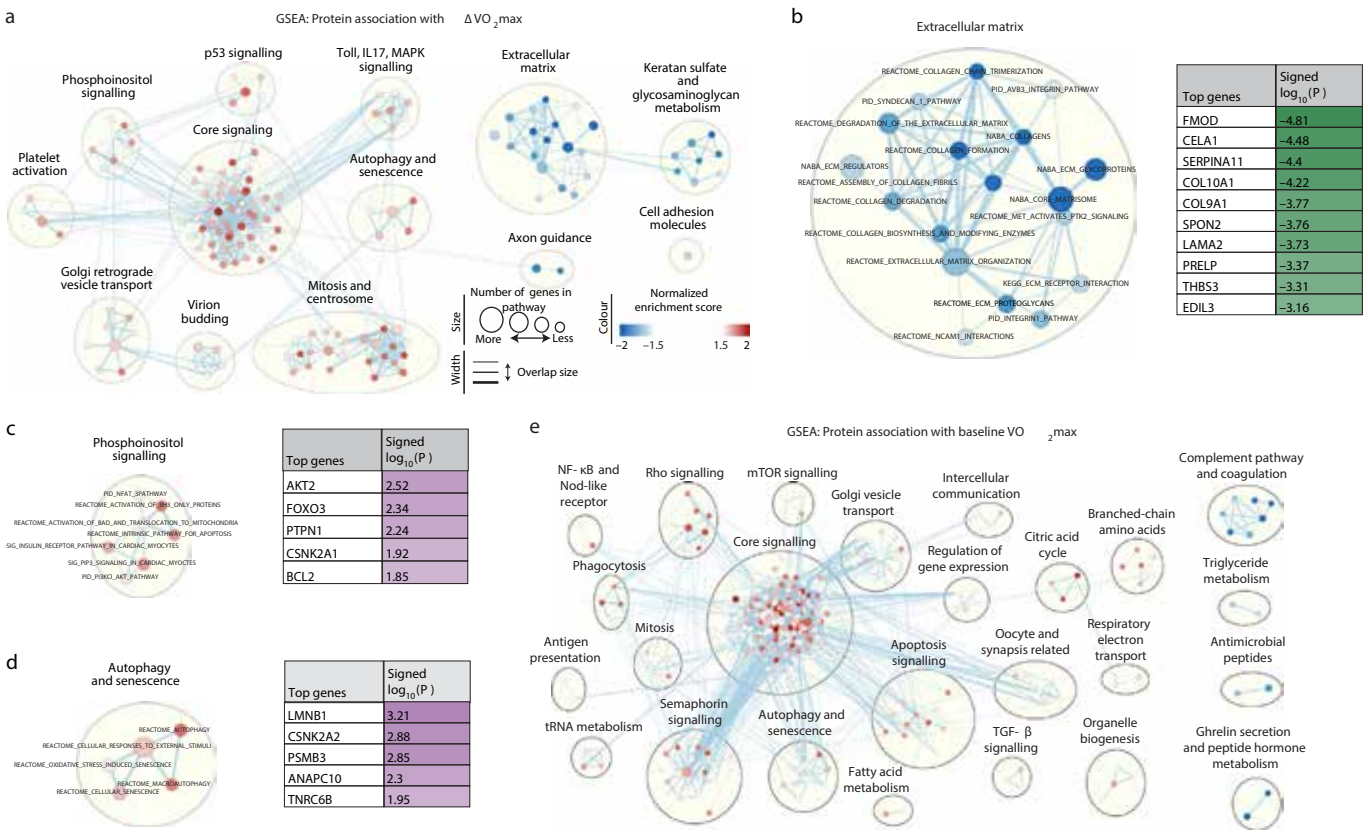


Fig. 4 | GSEA for proteins associated with ΔVO$_2$max or baseline VO$_2$max. Overview of overrepresented biological pathways and their connectivity using Cytoscape v3.7.1. a, Network visualization of GSEA results using the complete dataset of protein–ΔVO$_2$max associations. Red dots indicate pathways with over-represented positive protein-ΔVO$_2$max associations, and blue dots indicate over-represented negative protein-ΔVO$_2$max associations. A larger circle size denotes a larger number of genes in a pathway, and darker shades indicate a higher degree of enrichment. Clusters indicate biological pathways with shared proteins and biological function.b–d, Selected clusters of biological pathways with annotation, froma; the top contributing proteins to enrichment score are shown in a table. e, Network visualization of GSEA results using the complete dataset of protein–baseline VO$_2$max associations.

groups. Only five proteins, T132B, ATF6A, COL9A1, INS and PIANP, were associated with both baseline VO$_2$max and ΔVO$_2$max.

Plasma proteins improve prediction of ΔVO$_2$max responses. Given the vast heterogeneity in VO$_2$max changes that occur with exercise training, as described above, and that clinical factors account for a limited amount of the variance in VO$_2$max trainability[15], we sought to determine whether baseline plasma proteins could improve our ability to predict VO$_2$max changes in response to exercise training. Because baseline VO$_2$max and VO$_2$max changes with exercise training are minimally correlated, we tested to see whether proteins could help predict VO$_2$max changes relative to one's baseline VO$_2$max level (ΔVO$_2$max/baseline VO$_2$max). We selected a relative VO$_2$max change threshold of 15%, given that the median value among the cohort was ~16% (4.9 ml O$_2$ kg^{-1} min^{-1}) and a 15% change represented > 1 metabolic equivalent (1 MET), a clinically meaningful unit that has been related to >10% relative risk reduction in CVD and all-cause mortality in a series of longitudinal cohorts[3].

We first performed receiver–operating characteristic (ROC) analyses using a clinical trait model that included age, sex, race and BMI for relative VO$_2$max changes > 15%. The area under of the curve (AUC) was 0.62 (P = 0.91) (Fig. 5). Feature selection and elastic net regression modelling of the 5,000 proteins yielded a final panel of 56 proteins (Supplementary Table 12). We next added our protein panel to the clinical trait model, and the AUC significantly increased to 0.81 (P = 0.00018). With regard to the operator characteristics, we found 79% sensitivity, 71% specificity, positive predictive value of 66% and negative predictive value of 83% for relative VO$_2$max changes > 15%. In a subsequent model that included the same clinical traits but only the group of proteins that both overlapped with an antibody-based proteomics platform (see 'Complementary data to support aptamer specificity') and demonstrated moderate to strong correlation between both platforms (7/10 proteins; SELE, TCL1A, COMP, CREG1, STC1, IL1RL2, LILRA2; ρ = 0.41–0.91), the operator characteristics were similar but performed slightly worse (AUC = 0.75, Extended Data Fig. 2), suggesting that there is added information provided by the remaining protein targets in our main model.

Association of VO$_2$max-related proteins and mortality. We previously performed proteomics profiling in the Framingham Heart Study (FHS) Offspring Study using first a 1.1 k-plex (n = 821 participants) and then an updated 1.3 k-plex version (n = 1,092) of the aptamer-based proteomics platform used in HERITAGE[28,29]. The clinical characteristics of the FHS sample are presented in Supplementary Table 13. Among the 102 proteins that were associated with ΔVO$_2$max in HERITAGE, 20 were available in both batches of FHS. Thirty-six out of the 147 proteins associated with baseline VO$_2$max were available in the FHS.

Of 1,909 FHS participants, 551 died after a mean (s.d.) follow-up of 13.6 (5.6) years. In age- and sex-adjusted analyses, 12 out of 36 proteins associated with baseline VO$_2$max and 9 out of 20 proteins associated with ΔVO$_2$max were also associated with incident

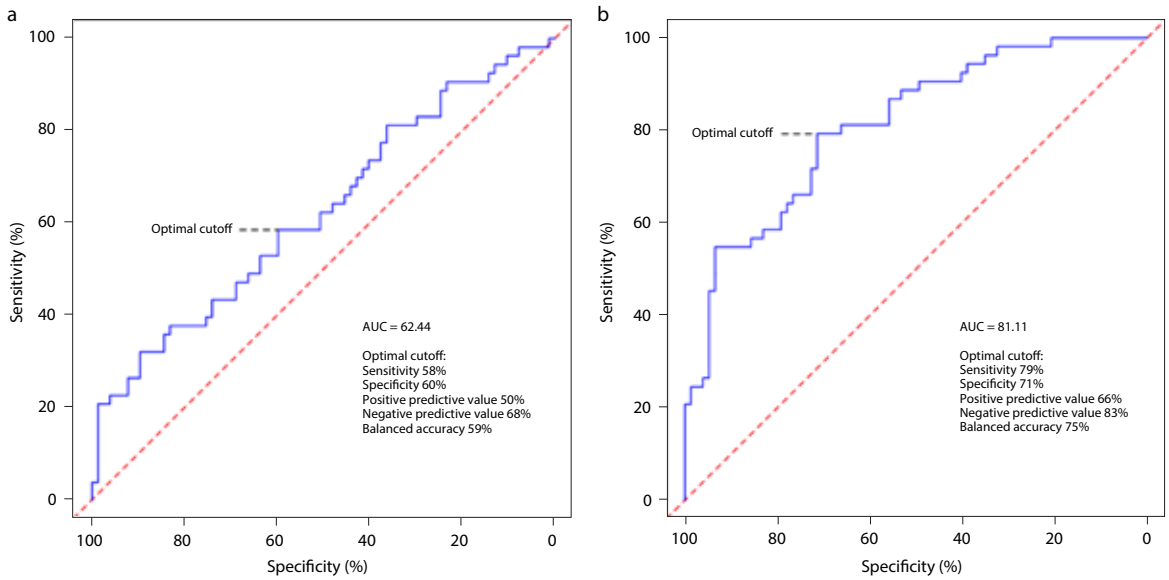


Fig. 5 | ROC curves for relative V O_2max changes with exercise training > 15%. a, The clinical trait score (age, sex, BMI and race) had a modest AUC. b, Addition of the protein score significantly improved the AUC. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy at the optimal cutoff are included.

all-cause mortality (FDR $q < 0.1$; Table 2). We next performed stepwise regression using these protein sets (12 and 9 proteins, respectively) to estimate the percentage variation in all-cause mortality explained by each protein beyond age, sex and batch. Among the proteins associated with baseline VO_2max, gelsolin (GSN) was the most significantly associated with all-cause mortality (hazard ratio (HR), 0.71; FDR $q = 9.1 \times 10^{-13}$) and explained 3.4% of the variation beyond age and sex. Among proteins associated with ΔVO_2max, macrophage metalloelastase (MMP12) was the most significantly associated with all-cause mortality (HR, 1.34; FDR $q = 1.2 \times 10^{-7}$), explaining 1.8% of the variation in outcome.

Complementary data to support aptamer specificity. We tested the reproducibility of our top aptamer-based findings in HERITAGE specific samples using Olink's antibody-based proteomics platform (Olink Explore). Clinical characteristics of the random sample from HERITAGE are shown in Supplementary Table 14. Among the 21 proteins significantly associated with incident all-cause mortality, 12 protein targets were available on both platforms. Nine out of 12 of the protein targets were highly correlated. In addition, among the top protein targets associated with either baseline or ΔVO_2max that did not overlap with our all-cause mortality findings (Supplementary Table 15 and Tables 2 and 3 in Supplementary Data), an additional 13 proteins were available on both platforms. Ten of 13 assays demonstrated strong correlations. Taken together, 19 out of 25 of our top aptamer-based protein findings from HERITAGE were well correlated with an equivalent antibody-based assay (both sets of protein correlations shown in Fig. 6).

In addition, we leveraged mass spectrometry (MS)-based and genetic assays to support the specificity of the aptamer assays for our most significant findings. Among the 21 proteins significantly associated with incident all-cause mortality, genome-wide significant associations at *cis* loci (within 1 Mb of the transcription start site for the cognate gene of the protein) were identified for 17, consistent with the specificity of the aptamer–protein relationship. Aptamer specificity for two additional proteins (B2M and MB) was confirmed by MS[30]. Among the top 25 findings in both our baseline VO_2max and ΔVO_2max analyses, 23 and 24 were available for testing across genetic and MS-based analyses, respectively. The specificity of 11/23 proteins associated with baseline VO_2max and 12/24 proteins associated with ΔVO_2max was supported by these tests (Supplementary Table 15).

Discussion

VO_2max—as a direct measure of CRF—reflects the body's ability to transfer oxygen to skeletal muscle during sustained physical activity, and is thus a quantifiable measure of functional capacity. It has emerged as an important prognostic marker of future health risk that adds value beyond traditional risk factors[3]. While both baseline VO_2max and the adaptive changes in VO_2max in response to regular exercise provide valuable information about health status, these traits are largely unrelated to each other, a fact that underscores our limited understanding of their biologic basis and links to long-term health outcomes. Here, we performed large-scale plasma proteomic profiling in over 650 individuals with directly measured VO_2max before and after an endurance-exercise intervention to illuminate the biochemical features of baseline CRF and its adaptation to regular exercise. These analyses produced four notable findings. First, there was a broad and diverse set of circulating proteins associated with both baseline VO_2max levels and its changes in response to exercise training. Second, there was minimal overlap between the proteomic profiles of these distinct clinical traits. Third, the addition of a plasma protein score to baseline clinical traits improved the predictive accuracy of clinically significant improvements in VO_2max to exercise training. Finally, key proteins that are correlated with baseline VO_2max or ΔVO_2max were also associated with incident all-cause mortality in a separate population-based cohort.

Proteins are important regulators of biologic processes and, like CRF, reflect an individual's current health state as well as future risks[22]. The plasma proteome encompasses proteins from all tissues, making it an attractive medium to study the integrative biology of CRF. Indeed, we identified circulating proteins that spanned many of the organ systems involved in determining VO_2max, including the nervous, musculoskeletal, pulmonary, haematologic and circulatory systems. These included tissue-specific, structural and functional proteins (for example, striated muscle, Fig. 3) and proteins with signal peptide sequences (for example, secreted proteins;

Table 2 | Proteins associated with baseline or ΔVO$_2$max in HERITAGE and all-cause mortality in the FHS Offspring Study

Gene name	Protein name	Adjusted HR	95% CI		FDR q value	Variation explained by protein (%)
	Baseline VO$_2$max					
GSN[a]	Gelsolin	0.71	0.65	0.78	9.1×10^{-13}	3.00
CRP[a]	C-reactive protein	1.24	1.13	1.36	8.2×10^{-5}	
B2M[a]	β2-microglobulin	1.21	1.09	1.33	1.6×10^{-3}	1.00
ECM1[a]	Extracellular matrix protein 1	0.84	0.77	0.93	2.9×10^{-3}	
MB[a–c]	Myoglobin	0.87	0.79	0.96	1.7×10^{-2}	0.22
FCGR3B[a–c]	Low-affinity immunoglobulin gamma Fc region receptor III-B	1.13	1.04	1.23	1.7×10^{-2}	
ACP5[a–c]	Tartrate-resistant acid phosphatase type 5	1.14	1.03	1.27	3.1×10^{-2}	0.17
PLG[a]	Plasminogen	0.90	0.82	0.98	4.4×10^{-2}	0.45
NRCAM[a,b]	Neuronal cell adhesion molecule	0.90	0.83	0.98	4.6×10^{-2}	–
CFB[a]	Complement factor B	1.11	1.01	1.22	5.4×10^{-2}	–
ENPP7[a–c]	Ectonucleotide pyrophosphatase/phosphodiesterase family member 7	1.11	1.01	1.21	5.4×10^{-2}	–
NRXN3[a]	Neurexin-3-β	0.90	0.83	0.99	5.4×10^{-2}	–
ΔVO$_2$max						
MMP12[a–c]	Macrophage metalloelastase	1.34	1.22	1.48	1.2×10^{-7}	1.80
FAP[a–c]	Prolyl endopeptidase FAP	0.78	0.72	0.85	3.8×10^{-7}	
ANGPT2[a–c]	Angiopoietin-2	1.21	1.10	1.33	6.7×10^{-4}	0.47
STC1[a–c]	Stanniocalcin-1	1.19	1.09	1.30	1.8×10^{-3}	0.74
CCL27[a,b]	C–C motif chemokine 27	1.16	1.06	1.28	7.3×10^{-3}	–
IL11RA[a]	Interleukin-11 receptor subunit α	0.86	0.79	0.94	7.3×10^{-3}	0.54
ERBB3[a,b]	Receptor tyrosine-protein kinase erbB-3	0.86	0.78	0.94	8.3×10^{-3}	0.21
ACAN[a,c]	Aggrecan	0.87	0.80	0.96	1.7×10^{-2}	–
IMDH2	Inosine-5 -monophosphate dehydrogenase	1.12	1.03	1.23	3.3×10^{-2}	–

Cox proportional hazards analysis was performed for both the proteins associated with baseline VO$_2$max and those associated with VO$_2$max and all-cause mortality, adjusting for age, sex and batch. Proteins from each analysis that were statistically significant (FDR q< 0.1) were brought forwards in stepwise regression. The percent variation in all-cause mortality beyond age and sex is listed in the final column for those proteins retained in the final model.[a]Aptamer specificity supported by pQTLs and/or MS-based proteomics in population-based data (Supplementary Table 15). [b]Aptamer targets available for comparison on Olink Explore platform in HERITAGE subset (n= 88). [c]Proteins with Spearman correlation > 0.4 on aptamer and antibody-based platforms in HERITAGE subset.

Supplementary Table 2), as well as several proteins of uncertain function or not predicted to be secreted. Although these latter proteins may reflect tissue leakage or aberrantly secreted proteins, recent evidence suggests that traditional annotation methods may not fully account for proteins released into circulation via extracellular vesicles[31]. Indeed, our finding that a number of glycolytic enzymes, including fructose bisphosphate aldolase A (ALDOA), β-enolase 3 (ENO3) and lactate dehydrogenase (LDHB and LDHA), were present in the blood are consistent with those from Whitham et al.[31], who demonstrated a rise in plasma levels during acute bouts of exercise. The mechanistic relevance of these findings remains unknown, and additional research is needed to understand whether these enzymes have unanticipated functional effects in circulation or are biomarkers of physiologic states.

Among a group of classically secreted proteins, we identified several relevant to bone homeostasis that were positively associated with baseline VO$_2$max (Extended Data Figure 1). This group included BMP8B, an adipokine that regulates cartilage and bone development and has also been shown to induce brown-adipose-tissue thermogenesis[32] and adipocyte neurovascular remodelling[33], and SMOC1, a regulator of osteoblast differentiation relevant in physiologic cardiac hypertrophy[34]. We cannot localize the tissue origin of these circulating proteins, but our findings highlight the emerging paradigm of bone as an important endocrine organ involved in tissue crosstalk and exercise adaptation and motivate further interrogation of our data[35].

Few data describing the plasma proteomic profiles of baseline VO$_2$max exist[22,36], and to our knowledge this is the first study to investigate large-scale proteomic relationships with longitudinal VO$_2$max adaptations. Santos-Parker and colleagues[36] performed aptamer-based proteomics using a smaller-scale (1.1 *k*-plex) platform among a group of 47 sedentary or exercise-trained young men and women, and older men. The authors performed gene network and gene ontology (GO)-based annotation to identify biological processes associated with those in the exercise-trained state. More recently, Williams et al.[22] applied aptamer-based proteomic profiling in HERITAGE to generate a predictive model of cross-sectional VO$_2$max based on 115 proteins, using a training set that included 50% of samples from participants at baseline and 50% after completing exercise training.

While there was overlap among some of the broad biologic processes identified by Santos-Parker et al. (for example, autophagy and vasculogenesis) or individual proteins found by Williams et al. (~23% of our findings overlapped), our baseline VO$_2$max findings differed from these for several reasons. First, in contrast to these studies, our analyses were performed separately using only pretraining or post-training measures of VO$_2$max. Our baseline analyses did not include values obtained after the HERITAGE exercise intervention, which may reflect adaptive changes in VO$_2$max, a trait that is uncorrelated to its intrinsic value[13]. In addition, we used absolute values of VO$_2$max (ml O$_2$ min^{-1}) and adjusted for clinical characteristics in contrast to the univariate analyses of weight-adjusted

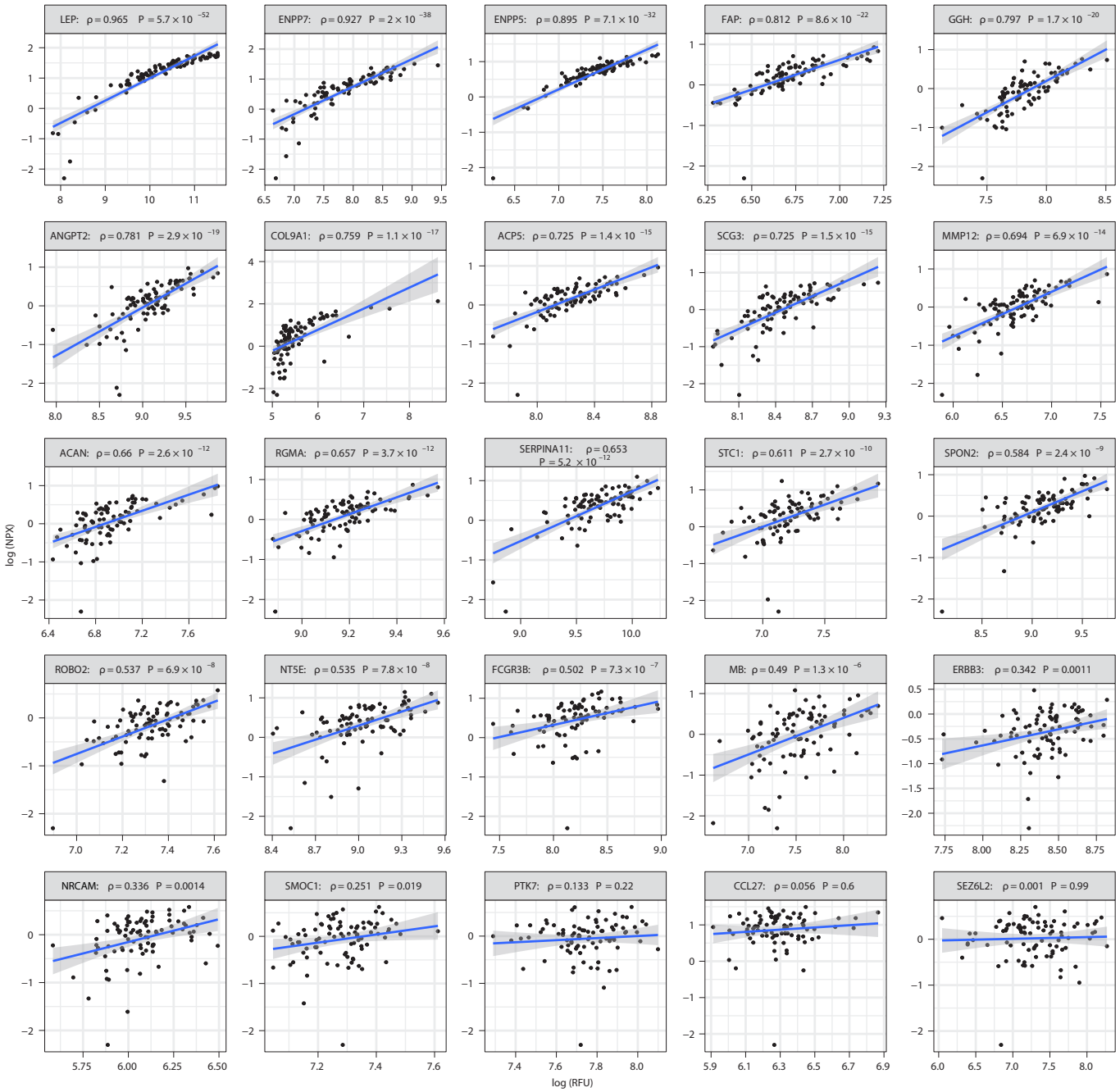


Fig. 6 | Spearman's correlations between aptamer-based and antibody-based assays among top findings. Spearman's correlations between protein levels measured by an aptamer-based method (log (RFU); x axis) and antibody-based method (log (NPX); y axis).

VO_2max (ml O_2 kg^{-1} min^{-1}) performed by Williams et al.[22]. Adjustments for age, sex and race probably significantly contributed to the differences between our groups' findings, owing to their relationships with CRF as previously documented and underscored in our interaction analyses[37–39].

Interestingly, when we performed additional adjustments for body-composition measures, we found that proteins closely associated with adiposity (for example, C-reactive protein, leptin and insulin) were no longer significant after adjusting for body fat percentage, but remained highly associated with VO_2max in a model adjusted for fat-free mass, similar to our main model using BMI. Although the main influence of body mass on VO_2max is mediated by fat-free mass, these data support prior findings that adipose tissue may contribute to VO_2max beyond differences in lean body weight[40]. Overall, there was modest overlap between the proteins related to baseline VO_2max in the models adjusted for body fat percentage and fat-free mass compared with the BMI-adjusted model (61 proteins, 48% overlap and 15 proteins, 56% overlap, respectively), whereas there was only one common protein (insulin-like growth factor binding protein 1 (IGFBP1)) among the fat-free-mass-adjusted and body-fat-percentage-adjusted models (Supplementary Table 4). These findings, coupled with the attenuation of striated-muscle-specific protein associations with baseline VO_2max after adjustment for lean body mass, highlight the importance of using standardized body size and composition adjustments for VO_2max when comparing results across studies.

We believe that the limited number of derivation proteins that achieved statistical significance in the external validation cohort

reflects the large differences in sample size between the two studies ($n = 745$ in HERITAGE versus $n = 91$ in the validation study) and the directional consistency of protein–VO_2max relationships (79/107) better reflects the stability of our findings across these studies. Further, given the known age- and body-size-related effects on proteomic profiles, as demonstrated in HERITAGE, we believe that large differences in the clinical characteristics between the two studies—even after restricting the validation cohort to age- and BMI-specific limits—impact the interpretation of our findings. We encourage additional validation of our findings; however, we are unaware of any other longitudinal, large-scale proteomic studies that include directly measured VO_2max at the moment.

The distinct proteomic profiles of baseline VO_2max and its exercise-induced changes that we observed are consistent with prior clinical observations demonstrating a lack of correlation between these traits[13,14]. The molecular mechanisms that underlie these differences are not well understood, and prior efforts to characterize CRF using candidate gene analyses[41], gene-expression data for skeletal muscle[42] and genome-wide association (GWAS) studies[43] have been limited by small sample sizes, lack of replication and the inherent challenges in applying reductionist strategies to describe a complex trait.

Using GSEA, we found nonrandom associations with baseline VO_2max in pathways related to hematopoiesis and angiogenesis (pathway participants included: chitinase 1 (CHIT1), haeme oxygenase 2 (HMOX2), cAMP-dependent protein kinase A (PRKACA), extracellular matrix protein 1 (ECM1)), the complement and coagulation systems (CD55, complement factor B precursor (CFB), cofilin-1 (CFI), plasminogen precursor (PLG), heparin cofactor 2 (SERPIND1)) and metabolic processes, including glycolysis, as described above (Supplementary Table 11a,b). These findings are consistent with those recently published from HERITAGE using integrative genomic analyses from GWAS and skeletal-muscle expression data in participants of European descent[44]. There, Ghosh et al. identified several gene loci that highlighted key determinants of CRF that we found using GSEA and through manual annotation (for example, skeletal muscle function (*SGCG*, *DMRT2*), cardiovascular physiology (*CASQ2*, *ATE1*) and hematopoiesis (*PICALM*)).

In contrast to our baseline VO_2max findings, we observed pathway enrichment reflecting proteins involved in extracellular matrix regulation (collagen alpha-1 (III) chain (COL3A1), COL9A1 COL10A1, aggrecan core protein (ACAN) and macrophage metalloelastase (MMP12)), key signalling pathways (for example, platelet-derived growth factor receptor B (PDGFRB) and hypoxia-induced factor 1 (HIF-1) signalling) and autophagy (for example, guanine nucleotide exchange factor (VAV3), cofilin-1 (CFL1)), among others, that were related to VO_2max responses to the exercise programme (Supplementary Table 10). These pathways were also present in a group of 16 over-represented Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in GSEA previously performed using GWAS from HERITAGE[45]. While many of the proteins encoded by the relevant genes from HERITAGE genomic analyses were intracellular and were not captured on our plasma proteomics platform, our shared findings regarding relevant pathways point to possible biologic underpinnings that reflect or possibly mediate the differences between these two traits. Ongoing efforts to incorporate additional molecular profiling data in the study of fitness traits, including the NIH-sponsored initiative, Molecular Transducers of Physical Activity Consortium (MoTrPAC: NCT03960827), will further advance our understanding of these processes.

We also identified five circulating proteins that were associated with both VO_2max traits. Although variants in *TMEM132B* have been associated with lean body mass[46], and insulin may also be correlated with both traits through its relationship to body composition, the relationships of COL9A1, PIANP and ATF6A with VO_2max are unclear and remain the subject of future exploration.

Our protein biomarker analyses highlight the current lack of predictive capacity for exercise-induced VO_2max responses and the potential for large-scale plasma protein profiling for biomarker discovery. Although individual clinical traits such as age, sex, race and BMI have all been shown to influence VO_2max, their collective ability to predict a clinically meaningful response in VO_2max to exercise training was modest, and no other readily available biomarkers exist. The addition of our protein score helped identify at a high percentage (negative predictive value = 83%) those individuals unable to modestly improve their VO_2max despite undergoing a standardized, supervised exercise training programme. If validated in an external cohort, these findings would help with the early identification of individuals who may benefit from alternative lifestyle interventions or additional therapeutics to improve their CRF.

Finally, our observation that plasma proteins related to both baseline VO_2max and its trainability are also associated with future mortality risk highlights the potential value of biochemical profiling to better understand the mechanistic links between CRF and long-term health outcomes. The strongest relationship among both sets of proteins was gelsolin (Table 2), both a secreted and intracellular protein with multiple cellular functions. Gelsolin was positively associated with baseline VO_2max ($\beta = 56.3$; FDR = 0.014) and inversely associated with incident all-cause mortality (HR = 0.71; 95% CI, 0.65–0.78), explaining ~3% of the variation in mortality after adjustment for age and sex in stepwise regression. Prior groups have linked lower plasma gelsolin levels to adverse outcomes in people with sepsis[47] and end-stage renal disease[48], and most recently higher gelsolin levels were associated with a decreased risk of congestive heart failure after adjusting for established risk factors[49]. Our data demonstrating its inverse association with all-cause mortality in a large population-based cohort extend these findings. Whether gelsolin is a biomarker or potential mediator of CRF and long-term health remains unclear. Gelsolin's most well-studied role relates to intracellular actin filament severing and cytoskeletal remodelling[50]; however, its secreted form predominantly comes from striated muscle and has been shown to function as an extracellular scavenger of actin[51] and inflammatory intermediates[52], as well as a participant in signal transduction pathways relevant to CRF, including the PI3K pathway[53]. Additional research into gelsolin's role in cardiometabolic health is warranted by these recent findings.

There are several limitations to our work. First, HERITAGE is a single-arm study and thus VO_2max adaptations may reflect unmeasured factors beyond the exercise-training stimuli. Leisure-time physical activity was not measured; however, all participants were sedentary for 6 months prior to enrolment. The aptamer-based platform that we utilized targets ~5,000 proteins; however, this technology is biased towards circulating proteins and does not provide complete coverage of the plasma proteome. Further, affinity-based assays, such as aptamer technology, are subject to nonspecific binding and may have limitations in their performance in response to post-translational protein modifications[54]. To address these concerns, we measured protein levels of 25 of our top findings using an orthogonal, antibody-based platform in a random subset of 88 HERITAGE samples and found that 18 out of 25 protein targets were correlated with our aptamer-based results. Among the 7 proteins with a Spearman correlation < 0.5, 2 aptamer targets (SMOC1 and ERBB3) have variants in *cis* (located within 1 Mb of the transcription start site of the gene encoding the protein) that are highly associated with protein levels in internal HERITAGE genetic-protein analyses (*SMOC1*, $P = 5.9 \times 10^{-8}$; *ERBB3*, $P = 2.16 \times 10^{-6}$). In addition, five (CCL27, PTK7, SMOC1, NRCAM and ERBB3) aptamer measurements had *cis* genotype-protein quantitative trait loci (*cis*-pQTL) relationships from publicly available and existing population-based human genetics studies, and one protein (MB) was validated using a multiple-reaction-monitoring MS-based method (Supplementary Table 15). Although we cannot

resolve the reason for the lack of a stronger correlation between these target proteins, these additional data support the specificity of our aptamer-based findings. Ultimately, we recognize the need for additional confirmation to validate the remaining analytes in the platform. Efforts to do so are ongoing[22], and all of our primary data have been made available to the broader scientific community for subsequent efforts. The proteomics platform includes a broad group of proteins; however, we are unable to identify their tissue origin. We limited the number of adjustments in our analyses relating proteins to all-cause mortality because our central goal was to assess the presence of shared protein biology between CRF and long-term health outcomes, thus these findings cannot explain the specific mechanisms through which this occurs nor can they be used as biomarkers of risk prediction without additional work. We also limited our analyses of VO_2max changes to linear methods, thus there may be additional insights yielded by using nonlinear methods. Our tests for interaction among race, sex and generation among protein–VO_2max relationships may not have been sufficiently powered, and our use of nominal statistical significance may have yielded false positive results, particularly given that the great majority of interactions were directionally consistent between groups.

In summary, we identified a large number of circulating proteins that are associated with VO_2max and highlight distinct profiles that exist for its baseline state as well as its adaptation to endurance exercise training. While our findings highlight specific proteins and biochemical pathways associated with these traits, further analyses of these data should yield additional biologic insights and motivate studies in model systems to both identify the sources of these proteins and evaluate their functional significance.

Methods

HERITAGE Family Study. The HERITAGE Family Study design and its participants have been described[55]. Briefly, family units of African Americans and people of European descent, totalling 763 sedentary participants (62% of European descent) between the ages of 17 and 65 years, were enroled in a 20-week training study of graded endurance exercise training across 4 clinical centres in the United States and Canada. Participants were healthy but sedentary over the previous 3 months and were free from apparent cardiometabolic disease. A total of 745 participants who had baseline measures of VO_2max and plasma samples were included in cross-sectional analyses, whereas 654 participants who completed exercise training and had complete data were used for longitudinal analyses. Written informed consent was obtained from all participants in the HERITAGE Family Study. HERITAGE study consent was reviewed and the research performed in these analyses was approved by Beth Israel Deaconess Medical Center's institutional review board.

Cardiopulmonary exercise testing and VO_2max. Two maximal CPETs were performed on separate days, at least 48 hours apart, before and after the 20-week exercise training programme, using a cycle ergometer (model 800S, SensorMedics) connected to a metabolic cart (model 2900, SensorMedics). Standard gas-exchange measures were obtained as an average of 20-second intervals. The criteria used for the attainment of VO_2max were defined as: a respiratory exchange ratio > 1.1, plateau in VO_2 uptake (change of < 100 ml/min in the last 3 consecutive 20-second averages) and a HR within 10 beats/minute of the maximal level predicted by age. All participants met at least one of these criteria in one of the two tests[12], but most met two or more[56]. The average of the two measurements before and after exercise training were used as VO_2max unless the values differed by more than 5%, in which case the higher value was used. The correlation between VO_2max measurements between the two tests (r = 0.97), coefficient of variations (CVs, 5%) and reproducibility among clinical centres were excellent[57]. We used absolute (ml O_2 min^{-1}) rather than weight-adjusted (ml O_2 kg^{-1} min^{-1}) measures of VO_2max so that body mass changes that occurred after exercise testing were not incorporated into our assessment of ΔVO_2max.

Exercise training protocol and plasma sampling. Participants exercised 3 times per week for 20 weeks, beginning at 30 minutes/session and increasing to 50 minutes/session for the final 6 weeks of the programme. Exercise intensity increased from the heart rate associated with 55% VO_2max obtained during baseline CPET to the heart rate associated with 75% VO_2max over the final 8 weeks of training. Cycle ergometers were electronically programmed to maintain a training heart rate by adjusting the power output. Each exercise session for all participants was continuously monitored by trained staff. Fasting plasma samples were collected in EDTA tubes from peripheral intravenous catheters prior to the beginning of the exercise training programme and at 24 hours following completion of the final exercise session.

Proteomic profiling. *Aptamer-based method.* Detailed analytic methods of the SOMAscan assay have been described[19–21]. Briefly, archived plasma samples stored at −80 °C from HERITAGE were diluted in 3 different concentrations (40%, 1% and 0.05%) and incubated with a mixture of fluorescently labelled single-stranded DNA aptamers (~5,000 SOMAmer). Plasma samples had either 0 freeze–thaw cycles or 1 freeze–thaw cycle prior to proteomics profiling. Protein–aptamer complexes were isolated from unbound or nonspecifically-bound proteins using a two-step, streptavidin-bead-based immobilization process. Aptamers eluted from the target proteins were quantified using the degree of fluorescence on a DNA microarray chip. Samples were normalized to 12 hybridization control sequences within each microarray and across plates, using the median signal for each dilution. We have previously reported median intra- and interassay CVs for the SOMAscan assay of ~5% (ref. [58]).

Antibody-based method. We subsequently performed additional proteomics profiling using an antibody-based technology (Olink) on a random sample (n = 88) from the HERITAGE study to determine the reproducibility of our aptamer-based results. Briefly, the Olink plasma extension assay technology uses DNA oligonucleotide-labelled antibody pairs to bind target proteins; 384 assays are performed on 4 separate panels with different dilutions for different dynamic ranges of target proteins (total proteins assayed = 1,536). After incubation with plasma samples, the oligonucleotide pairs hybridize and are extended by DNA polymerase to create a unique DNA barcode that is subsequently read out using next-generation sequencing. The median intra-assay CV for the 1,536 proteins was 10.25%, as assessed by multiple replicates of a pooled sample included in the experiment.

Genome-wide association studies. We also leveraged existing GWASs of proteins to help to determine aptamer specificity. Genotypes were available for 1,421 participants in the Malmo Diet and Cancer Study and 759 participants in the FHS with existing SOMAscan data[59]. A meta-analysis of genome-wide association analyses was performed to identify variants associated with circulating protein levels within 1 MB of the cognate gene, which were considered *cis*. Analyses were conducted on unrelated individuals. The methods used to generate publicly available genetics analyses for SOMAscan data have been described[30,60,61].

Framingham Heart Study. Participants in the FHS Offspring cohort who attended the fifth examination (1991–1995) and who had previously underwent plasma proteomic profiling with the SOMAscan single-stranded DNA aptamer-based platform (1.1 or 1.3 k-plex assays) were included in this study[28,29]. A total of 1,909 participants were included in analyses. Clinical characteristics were obtained from FHS investigators.

Validation cohort. The clinical characteristics and methods to derive baseline VO_2max from this randomized clinical exercise trial have been described[23]. Briefly, 300 sedentary adults with abdominal obesity were randomized into 3 exercise arms and a control group. Of the 217 participants who completed the 24-week exercise intervention, 216 had baseline VO_2max data and were available as a validation cohort. Given substantial differences—by design—in clinical characteristics between the validation and HERITAGE study cohorts, we restricted our analysis to subjects in the validation study with BMI < 40 and age < 55 (n = 91), to more closely approximate HERITAGE participants.

Statistical analysis. Baseline clinical characteristics of participants in the HERITAGE Family Study, validation study and FHS are reported as means ± s.d., proportions, or medians (interquartile range) according to visual inspection of normality. A two-sample Student's t-test was used to compare cases and controls in FHS. All protein values were natural-logarithmically transformed for subsequent analyses. Correlations between aptamer-based and antibody-based proteomics assays were assessed using the Spearman correlation coefficient. Linear regression was performed to determine the relationship between baseline protein values and both baseline VO_2max (ml O_2 min^{-1}) as well as the changes in VO_2max (ΔVO_2max, post-training VO_2max – pretraining VO_2max). Covariates in regression models included age, sex and baseline values of BMI, body fat percentage, fat-free mass (kg),and VO_2max (Δ model only). Protein levels were standardized to mean = 0 and multiples of 1 s.d. We used the Benjamini–Hochberg procedure to correct for multiple comparisons and employed a FDR < 0.1 to determine statistical significance for these hypothesis-generating analyses.

We tested for the interactions of generation, sex and race with protein level on baseline- and ΔVO_2max and adjusted for the other covariates, given previously reported differences in VO_2max trainability among these groups[13].

To evaluate the predictive utility of protein biomarkers for relative VO_2max changes (ΔVO_2max/baseline VO_2max) after exercise training, we performed the following analyses. First, we implemented a clinical trait model that included age, sex, race and BMI for relative VO_2max changes > 15%. We then added more than 5,000 proteins to train a more comprehensive model. The maximum number of missing values per protein within the entire dataset was ≤7, and the

total number of missing values was <2%. The data were randomly split into a training set (80% of cohort) that uses crossvalidation and a test set (20%) that was not used for model development. All preprocessing steps were first applied to the training set. The same steps were then carried out for the test set. We used a *k*-nearest neighbour algorithm to impute missing values ($k = 10$)[62]. All continuous variables were zero-centred and scaled (s.d. = 1). Scaling in the test set was applied using the same scaling factors calculated from the training set. The initial set of more than 5,000 predictors (proteins, age, sex, race and BMI) was reduced using a constraint-based feature selection algorithm for identifying minimal feature subsets (MMPC algorithm[63]). We then fit elastic net logistic regression models on the basis of the remaining predictors. The hyperparameters of the elastic net were optimized for the AUC using a global optimization algorithm. Receiver–operating characteristics of the protein score were subsequently calculated, with sensitivity, specificity, positive predictive value and negative predictive value generated. The training performance in the results is the result of repeated tenfold cross validation within the 80% training datasets.

GSEA using the full proteomics dataset was performed using the Molecular Signatures Database canonical pathways collection (MSigDB, http://software.broadinstitute.org/gsea/msigdb/collections.jsp), which includes a total of 2,199 curated gene sets from domain experts[64]. Signed log-transformed *P* values were computed from the regression models using the coefficient estimates and *P* values for protein–VO_2max associations. The full proteomic dataset was then ranked by their signed *P* values and used as input for GSEA (v4.0.3, with default parameters). GSEA results were exported to Cytoscape for visualization with the Enrichment Map tool using the following thresholds for gene set significance ($P < 0.05$, FDR $q < 0.15$, overlap index > 0.5)[65].

For the FHS participants, we performed Cox proportional-hazard regression to model all-cause mortality using the proteins that were significantly associated with baseline or ΔVO_2max and also available in FHS. In age-, sex- and batch-adjusted models, proteins that were associated with baseline or ΔVO_2max using a FDR $q < 0.1$ were brought forward for stepwise regression to estimate the percentage variation in all-cause mortality explained by each protein. *Cis* variants were identified using a linear regression model to assess the associations of variants with proteins that had statistically significant relationships with baseline and ΔVO_2max; statistical significance was set at $P < 5 \times 10^{-8}$. All statistical analyses were performed using R version 3.6.2 (R Core Team, R Foundation for Statistical Computing).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Deidentified, individual-level proteomics and phenotypic data that support the HERITAGE findings within this paper are available at https://motrpac-data.org/related-studies/heritage-proteomics. Overlapping aptamer-based and antibody-based proteomics data on the HERITAGE sample are included Supplementary Data Table 1. GWAS summary statistics for FHS and JHS are available through restricted access via the database of Genotypes and Phenotypes (dbGaP), a publicly available resource developed to archive data from human studies of genotype–phenotype relationships and can be accessed here (https://www.ncbi.nlm.nih.gov/gap/; FHS accession number: phs000363.v19.p13; JHS accession number: phs000964). FHS proteomics data have also been deposited in dbGaP and are available through the same accession number. JHS proteomics data have been deposited in the JHS Data Coordinating Center and are being deposited in dbGaP (accession number: phs002256.v1.p1); pending its receipt in dbGaP, all JHS data are available from the JHS Data Coordinating Center on request (JHSccdc@umc.edu). In addition, proteogenetics findings (precise SNP IDs) included in Supplementary Table 15 from FHS/MDCS meta-analysis and JHS have been provided in Tables 2 and 3 in the Supplementary Data, respectively. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

J.M.R., M.A.S., C.B. and R.E.G. conceptualized the study. J.M.R., B.P., D.S., T.R., S.D., M.J.K., C.S., P.M.J.B, R.R. and R.E.G designed research, performed biochemical experiments and analysed the proteomics data. J.M.R., U.A.T. and D.H.K. performed genetics analyses. J.L.B., C.B., S.A.C., S.G. and L.L.J. provided technical assistance and/or conceptual advice. J.M.R. and R.E.G. wrote the manuscript with assistance from the coauthors.

Competing interests

The authors declare no competing interests.

Additional information

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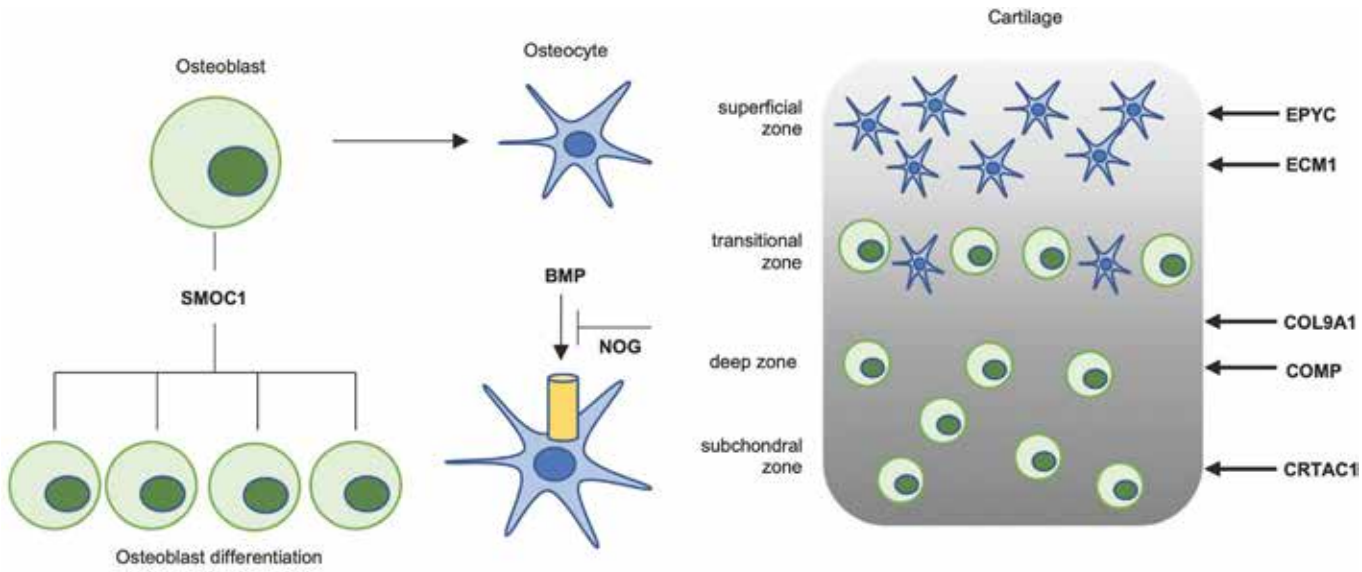
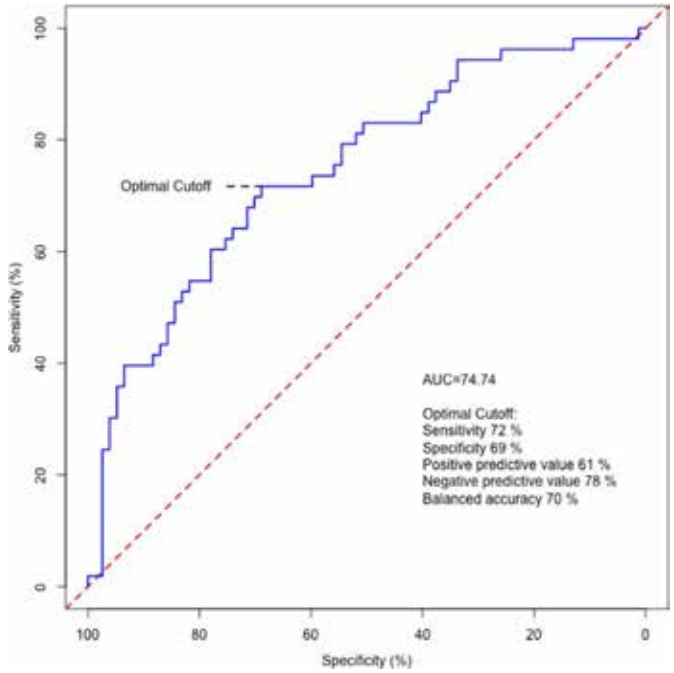


Entrez Gene Symbol	Target Protein Name
SMOC1	SPARC-related modular calcium-binding protein 1
BMP8B	Bone morphogenetic protein 8B
NOG	Noggin
ECM1	Extracellular matrix protein 1
EPYC	Epiphycan
COL9A1	Collagen alpha-1 (IX) chain
COMP	Cartilage oligomeric matrix protein
CRTAC1	Cartilage acidic protein

Extended Data Fig. 1 | Secreted proteins positively related to bone homeostasis and baseline V O_2max. Functional representation of proteins' role in bone metabolism and homeostasis. Left and middle: SMOC1 regulates osteoblast differentiation. BMPs are related to bone formation via the TGF-ß pathway and are mediated by extracellular signalling molecules such as NOG. Right: simplified schematic of proteins related to cartilage formation and their location within cartilage tissue.



Extended Data Fig. 2 | Receiver-operating characteristic curve for relative V O_2max changes with exercise training > 15% using overlapping targets between aptamer- and antibody-based proteomic platforms. 7/10 overlapping proteins on both platforms demonstrated moderate-strong correlation (SELE, TCL1A, COMP, CREG1, STC1, IL1RL2, LILRA2; ρ= 0.41–0.91) and were used in modeling.

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection in this study.
Data analysis	Data analyses and machine learning were performed using open source packages from R 3.6.2 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). Gene set enrichment analysis (GSEA v4.0.3) was performed using the Molecular Signatures Database canonical pathways collection (MSigDB v7.0, http://software.broadinstitute.org/gsea/msigdb/collections.jsp). GSEA results were exported to Cytoscape (v3.7.1) for visualization with the Enrichment Map tool (v3.3.1). Predictive modeling was performed using: MMPC algorithm: MXM_1.4.5 (R library); global optimization algorithm: pso_1.0.3 (R library); k-nearest neighbor algorithm to impute missing values: impute_1.60.0 (R package); data preprocessing: caret_6.0-85 (R package); and elastic net: glmnet_3.0-2 (R package).

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

De-identified, individual level proteomics and phenotypic data that support the HERITAGE findings within this paper are included in the Data Supplement Table 1. Overlapping aptamer-based and antibody-based proteomics data on the HERITAGE sample are included in Supplementary Data Table 1. GWAS summary statistics in FHS and JHS are available through restricted access via the database of Genotypes and Phenotypes (dbGaP), a publicly available resource developed to archive data from human studies of genotype-phenotype relationship and can be accessed here (https://www.ncbi.nlm.nih.gov/gap/; FHS accession number: phs000363.v19.p13; JHS accession number: phs000964). FHS proteomics data have also been deposited in dbGaP and are available through the same accession

number. JHS proteomics data have been deposited in the JHS Data Coordinating Center and are being deposited in dbGaP; pending its receipt in dbGaP, all JHS data are available from the JHS Data Coordinating Center on request (JHSccdc@umc.edu). In addition, proteogenetics findings (precise SNP IDs) included in the Supplemental Table 15 from FHS/MDCS meta-analysis and JHS have been provided in Supplementary Data Tables 2 and 3, respectively.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizing was not performed for these analyses. All HERITAGE study participants with complete clinical data were used for baseline analyses (N = 745) and longitudinal analyses (N = 654)
Data exclusions	Individuals with baseline VO2max measures who were missing post-training VO2max measures (N = 91) were excluded from longitudinal analyses. The clinical characteristics of individuals with baseline VO2max and those with complete longitudinal data are described in Table 1.
Replication	We derived protein-baseline VO2max relationships in the HERITAGE offspring and replicated our findings in the parents subgroup. We subsequently provided an external chronic exercise study to replicate our baseline VO2max analyses. The reproducibility of VO2max measures in the HERITAGE population has been described and is referenced in the manuscript. We validated aptamer specificity of our top findings using antibody-based assays. Quality control measures for proteomics data are also included in the manuscript.
Randomization	Randomization is not applicable for these secondary analyses of a single-arm exercise study. We adjusted for known clinical factors related to VO2max including age, sex, race, and body mass/composition. In addition we tested for these clinical factors effects on protein-VO2max relationships.
Blinding	All subjects in the HERITAGE study underwent the same standardized, exercise intervention. Proteomic analyses were performed in a blinded manner on de-identified samples and then data were returned to the HERITAGE principal investigators prior to analyses.

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Methods

n/a	Involved in the study
☒	☐ ChIP-seq
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Population characteristics	The HERITAGE Family Study enrolled white and black participants from two-generation biologic families (parents and offspring; ages 17-65) from 4 clinical centers in the US and Canada. Participants were sedentary (over the past 3 months) and free from overt cardiometabolic disease. The Framingham Heart Study Offspring cohort included 5124 persons ages 5-70 who have been followed longitudinally every 4-8 years from 1971 until now. Participants who attended the 5th examination (1991-1995) and who underwent plasma proteomic profiling were including in this study. This included 821 participants from a case-control cohort (311 incident CVD cases and 588 control) who were free of prevalent CVD; and a random sample of 1014 participants. We included all available subjects with proteomics data, however both known and unknown group differences exist between cases and controls. The validation study (Ross et al.) included 300 abdominally obese adult (mean age = 51 years), sedentary white men and women. Thus, our discovery cohort (HERITAGE) findings are most applicable to sedentary but otherwise healthy individuals whereas the validation cohort findings are most relevant to abdominally obese individuals. No other selection biases exist.
Recruitment	HERITAGE participants were recruited using community based outreach and advertisements. Participants in the FHS Offspring

Recruitment	Cohort were recruited from families of the original FHS cohort. Men and women between 35-65 years old were recruited from the Kingston, Ontario region for participation in the validation study (Ross et al.)
Ethics oversight	HERITAGE, FHS, and the Validation exercise study consents were reviewed and the research performed in these analyses was approved by Beth Israel Deaconess Medical Center's institutional review board. Given these analyses involved the integration of peripheral blood sampling and clinical traits using de-identified data, we are not aware of any risks to the participants.

Note that full information on the approval of the study protocol must also be provided in the manuscript.