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Candela Diaz-Canestro, Kit Cheung, Enrique Roche, Jose Manuel Sarabia, Michael Andrew Tse & Aimin Xu

Multi-Omics Signatures of Circulating Factors Associated with Cardiorespiratory Fitness Adaptations in Individuals with Prediabetes

Candela Diaz-Canestro,[1,2,3*] Kit Cheung, [1,2,3] Enrique Roche,[4,5,6] Jose Manuel Sarabia,[5,7] Michael Andrew Tse,[8] Aimin Xu[2,3,9*]

[1]Center for Molecular Cardiology, University of Zurich, Zurich, Switzerland. [2]State Key Laboratory of Pharmaceutical Biotechnology, the University of Hong Kong, Hong Kong, China; [3]Department of Medicine, the University of Hong Kong, Hong Kong, China. [4]Department of Applied Biology-Nutrition and Institute of Bioengineering, Miguel Hernández University (UMH), 03202 Elche, Spain. [5]Alicante Institute for Health and Biomedical Research (ISABIAL). 03010-Alicante, Spain. [6]CIBER for the Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III (ISCIII), Madrid 28029, Spain. [7] Department of Sports Science, Sports Research Center, Miguel Hernandez University, Elche, Spain.[8]Active Health Clinic, Centre for Sports and Exercise, the University of Hong Kong, Hong Kong, China., [9]Guangdong-Hong Kong Joint Laboratory for Metabolic Medicine, the University of Hong Kong, Hong Kong, China.

*Correspondence: candela.diazcanestro@uzh.ch, amxu@hku.hk

Background: Patients with insulin resistance exhibit reduced cardiorespiratory fitness (CRF), assessed by peak oxygen consumption (VO_2peak), compared with healthy age-matched individuals. Although high-intensity interval training (HIIT) can substantially improve VO_2peak, there is considerable interindividual variability in this response. Therefore, further research is needed to elucidate the molecular mechanisms underlying the heterogeneous response of VO_2peak to HIIT in individuals with prediabetes.

Methods: Proteomic analyses of serum samples, along with fecal metagenomic and targeted metabolomic profiling, were conducted in medication-naïve, overweight and obese Chinese men with prediabetes (n = 35; aged 24–62 years). All participants underwent a 12-week HIIT intervention, and biological samples were collected both before and after the

intervention to evaluate exercise-induced alterations in circulating proteins, gut microbial composition, and metabolite profiles.

Results: After 12 weeks of HIIT, mean VO_2peak increased by 0.47 L/min with individual responses ranging from 0 to 1.7 L/min. Baseline levels of short-chain fatty acid (SCFA)-producing genera, including *Prevotella* (β = 105.65, P = < 0.001, FDR = 0.034), *Coprococcus* (β = 50.22, P = 0.01, FDR = 0.39), and *Hungatella* (β = 40.72, P = 0.025, FDR = 0.50) , were positively associated with ΔVO_2peak. In contrast, baseline levels of the erythropoiesis-stimulating hormone erythropoietin (EPO) (β = −279.03, P = 0.024, FDR = 0.99) were negatively associated with ΔVO_2peak. Exercise-induced changes in growth hormone 1 (β = 63.97, P = 0.04, FDR = 0.99) were positively associated with ΔVO_2peak, whereas exercise-induced changes in BTB and CNC Homology 1 (β = −250.82, P = 0.01, FDR = 0.99), a repressor of heme oxygenase-1, were negatively associated with ΔVO_2peak. In multiple linear regression analysis including clinical variables, percentage lean mass (β = 64.17, P = 0.0005) was the strongest variable associated with **Δ**VO_2peak. The clinical model explained 27% of the variance which increased to 37% (P = 0.002) upon inclusion of exercise-associated circulating factors such as EPO.

Conclusions: Our findings reveal that baseline proteomic and metagenomic signatures are associated with VO_2peak adaptations. These multi-omics signatures may support the clinical implementation of personalized exercise interventions to improve CRF in individuals with prediabetes.

Research Insights

What is currently known about this topic?

- Patients with insulin resistance exhibit reduced cardiorespiratory fitness compared with healthy age-matched individuals.
- There is considerable interindividual variability in the cardiorespiratory fitness response to exercise training.

- Previous studies have focused on proteomic profiles associated with VO_2peak and VO_2peak trainability in healthy populations.

What is the key research question?

- What molecular signatures underlie VO_2peak and its trainability in individuals with impaired glucose homeostasis?

What is new?

- Baseline levels of short-chain fatty acid–producing genera were positively associated with ΔVO_2peak, whereas erythropoietin was negatively associated with ΔVO_2peak.
- Exercise-induced changes in growth hormone 1 were positively associated with ΔVO_2peak, whereas exercise-induced changes in BTB and CNC Homology 1, a repressor of heme oxygenase-1, showed a negative association with ΔVO_2peak.
- Adding proteomic markers, such as erythropoietin, to the multivariable regression model increased the explained variance for ΔVO_2peak to 37%.

How might this study influence clinical practice?

- These findings may facilitate the clinical implementation of personalized exercise interventions to improve CRF in individuals with prediabetes.

Introduction

Cardiorespiratory fitness (CRF), commonly assessed by peak oxygen consumption (VO_2peak), is inversely associated with cardiovascular (CV) and all-cause mortality, independent of established risk factors [1]. Extremely high CRF has been linked to the greatest survival, suggesting that an increase in VO_2peak will improve health without an apparent upper limit [2]. Individuals with insulin resistance exhibit reduced VO_2peak compared to healthy age-matched counterparts [3], and they are at higher risk of

developing cardiovascular diseases compared with individuals with normal insulin sensitivity[4]. Therefore, interventions aimed at improving CRF in individuals with insulin resistance are of particular clinical importance.

Endurance exercise training (ET) is recognized as the most effective lifestyle intervention for enhancing VO_2peak, with high-volume high-intensity interval training (HIIT) often producing greater improvements than traditional ET [5]. In this regard, training dose is emerging as the primary determinant of VO_2peak improvement, and increasing the ET dose has been shown to reduce non-response in VO_2peak among healthy individuals [6]. Despite this, substantial interindividual variability in VO_2peak trainability persists, representing a critical challenge for cardiovascular prevention in metabolic diseases. This variability raises important questions regarding differential responses to HIIT, as a blunted increase in cardiorespiratory fitness may reflect a residual cardiometabolic risk despite exercise adherence. Individuals with insulin resistance often exhibit, reduced blood volume, mitochondrial impairments, chronic low-grade inflammation, endothelial dysfunction, and altered muscle perfusion [7, 8]. These factors collectively may attenuate physiological adaptations to exercise [7, 9]. On the other hand, individuals with type 2 diabetes mellitus are typically prescribed multiple pharmacological treatments, which can increase variability and make it difficult to distinguish whether observed outcomes, in particular cardiometabolic outcomes, are attributable to the medications or the intervention. In addition, certain medications, such as antihypertensive drugs, may impair hematological adaptations, thereby limiting increases in VO_2peak induced by ET [10] Consequently, it remains important to investigate VO_2peak adaptations to HIIT in medication naïve individuals with insulin resistance.

This adaptive process is largely mediated by 'exerkines', exercise-induced signaling molecules released by tissues such as skeletal muscle, adipose tissue, liver, bone, and the cardiovascular system that mediate inter-organ

communication and contribute to the molecular and physiological adaptations to exercise [11]. In our previous study, we identified and characterized circulating proteins responsive to HIIT in individuals with prediabetes [12]. In addition, genomic and proteomic studies—such as those conducted within the HERITAGE Family Study—have primarily focused on proteomic profiles associated with VO_2peak and VO_2peak adaptations in healthy populations [13, 14]. The Molecular Transducers of Physical Activity Consortium (MoTrPAC) aims to enroll 2,400 healthy participants across North America to elucidate molecular responses to ET [15]. However, a multi-omic approach is required to investigate the complex, multi-layered regulation of HIIT and decipher how the systemic release of circulating proteins coordinately modulates VO_2peak adaptations and to identify baseline molecular signatures that associated with VO_2peak changes in individuals with prediabetes.

To address these gaps, we examined the relationships between circulating proteins, fecal metagenomics, and metabolomics with VO_2peak at baseline and following 12-week HIIT. Furthermore, multivariable linear regression models were applied to examine the associations of baseline clinical and proteomic variables with VO_2peak adaptations.

Methods

Experimental model and subject details

Ethics statement

This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 15-370) and endorsed the principles of the Declaration of Helsinki. Written informed consents were obtained from all participants.

Study participants

Overweight and obese Chinese men were recruited from the local community via flyers and online advertisements, as previously described [16]. A total of

35 participants who completed the exercise intervention and had samples available at different time points were included in the proteomic analysis, whereas 20 participants were included in the metagenomic and metabolomic analyses (Supplementary Figure 1). All participants recruited were instructed to continue their normal routine and not make any changes to their habitual physical activity and diet.

Method details

Participants selection

The inclusion and exclusion criteria were previously discussed [16]. Briefly, inclusion criteria comprised: non-smoking Chinese men aged 20–60 years with overweight/obesity (BMI > 23 kg/m^2) and stable body weight, diagnosed with prediabetes (impaired glucose tolerance and/or impaired fasting glucose according to the American Diabetes Association guidelines criteria). Eligible individuals were not on any chronic medication. Participants were excluded if they had acute or chronic illness, inflammatory or infectious conditions, neurological, musculoskeletal, or cardiopulmonary disorders affecting exercise safety or tolerance, recent structured exercise or dietary programs, or mental illness limiting their ability to participate or understand the study. Additionally, participants who did not complete ≥85% of the prescribed training frequency were excluded from further analyses.

HIIT protocol

The 12-week HIIT consisted of three sessions per week on non-consecutive days at the Active Health Clinic, Center for Sports and Exercise, The University of Hong Kong, supervised by certified exercise specialists in a one-to-one manner, as previously described [16]. Participants were required to take part in at least 85% of all the exercise sessions for inclusion into the analysis. Each session consisted of a 70-minute high-intensity combined aerobic and resistance interval training session included a 10-minute warm-up, followed by participants rotating through three 10-minute stations: high-

intensity treadmill intervals, high-intensity resistance and calisthenics intervals, and high-intensity stationary bike intervals. There were 3–4 minutes of recovery between stations. Each session ended with 10–15 minutes of cool-down and stretching. Intensity was adjusted using real-time heart rate telemetry, and participants were encouraged to work at 80–95% of their maximum heart rate (HRmax).

The treadmill interval station consisted of 3–4 bouts of 2 minutes of running at 85–95% of VO_2peak, interspersed with 30–45 seconds of active recovery at 50% VO_2peak. Speed and incline were adjusted individually to ensure progressive overload. The stationary bike station included 4–5 bouts of 45–60 seconds of high-intensity cycling at 90–95% of peak work capacity, interspersed with 60–75 seconds of active recovery at 30% of peak work capacity. Resistance and cadence were adjusted according to individual progression. The resistance and calisthenics station consisted of 2–3 sets of high-intensity exercises such as squats, kettlebell swings, planks, and burpees, with 30 seconds of rest between sets.

Collection of clinical data and VO_2peak

Fasting serum samples were collected at baseline and 48-72 h after the final exercise session to prevent the influence of the acute effects of exercise. Body composition was assessed via whole-body dual-energy X-ray absorptiometry (DXA) scans (Explorer S/N 91,075, Hologic Inc., Waltham, MA, USA). A graded exercise test using the Balke treadmill protocol [17] was performed on a motor-driven, electronically controlled treadmill (TrackMaster, Full Vision Inc., Newton, KS, USA) to assess VO_2peak before and after the exercise intervention. VO_2peak was assessed using a Medgraphics Ultima™ CardiO2® gas exchange analysis system (MGC Diagnostics, USA). Gas exchange data were collected breath-by-breath and averaged over 30-second intervals. The system was calibrated prior to each test using certified calibration gases and a 3-L syringe according to manufacturer guidelines. Measurement error analysis showed a typical error of measurement of 1.14 $mL \cdot kg^{-1} \cdot min^{-1}$ and a

coefficient of variation of 2.5%, with Bland–Altman limits of agreement ranging from −2.75 to 3.56 $mL \cdot kg^{-1} \cdot min^{-1}$. After sufficient familiarization time, participants started walking at a constant speed of 5.3 km/h. The treadmill gradient began at 0% (no gradient) for the first minute, then it was increased to 2% in the second minute, after which the treadmill gradient was increased by 1% every minute thereafter until test completion. Heart rate, rate of perceived exertion (RPE) and gas analysis were measured throughout in order to assess exertion and heart rate function via measurement of oxygen consumption.

Olink Proteomics

Antibody-based technology (Olink Proteomics AB, Uppsala, Sweden) was used for proteomics profiling in serum samples as previously described [12]. The selection of these panels was strategically aimed at identifying a broad spectrum of circulating proteins—including markers of vascular remodeling, metabolism, and systemic inflammation—to elucidate the molecular crosstalk between exercise and cardiovascular health in individuals with prediabetes. Specifically, protein biomarkers were detected via the Olink Explore 384 cardiometabolic and inflammation panels. After excluding 46 proteins that were not detected in >50% of the samples and 3 proteins that were duplicated, the final analysis included 688 proteins (352 from the cardiometabolic panel, 333 from the inflammatory panel and 3 included in both panels, Supplementary Figure 1). The median intra-assay coefficient of variability (CV) was 12.5%, as assessed by multiple replicates of a pooled sample included in the experiment. Values were presented as normalized protein expression (NPX) units on a log2 scale.

Fecal DNA Sequencing and Targeted Metabolomics Profiling

Fecal genomic DNA was sequenced on the Illumina HiSeq 4000 platform (Illumina, San Diego, California, USA; paired-end; insert size: 350 bp; read length: 150 bp) by BGI (Hong Kong S.A.R., China). Genus-level microbiome data were analyzed using a compositional data framework. Low-prevalence

taxa (<20% prevalence across samples) were excluded prior to analysis. To address zero values, a small pseudocount (1×10^{-6}) was added to all taxa, followed by renormalization. We also applied a centered log-ratio (CLR) transformation, which is widely recommended for compositional microbiome data. The CLR transformation expresses each taxon relative to the geometric mean of all taxa within a sample:

$$\mathrm{CLR}(x_i) = \log\left(\frac{x_i}{g(x)}\right)$$

$$\mathrm{CLR}(x_i) = \log\left(\frac{x_i}{g(x)}\right)$$

Targeted metabolomics profiling of fecal samples was performed by Metabo-Profile (Shanghai, China) as previously described [16]. Briefly, 10 mg of lyophilized feces was homogenized with 1 M NaOH and methanol, and the resulting supernatants were combined. Samples were derivatized with methyl chloroformate (MCF; Sigma-Aldrich, Stockholm, Sweden) using a MultiPurpose Sample PrepStation 2 (Gerstel, Germany), followed by the addition of internal standards. Derivatized samples were analyzed in randomized order using gas chromatography coupled to time-of-flight mass spectrometry (GC/TOFMS; Pegasus HT, Leco Corp., USA) equipped with an Rxi-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent), as previously described by Zhao et al [18]. Quality control (QC) samples were prepared identically to study samples and injected after every 14 samples to monitor analytical reproducibility and instrument stability. Raw data were processed using ChromaTOF software (v4.50, Leco Co., CA, USA), including baseline correction, smoothing, noise reduction, deconvolution, library searching, and peak area integration. Metabolites were identified by comparison of mass spectral similarity and Kovats retention index values against an in-house alkyl chloroformate derivative library, using a similarity score cutoff of >70%.

Absolute metabolite concentrations (nmol/mL) were normalized to internal standards and fecal dry weight, and subsequently log2-transformed to improve normality. Metabolites detected in fewer than 50% of samples were excluded. Missing values, including values below the limit of detection, were imputed using half of the minimum observed value for each metabolite. No additional batch correction was applied, as QC samples demonstrated stable analytical performance throughout the acquisition sequence.

Statistical Analysis

Main statistical analyses were performed using R software. Continuous variables are presented as mean ± SEM. Paired comparisons were performed using paired Student's t-tests for normally distributed variables and Wilcoxon signed-rank tests for non-normally distributed variables. Assumptions of multiple linear regression, including linearity, homoscedasticity, normality of residuals, independence, and absence of multicollinearity, were evaluated using residual plots and variance inflation factors. Linear regression analyses were conducted to determine the relationship between ΔVO_2peak and post-training VO_2peak and (i) baseline levels of proteins, fecal bacteria, and metabolites, and (ii) intervention-induced changes in proteins, fecal bacteria, metabolites, and (iii) VO_2peak, and baseline levels of proteins, fecal bacteria, and metabolites ($p < 0.05$). Covariates included in the regression models were age, baseline body weight, fat mass, and baseline VO_2peak (for analyses of ΔVO_2peak). Multivariable linear regression models were specified a priori. Model 1 included age, lean mass and baseline VO_2peak; and Model 2 further included erythropoietin (EPO). EPO was selected a priori based on its established physiological relevance to oxygen transport and exercise adaptation. This sequential approach was used to evaluate the stability of estimates and potential confounding. Estimates are reported as coefficients with 95% CIs. Two-sided $P<.05$ indicated statistical significance. To minimize model overfitting, the number of predictors included in each model was restricted considering the sample size and commonly recommended

observations-to-predictor ratios. Multicollinearity was low for all predictors (all VIF < 1.2). Visual inspection of residual diagnostic plots demonstrated acceptable adherence to assumptions of linearity, homoscedasticity, normality of residuals, and absence of influential observations.

Results

Cohort characteristics

Clinical characteristics of thirty-five medication-naïve overweight and obese men with prediabetes enrolled in 12-week HIIT intervention are shown in Table 1. Notably, no significant differences were observed between the two cohorts included in this study (Supplementary Table 1). Following the intervention, mean VO_2peak increased by 0.47 L/min with individual responses ranging from 0 to 1.7 L/min (Figure 2). This heterogeneity in VO_2peak response (ΔVO_2peak) prompted us to explore the association between multi-omic changes and ΔVO_2peak.

Baseline multi-omic signatures associated with VO_2peak trainability

We next examined the relationship between baseline multi-omic signatures and changes in absolute VO_2peak (ΔVO_2peak, ml/min) to identify potential factors influencing the physiological adaptations to HIIT. Regression analyses were adjusted for age, body weight, % body fat, and baseline VO_2peak. The final analysis included 688 proteins (352 from the cardiometabolic panel and 333 from the inflammatory panel and 3 included in both panels). We identified nine proteins positively associated with ΔVO_2peak, and five negatively associated (Figure 3A). Among the positively associated proteins, soluble epoxide hydrolase (sEH)—encoded by the *EPHX2* gene—acts as a modulator of vascular tone by metabolizing epoxyeicosatrienoic acids, signaling molecules involved in vascular relaxation, into less active or inactive dihydroxy-eicosatrienoic acids. Among the negatively associated proteins, several were linked to erythropoiesis and inflammation. These include erythropoietin (EPO), the hormone regulating red blood cell production; scavenger receptor cysteine-rich family member with five domains (SSC5D),

a member of the highly conserved scavenger receptor cysteine-rich superfamily of cell-surface and/or secreted proteins involved in immune signaling [19]; and V-set and transmembrane domain-containing 2-like (VSTM2L), a peripheral regulator of insulin action [20]. Regarding gut microbiota, baseline levels of bacterial genera associated with the production of short-chain fatty acids (SCFAs) were positively associated with ΔVO_2peak (Figure 3B), including *Prevotella*. *Coprococcus* and *Hungatella*, and fecal metabolomics identified ten metabolites positively associated with ΔVO_2max, and three negatively associated (Figure 3C). Among the positively associated metabolites, arachidonic acid, an omega-6 polyunsaturated fatty acid, and a higher sarcosine-to-dimethylglycine ratio were linked to greater improvements in ΔVO_2peak. Conversely, lower baseline levels of valeric acid were associated with greater improvements in VO_2peak, suggesting a potential relationship between specific SCFA-related metabolic states and variability in adaptations to HIIT.

Multi-omic changes associated with VO_2peak trainability

We examined the relationship between multi-omic fold changes and ΔVO_2peak to identify potential mediators of VO_2peak adaptations to HIIT. We identified one exercise-induced protein that was positively associated with ΔVO_2peak and ten that were negatively associated (Figure 4A). We observed that growth hormone (GH1) was positively associated with ΔVO_2peak. Among the negatively associated proteins, we identified mitogen-activated protein kinase 9 (MAPK9), a stress-activated kinase that regulates gene expression by modifying transcription factors [21], and BTB and CNC homology 1 transcription regulator (BACH1), a transcription factor that represses antioxidant genes [22].

Regarding gut microbiome, exercise-induced changes in three bacterial genera were negatively associated with ΔVO_2peak (Figure 4B), including *Prevotella, Bacteroides and Fusicatenibacter*. In addition, fecal metabolomics revealed three metabolites positively associated with ΔVO_2peak and seven

metabolites negatively associated (Figure 4C). Among the positively associated metabolites, a greater increase in the L-serine/glycine ratio was linked to larger improvements in ΔVO_2peak, suggesting a potential shift with amino-acid-dependent energy pathways. In contrast, the odd-chain saturated fatty acid nonadecanoic acid (C19:0) was negatively associated with ΔVO_2peak, indicating possible lipidomic signatures reflecting less favorable adaptations to HIIT.

Baseline multi-omic signatures associated with VO_2peak

We next examined the relationship between baseline serum proteins and VO_2peak to explore factors associated with absolute VO_2peak using regression analyses adjusted for age, weight, % body fat. We identified eight proteins positively associated with VO_2peak, and five proteins showing a negative association (Figure 5A). Notably, the protein with the strongest positive association (based on p value) with baseline VO_2peak was pancreatic secretory granule membrane major glycoprotein GP2 (GP2), a secreted protein expressed in zymogen granules of pancreatic acinar cells as well as in plasma membrane of intestinal M cells. Conversely, inflammatory markers, including Signaling Threshold-Regulating Transmembrane Adaptor 1 (SIT), Immunoglobulin Superfamily Member 8 (IGSF8) and Oncostatin M Receptor (OSMR) were negatively associated with VO_2peak. Regarding fecal bacteria and metabolites, two bacterial genera and three metabolite were positively associated with VO_2peak and one bacterial genera and two metabolites showed negative associations (Figure 5B and 5C). Notably, *Faecalibacterium*, a bacteria genus associated with anti-inflammatory effects [23], showed the strongest positive correlations with VO_2peak, whereas D-2-Hydroxyglutaric Acid (D-2-HG), a metabolite associated with cancer, showed negative correlations with VO_2peak.

Multiple linear regression model

In the clinical model (model 1), % lean mass emerged as the only baseline variable significantly associated with ΔVO_2peak together explaining 27% of

the variance. We then assessed whether selected proteomic markers provided additional explanatory information beyond clinical variables. The inclusion of EPO (model 2) increased the adjusted R^2 from 0.27 to 0.39 (Table 2), which may indicate that the proteomic profile contributes to explaining interindividual differences in VO_2peak response.

Discussion

This study examined how baseline multi-omic profiles and their changes relate to VO_2peak trainability, and how baseline multi-omic signatures are associated with VO_2peak. Twelve weeks of HIIT significantly increased mean VO_2peak in individuals with prediabetes, although the magnitude of improvement varied substantially across participants. Baseline levels of SCFA-producing genera, including *Prevotella. Coprococcus and Hungatella*, were positively associated with ΔVO_2peak. In contrast, baseline EPO levels were negatively associated with changes in VO_2peak. In addition, a strong relation was observed between exercise-induced changes in GH1 and ΔVO_2peak, whereas exercise-induced changes in BTB and CNC Homology 1 showed a negative association with ΔVO_2peak. The clinical model explained 27% of the variance in ΔVO_2peak, which increased to 37% with inclusion of proteomic features such as EPO.

The response of the cardiovascular system to chronic exercise is regulated by the secretion of specific proteins known as “exerkines” [11]. It has been proposed that HIIT induces a complex organ crosstalk mediated by exercise-responsive factors secreted from skeletal muscle, adipose tissue, liver, gut, among other organs [24]. A well-documented exercise-responsive factor is EPO, the primary hormone regulating erythropoiesis. EPO increases during the first 1–2 weeks of exercise training in healthy individuals and then returns to baseline with continued training [25], whereas it may remain elevated after 12 weeks of HIIT in individuals with prediabetes [12]. Interestingly, participants in our cohort with elevated baseline EPO exhibited blunted VO_2peak responses, which may reflect a compensatory relationship with

hemoglobin and hematocrit, as previously observed in individuals with hematological disorders [26] and in type 2 diabetes [27]. Furthermore, elevated basal EPO might also serve as an indicator of chronic systemic stress or inflammation-induced EPO resistance, which effectively constrains cardiovascular plasticity [28]. Furthermore, exercise-induced changes in BACH1, a repressor of heme oxygenase-1 (HO-1), were negatively correlated with ΔVO_2peak. HO-1 is a rate-limiting enzyme in heme catabolism that contributes to the regulation of erythropoiesis and red blood cell homeostasis [29, 30]. Central hemodynamic factors, including red blood cell mass and blood volume expansion, are fundamental adaptations to ET limiting changes in VO_2peak in healthy individuals [31]. Collectively, these findings raise the possibility that baseline hematological status, or exercise-induced changes in proteins regulating erythropoiesis, may constrain or facilitate improvements in aerobic capacity in individuals with prediabetes.

Recently, the gut microbiome has emerged as a potential modulator of exercise responses in individuals with prediabetes [16]. In addition, specific microbial genera have been associated with enhanced endurance performance and improved metabolism in elite athletes [32]. In mice, treatment with antibiotics for two weeks reduced endurance capacity compared to the control group [33]. Conversely, supplementation with *Bacteroides uniformis* increased endurance performance [34]. Our findings revealed that baseline microbiota may be associated with the changes in VO_2peak trainability. In this context, higher relative abundances of short-chain fatty acid (SCFA)-producing genera, including *Prevotella. Coprococcus and Hungatella*, were associated with greater ΔVO_2peak. Fecal SCFAs are increased in athletes compared to control subjects [35]. In mice, treatment with intracolonic infusion of SCFAs propionate improved running times compared to a control group, suggesting that SCFAs may increase aerobic performance [32]. Mechanistically, SCFAs may support energy production and enhance mitochondrial oxidative capacity, potentially influencing aerobic

metabolism [36]. Alternatively, these microbially derived metabolites may act as distal exerkines that prime the cardiovascular system for HIIT-induced adaptations [11]. Altogether, these findings suggest a potential association between baseline microbiome composition and heterogeneity in VO_2peak trainability, particularly in metabolically compromised individuals, such as those with or at risk of developing T2DM.

Our study also highlights a strong relationship between exercise-induced changes in GH1 and ΔVO_2peak. GH1 is an exercise-responsive hormone released by the anterior pituitary gland in response to acute and chronic exercise [12, 37, 38]. Administration of GH1 reduced adipose tissue and increased lean body mass (LBM) in older individuals [39]. Notably, LBM and VO_2peak have been previously associated in men and women of Hans Chinese ethnicity [40] and women of Caucasian ethnicity [41], suggesting that GH1 may regulate ΔVO_2peak by affecting body composition in individuals with prediabetes. Consistent with previous reports [42], we found that participants with higher baseline LBM demonstrated greater improvements in VO_2peak following HIIT. While exploratory, these findings are consistent with the possibility that higher LBM may facilitate aerobic adaptations to training in individuals with prediabetes.

This study has several limitations. First, the relatively small sample size and restrictive inclusion criteria, including the inclusion of men only, limit the generalizability of the findings to women and other populations. In addition, the absence of a sedentary control group limits the ability to distinguish exercise-induced effects from time-dependent changes, biological day-to-day variability and technical error. Although participants were required to achieve a minimum attendance rate of 85% for study inclusion, comprehensive adherence data were not available for all individuals, limiting the evaluation of the potential influence of training compliance on the observed adaptations. Another limitation is the focus on obesity-associated insulin resistance, with limited consideration of other phenotypes, such as

lean insulin-resistant individuals, who may exhibit stronger genetic contributions. The timing of post-intervention sampling may also represent a source of biological variability. Although a 48–72 h window following the final exercise session was selected to minimize acute exercise effects, it may still reflect differential recovery states across participants. Exercise-induced hematological adaptations may represent another source of biological variability, and the incorporation of direct cardiovascular measurements, including blood volume and cardiac parameters, may provide greater insight into the underlying molecular mechanisms. Finally, the use of nominal p-values without correction for multiple testing represents an important limitation, and the findings should therefore be interpreted as exploratory. Validation of the proposed model in an independent cohort is warranted. Future studies including larger sample sizes, multiple sampling time points, diverse ethnic groups, women, and sedentary control participants are needed to confirm these findings and further explore their potential clinical relevance.

In conclusion, the present study identified multi-omic signatures associated with VO_2peak and VO_2peak trainability in individuals with prediabetes. The identification of baseline proteomic and metagenomic signatures associated with VO_2peak trainability may facilitate the clinical implementation of personalized exercise interventions aimed at improving cardiorespiratory fitness in individuals with prediabetes.

Declarations

Ethics approval and consent to participate: This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 15-370) and endorsed the principles of the Declaration of Helsinki. Written informed consents were obtained from all participants.

Consent for publication: The authors consent for publication of the manuscript above, including accompanying images or data contained therein.

Availability of data and materials: Olink raw proteomics data are provided in Table S2 from previous publication [12]. Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

Competing interests: The authors declare no competing interests.

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Author contributions: C.D.-C. performed bioinformatics analysis, interpreted results, and wrote the manuscript. K.C. performed bioinformatics analysis A.X. conceived and supervised the study and edited the manuscript. E.R., J.M. S-M, M.A.T., and A.X. revised the manuscript critically for important intellectual contents.

References

1. Ladenvall P, Persson CU, Mandalenakis Z, Wilhelmsen L, Grimby G, Svardsudd K, Hansson PO: Low aerobic capacity in middle-aged men associated with increased mortality rates during 45 years of follow-up. Eur J Prev Cardiol 2016, 23(14):1557-1564.
2. Mandsager K, Harb S, Cremer P, Phelan D, Nissen SE, Jaber W: Association of Cardiorespiratory Fitness With Long-term Mortality Among Adults Undergoing Exercise Treadmill Testing. JAMA Netw Open 2018, 1(6):e183605.
3. Nyholm B, Nielsen MF, Kristensen K, Nielsen S, Ostergard T, Pedersen SB, Christiansen T, Richelsen B, Jensen MD, Schmitz O: Evidence of increased visceral obesity and reduced physical fitness in healthy insulin-resistant first-degree relatives of type 2 diabetic patients. Eur J Endocrinol 2004, 150(2):207-214.
4. Zheng Y, Ley SH, Hu FB: Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol 2018, 14(2):88-98.
5. Milanovic Z, Sporis G, Weston M: Effectiveness of High-Intensity Interval Training (HIT) and Continuous Endurance Training for VO2max

Improvements: A Systematic Review and Meta-Analysis of Controlled Trials. Sports Med 2015, 45(10):1469-1481.
6. Montero D, Lundby C: Refuting the myth of non-response to exercise training: 'non-responders' do respond to higher dose of training. J Physiol 2017, 595(11):3377-3387.
7. Patti ME, Corvera S: The role of mitochondria in the pathogenesis of type 2 diabetes. Endocr Rev 2010, 31(3):364-395.
8. Montero D, Diaz-Canestro C, Oberholzer L, Lundby C: The role of blood volume in cardiac dysfunction and reduced exercise tolerance in patients with diabetes. Lancet Diabetes Endocrinol 2019, 7(10):807-816.
9. DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, Hu FB, Kahn CR, Raz I, Shulman GI et al: Type 2 diabetes mellitus. Nat Rev Dis Primers 2015, 1:15019.
10. Guo M, Liu X, Dixon SM, Tse HF, Montero D: Effects of angiotensin II receptor type 1 blockade combined with endurance training on haemoglobin mass and aerobic capacity: a randomized placebo-controlled trial. Cardiovasc Res 2026, 121(18):2830-2833.
11. Jin L, Diaz-Canestro C, Wang Y, Tse MA, Xu A: Exerkines and cardiometabolic benefits of exercise: from bench to clinic. EMBO Mol Med 2024, 16(3):432-444.
12. Diaz-Canestro C, Chen J, Liu Y, Han H, Wang Y, Honore E, Lee CH, Lam KSL, Tse MA, Xu A: A machine-learning algorithm integrating baseline serum proteomic signatures predicts exercise responsiveness in overweight males with prediabetes. Cell Rep Med 2023, 4(2):100944.
13. Robbins JM, Peterson B, Schranner D, Tahir UA, Rienmuller T, Deng S, Keyes MJ, Katz DH, Beltran PMJ, Barber JL et al: Human plasma proteomic profiles indicative of cardiorespiratory fitness. Nat Metab 2021, 3(6):786-797.
14. Robbins JM, Rao P, Deng S, Keyes MJ, Tahir UA, Katz DH, Beltran PMJ, Marchildon F, Barber JL, Peterson B et al: Plasma proteomic changes in response to exercise training are associated with cardiorespiratory fitness adaptations. JCI Insight 2023, 8(7).
15. MoTr PACSG, Jakicic JM, Kohrt WM, Houmard JA, Miller ME, Radom-Aizik S, Rasmussen BB, Ravussin E, Serra M, Stowe CL et al: Molecular Transducers of Physical Activity Consortium (MoTrPAC): human studies design and protocol. J Appl Physiol (1985) 2024, 137(3):473-493.
16. Liu Y, Wang Y, Ni Y, Cheung CKY, Lam KSL, Wang Y, Xia Z, Ye D, Guo J, Tse MA et al: Gut Microbiome Fermentation Determines the Efficacy of Exercise for Diabetes Prevention. Cell Metab 2020, 31(1):77-91 e75.
17. Balke B, Ware RW: An experimental study of physical fitness of Air Force personnel. U S Armed Forces Med J 1959, 10(6):675-688.
18. Zhao L, Ni Y, Su M, Li H, Dong F, Chen W, Wei R, Zhang L, Guiraud SP, Martin FP et al: High Throughput and Quantitative Measurement of Microbial Metabolome by Gas Chromatography/Mass Spectrometry

Using Automated Alkyl Chloroformate Derivatization. Anal Chem 2017, 89(10):5565-5577.
19. Cardoso MS, Santos RF, Almeida S, Sa M, Perez-Cabezas B, Oliveira L, Tavares J, Carmo AM: Physical Interactions With Bacteria and Protozoan Parasites Establish the Scavenger Receptor SSC4D as a Broad-Spectrum Pattern Recognition Receptor. Front Immunol 2021, 12:760770.
20. Muzumdar RH, Huffman DM, Atzmon G, Buettner C, Cobb LJ, Fishman S, Budagov T, Cui L, Einstein FH, Poduval A et al: Humanin: a novel central regulator of peripheral insulin action. PLoS One 2009, 4(7):e6334.
21. Yang SH, Sharrocks AD, Whitmarsh AJ: Transcriptional regulation by the MAP kinase signaling cascades. Gene 2003, 320:3-21.
22. Dhakshinamoorthy S, Jain AK, Bloom DA, Jaiswal AK: Bach1 competes with Nrf2 leading to negative regulation of the antioxidant response element (ARE)-mediated NAD(P)H:quinone oxidoreductase 1 gene expression and induction in response to antioxidants. J Biol Chem 2005, 280(17):16891-16900.
23. Mao X, Ma J, Jiao C, Tang N, Zhao X, Wang D, Zhang Y, Ye Z, Xu C, Jiang J et al: Faecalibacterium prausnitzii Attenuates DSS-Induced Colitis by Inhibiting the Colonization and Pathogenicity of Candida albicans. Mol Nutr Food Res 2021, 65(21):e2100433.
24. Thyfault JP, Bergouignan A: Exercise and metabolic health: beyond skeletal muscle. Diabetologia 2020, 63(8):1464-1474.
25. Montero D, Breenfeldt-Andersen A, Oberholzer L, Haider T, Goetze JP, Meinild-Lundby AK, Lundby C: Erythropoiesis with endurance training: dynamics and mechanisms. Am J Physiol Regul Integr Comp Physiol 2017, 312(6):R894-R902.
26. Wenger RH, Kurtz A: Erythropoietin. Compr Physiol 2011, 1(4):1759-1794.
27. Lalande S, Hofman PL, Baldi JC: Effect of reduced total blood volume on left ventricular volumes and kinetics in type 2 diabetes. Acta Physiol (Oxf) 2010, 199(1):23-30.
28. Khankin EV, Mutter WP, Tamez H, Yuan HT, Karumanchi SA, Thadhani R: Soluble erythropoietin receptor contributes to erythropoietin resistance in end-stage renal disease. PLoS One 2010, 5(2):e9246.
29. Kitamuro T, Takahashi K, Ogawa K, Udono-Fujimori R, Takeda K, Furuyama K, Nakayama M, Sun J, Fujita H, Hida W et al: Bach1 functions as a hypoxia-inducible repressor for the heme oxygenase-1 gene in human cells. J Biol Chem 2003, 278(11):9125-9133.
30. Liu R, Zhang X, Nie L, Sun S, Liu J, Chen H: Heme oxygenase 1 in erythropoiesis: an important regulator beyond catalyzing heme catabolism. Ann Hematol 2023, 102(6):1323-1332.
31. Montero D, Cathomen A, Jacobs RA, Fluck D, de Leur J, Keiser S, Bonne T, Kirk N, Lundby AK, Lundby C: Haematological rather than skeletal muscle adaptations contribute to the increase in peak oxygen uptake

induced by moderate endurance training. J Physiol 2015, 593(20):4677-4688.
32. Scheiman J, Luber JM, Chavkin TA, MacDonald T, Tung A, Pham LD, Wibowo MC, Wurth RC, Punthambaker S, Tierney BT et al: Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. Nat Med 2019, 25(7):1104-1109.
33. Okamoto T, Morino K, Ugi S, Nakagawa F, Lemecha M, Ida S, Ohashi N, Sato D, Fujita Y, Maegawa H: Microbiome potentiates endurance exercise through intestinal acetate production. Am J Physiol Endocrinol Metab 2019, 316(5):E956-E966.
34. Morita H, Kano C, Ishii C, Kagata N, Ishikawa T, Hirayama A, Uchiyama Y, Hara S, Nakamura T, Fukuda S: Bacteroides uniformis and its preferred substrate, alpha-cyclodextrin, enhance endurance exercise performance in mice and human males. Sci Adv 2023, 9(4):eadd2120.
35. Barton W, Penney NC, Cronin O, Garcia-Perez I, Molloy MG, Holmes E, Shanahan F, Cotter PD, O'Sullivan O: The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level. Gut 2018, 67(4):625-633.
36. Marttinen M, Ala-Jaakkola R, Laitila A, Lehtinen MJ: Gut Microbiota, Probiotics and Physical Performance in Athletes and Physically Active Individuals. Nutrients 2020, 12(10).
37. Kraemer WJ, Marchitelli L, Gordon SE, Harman E, Dziados JE, Mello R, Frykman P, McCurry D, Fleck SJ: Hormonal and growth factor responses to heavy resistance exercise protocols. J Appl Physiol (1985) 1990, 69(4):1442-1450.
38. Wideman L, Weltman JY, Hartman ML, Veldhuis JD, Weltman A: Growth hormone release during acute and chronic aerobic and resistance exercise: recent findings. Sports Med 2002, 32(15):987-1004.
39. Rudman D, Feller AG, Nagraj HS, Gergans GA, Lalitha PY, Goldberg AF, Schlenker RA, Cohn L, Rudman IW, Mattson DE: Effects of human growth hormone in men over 60 years old. N Engl J Med 1990, 323(1):1-6.
40. Guo M, Diaz-Canestro C, Pugliese NR, Paneni F, Montero D: Lean body mass and the cardiorespiratory phenotype: An ethnic-specific relationship in Hans Chinese women and men. J Cachexia Sarcopenia Muscle 2024, 15(3):963-974.
41. Diaz-Canestro C, Pentz B, Sehgal A, Montero D: Sex dimorphism in cardiac and aerobic capacities: The influence of body composition. Obesity (Silver Spring) 2021, 29(11):1749-1759.
42. Bouchard C, An P, Rice T, Skinner JS, Wilmore JH, Gagnon J, Perusse L, Leon AS, Rao DC: Familial aggregation of VO(2max) response to exercise training: results from the HERITAGE Family Study. J Appl Physiol (1985) 1999, 87(3):1003-1008.

Figures legends

Figure 1. Multi-omic Changes in Response to High intensity Interval Training. Schematic diagram of the study design.

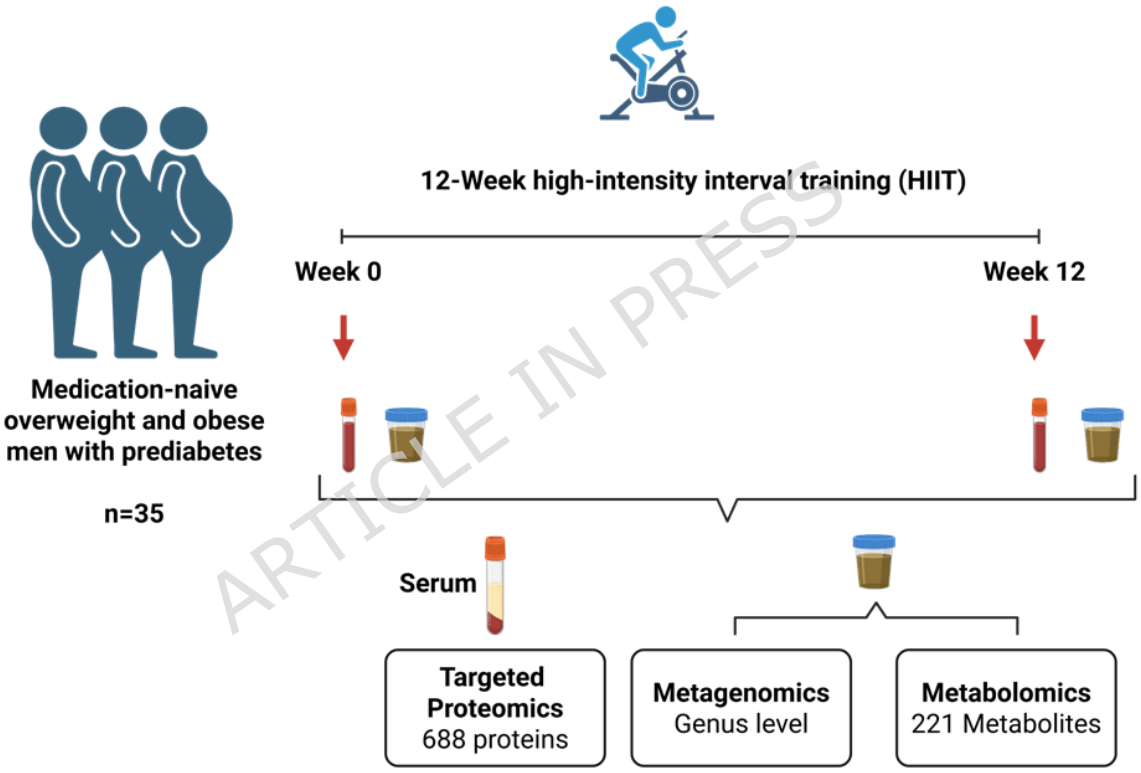
Figure 2. Individual responses to exercise training expressed as absolute and relative changes in VO_2peak. Bars represent individual participants ranked from the lowest to highest response. The left panel shows the absolute change in VO_2peak (ml/min), while the right panel shows the percentage change in VO_2peak following the intervention. In the right panel, blue bars indicate low responders (<5% increase in VO_2peak), grey bars indicate moderate responders (5–20% increase in VO_2peak), and red bars indicate high responders (>20% increase in VO_2peak) according to the predefined classification criteria.

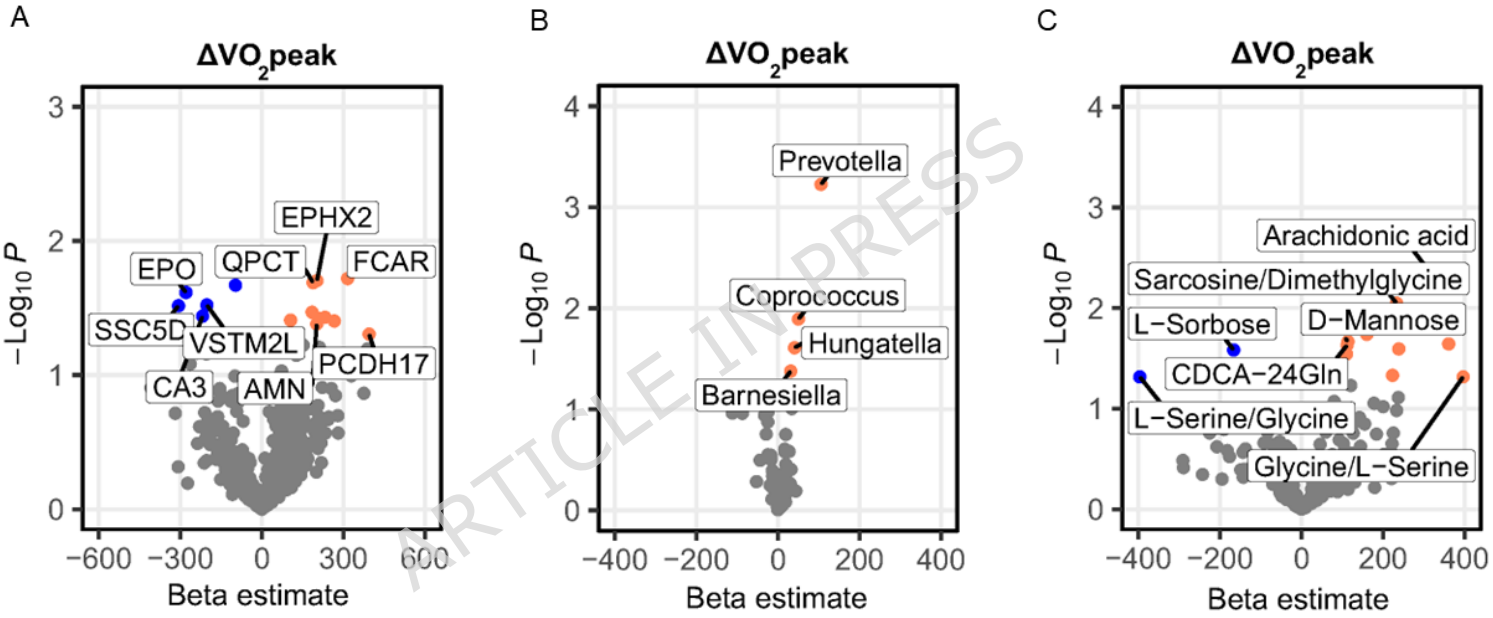
Figure 3. Baseline multi-omic signatures associated with changes in aerobic capacity (ΔVO_2peak). Baseline levels of proteins (A), fecal gut bacteria (B), and fecal metabolites (C) associated with changes in absolute VO_2peak (ΔVO_2peak) were identified using a linear regression model adjusted for age, weight, % fat, and baseline VO_2peak. Orange and blue dots indicate the direction of the association with metabolic outcomes, representing positive and negative associations, respectively, whereas grey dots indicate non-significant associations ($P > 0.05$).

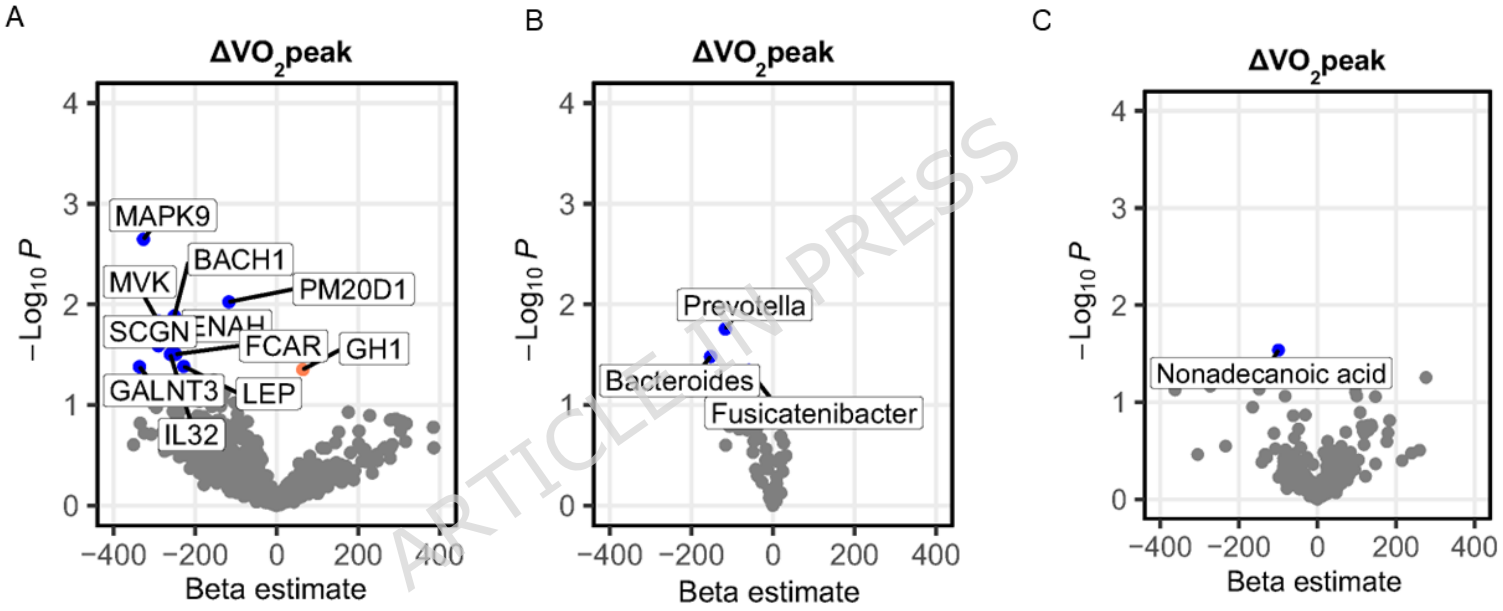
Figure 4. Multi-omic changes associated with changes in aerobic capacity (ΔVO_2peak). Changes in levels of proteins (A), fecal gut bacteria (B), and fecal metabolites (C) associated with changes in absolute VO_2peak (ΔVO_2peak) were identified using a linear regression model adjusted for age, weight, % fat, and baseline VO_2peak. Orange and blue dots indicate the direction of the association with metabolic outcomes, representing positive and negative associations, respectively, whereas grey dots indicate non-significant associations ($P > 0.05$).

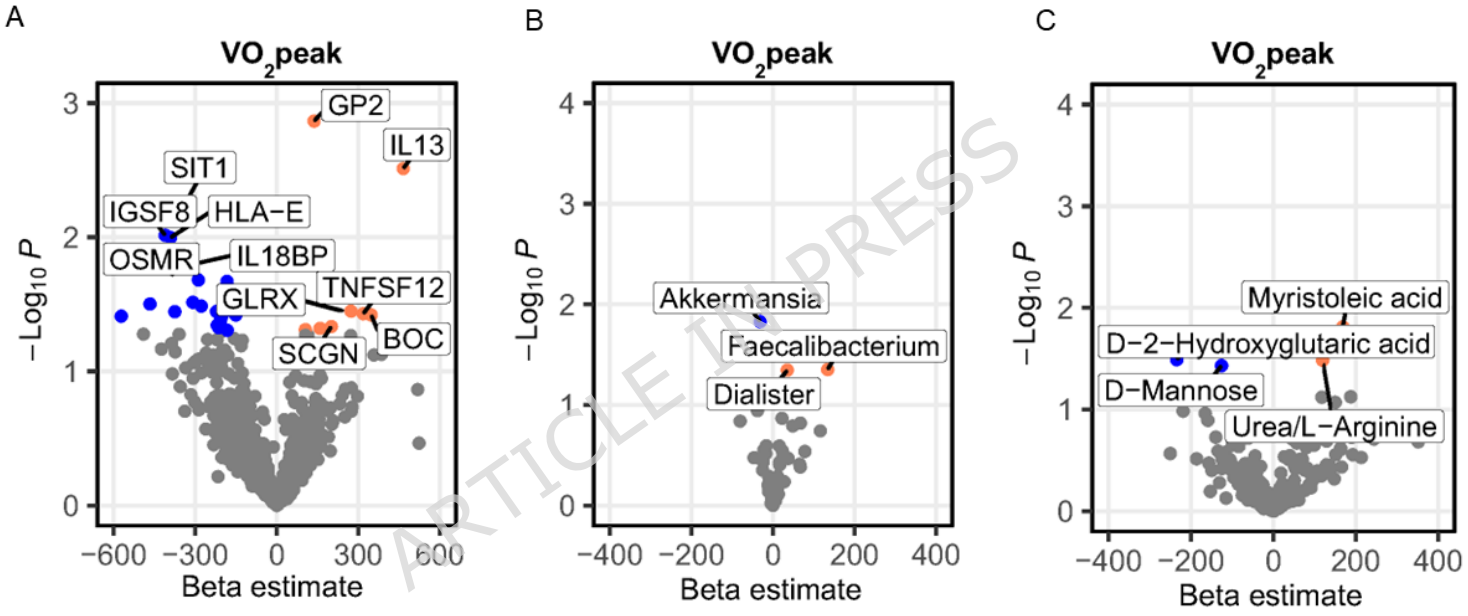
Figure 5. Baseline multi-omic signatures associated with aerobic capacity (VO_2peak). Baseline levels of proteins (A), fecal gut bacteria (B), and fecal metabolites (C) associated with absolute VO_2peak were identified

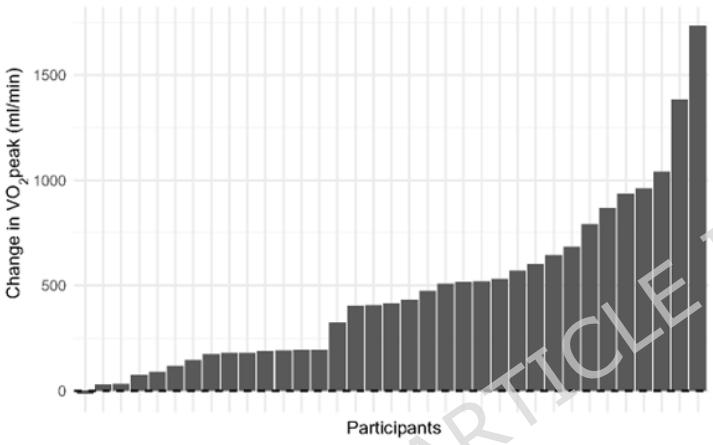
using a linear regression model adjusted for age, weight, % fat. Orange and blue dots indicate the direction of the association with metabolic outcomes, representing positive and negative associations, respectively, whereas grey dots indicate non-significant associations ($P > 0.05$).











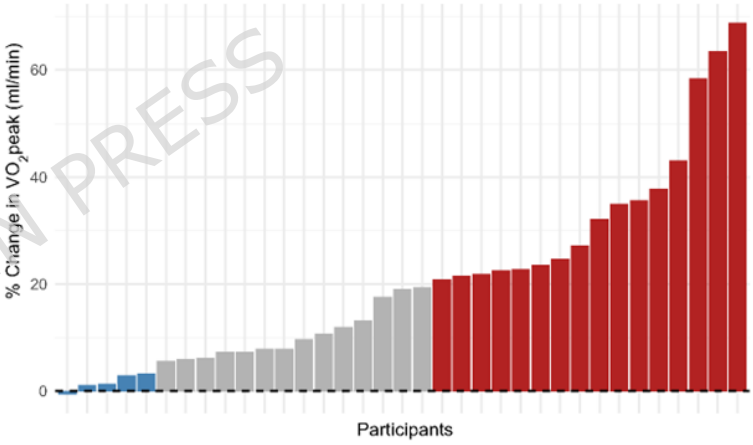


Table 1. Clinical Parameters in Response to 12 Weeks of High-Intensity Interval Training (N = 35)

Variable	0-Week	12-Week	p-Value
Age (years)	40.66 ± 1.76	—	—
Weight (kg)	87.82 ± 2.10	85.95 ± 2.10	**<0.001**
BMI (kg/m^2)	30.14 ± 0.71	29.7 ± 0.72	**<0.001**
Fat mass%	33.86 ± 0.79	31.98 ± 0.73	**<0.001**
Lean Mass%	62.01 ± 0.64	65.28 ± 0.73	**<0.001**
Fasting glucose (mM)	5.48 ± 0.08	4.94 ± 0.09	**<0.001**
2h OGTT glucose (mM)	8.21 ± 0.22	5.56 ± 0.31	**<0.001**
Fasting insulin (µU/ml)	12.96 ± 1.55	9.11 ± 1.37	**<0.001**
HOMA-IR	3.05 ± 0.36	1.93 ± 0.28	**<0.001**
Triglycerides (mM)	2.32 ± 0.12	1.74 ± 0.13	**<0.001**
Total cholesterol (mM)	5.35 ± 0.16	4.86 ± 0.14	**<0.001**
HDL-c (mM)	1.17 ± 0.04	1.26 ± 0.04	**<0.001**
LDL-c (mM)	3.59 ± 0.14	3.18 ± 0.11	**<0.001**
Systolic blood pressure (mmHg)	132.35 ± 2.63	131.47 ± 2.52	0.686
Diastolic blood pressure (mmHg)	81.94 ± 1.56	80.32 ± 1.79	0.188
Resting heart rate (bpm)	76.03 ± 1.65	73.65 ± 1.57	0.058
VO_2peak (ml/kg/min)	27.17 ± 0.64	33.33 ± 1.00	**<0.001**
VO_2peak (ml/min)	2380.78 ± 72.86	2852.33 ± 97.37	**<0.001**

Data are presented as mean ± SEM. Abbreviations: BMI, body mass index; HDL-c, high-density lipoprotein cholesterol; HOMA, homeostatic model assessment for insulin resistance; LDL-c, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; VO_2max, maximal oxygen consumption. Differences were assessed using a paired t-test or Wilcoxon signed-rank test, depending on data normality.

Table 2. Multiple Linear Regression Models Predicting Changes in Absolute VO_2peak

Variable	Model 1	Model 2
Age	-6.11 (95% CI -18.33 to 6.11; p = 0.316)	-5.54 (95% CI -16.91 to 5.83; p = 0.328)
Lean Mass	57.19 (95% CI 23.14 to 91.25; p = 0.002)	47.70 (95% CI 15.04 to 80.36; p = 0.006)
Baseline VO_2peak	-0.12 (95% CI -0.41 to 0.17; p = 0.401)	-0.11 (95% CI -0.39 to 0.16; p = 0.400)
EPO	—	-299.58 (95% CI -551.72 to -47.44; p = 0.022)

Model Fit Statistics

Statistic	Model 1	Model 2
R^2	0.277	0.395
Adjusted R^2	0.207	0.314
ΔR^2	—	0.119
Residual SE	353.2	328.3
F-statistic	3.95	4.90
Model p-value	0.017	0.004

Abbreviations: CI, confidence interval; EPO, erythropoietin; Residual SE, residual standard error; VO_2peak, peak oxygen consumption.

Notes: Values are presented as regression coefficients with 95% confidence intervals (CI) and corresponding p-values. Model 1 includes age, lean mass, and absolute VO_2peak. Model 2 additionally includes EPO. Statistically significant associations were considered at $p < 0.05$.