

Higher aerobic capacity is associated with a more favorable circulating redox profile: potential implications for cardiovascular health

Kinga Zujko-Kowalska

Medical University of Białystok

Małgorzata Elżbieta Zujko

Medical University of Białystok

Wojciech Łaguna

Medical University of Białystok

Marlena Dubatówka

Medical University of Białystok

Jacek Jamiołkowski

Medical University of Białystok

Paweł Sowa

Medical University of Białystok

Marcin Kondraciuk

Medical University of Białystok

Anna Szpakowicz

Medical University of Białystok

Łukasz Małek

Józef Piłsudski University of Physical Education in Warsaw

Karol Kamiński

karol.kaminski@umb.edu.pl

Medical University of Białystok

Article

Keywords: aerobic capacity, antioxidants, oxidative stress, physical activity, prevention

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10819652/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Disturbances in redox homeostasis may precede the clinical manifestation of cardiovascular disease. However, relationships between systemic redox status, objectively measured aerobic capacity, and habitual physical activity in apparently healthy individuals remain insufficiently understood. This study examined associations of aerobic capacity and self-reported leisure-time physical activity with systemic redox biomarkers, clinical and biochemical parameters, and body composition.

Methods

This cross-sectional analysis included 70 participants from the Białystok PLUS study (Polish Longitudinal University Study), which was registered at ClinicalTrials.gov (Identifier: NCT03197363). Aerobic capacity was assessed by cardiopulmonary exercise testing. Systemic redox status was evaluated using total antioxidant status (TAS), total oxidant status (TOS), and the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). The oxidative stress index (OSI) was calculated as the TOS-to-TAS ratio. Plasma growth differentiation factor 15 (GDF-15), a stress-responsive biomarker, was also measured. Body composition was assessed using dual-energy X-ray absorptiometry (DXA), and standard anthropometric measurements were performed. Associations between body-mass-normalized maximal oxygen uptake ($\text{VO}_{2\text{max}}/\text{kg}$) and redox biomarkers were examined using sequential linear regression models: unadjusted, adjusted for age and sex, and fully adjusted for age, sex, smoking status, and the lean mass-to-fat mass ratio (LM/FM).

Results

In the fully adjusted model, higher $\text{VO}_{2\text{max}}/\text{kg}$ was independently associated with higher TAS, greater GPx, SOD, and CAT activities, and lower OSI. Participants reporting at least 2 h/week of leisure-time physical activity had higher $\text{VO}_{2\text{max}}/\text{kg}$, $\text{VO}_{2\text{max}}$ normalized to lean mass, TAS, GPx and SOD activities, and LM/FM than those reporting less than 2 h/week. They also had lower waist-to-hip ratio, body fat percentage, OSI, and GDF-15.

Conclusions

These findings indicate that higher aerobic capacity and at least 2 h/week of self-reported leisure-time physical activity were associated with a more favorable systemic redox balance, potentially benefiting cardiovascular health. However, because of the cross-sectional design, causality, the underlying mechanisms, and clinical significance cannot be established. Longitudinal and intervention studies are

needed to determine whether improving aerobic capacity or increasing physical activity enhances redox homeostasis and whether these changes translate into improved cardiovascular health.

Clinical Trial Registration

The manuscript reports data from the Białystok PLUS study, which was registered at ClinicalTrials.gov (Identifier: NCT03197363).

Background

Oxidative stress plays an important role in the pathophysiology of cardiovascular disease by reducing nitric oxide bioavailability, promoting oxidative modification of lipids and proteins, and contributing to endothelial dysfunction and vascular inflammation [1]. Alterations in circulating redox biomarkers have also been observed in individuals with coronary artery disease [2]. Therefore, evaluating the associations of redox status with physical activity and aerobic capacity in adults without clinically manifest cardiovascular disease may contribute to a better understanding of mechanisms potentially relevant to primary prevention.

Regular physical activity is a key component of cardiovascular disease prevention. The World Health Organization recommends that adults perform 150–300 minutes of moderate-intensity or 75–150 minutes of vigorous-intensity aerobic physical activity per week, or an equivalent combination of both, and undertake muscle-strengthening activities on at least 2 days per week [3]. Despite these recommendations, nearly one-third of adults worldwide do not meet the recommended levels of physical activity [4]. Acute exercise may transiently increase the production of reactive oxygen species (ROS), while regulated ROS-mediated signaling contributes to exercise-induced adaptations. Regular exercise training may strengthen endogenous antioxidant defenses, although the redox response depends on exercise intensity and duration, training status, recovery, and the timing of biological sample collection [5, 6].

Physical activity and cardiorespiratory fitness are related but distinct concepts. Physical activity represents a behavioral exposure, whereas cardiorespiratory fitness is an objectively measurable physiological attribute [7]. Cardiopulmonary exercise testing (CPET) provides an objective assessment of the integrated responses of the cardiovascular and respiratory systems and skeletal muscles during exercise. Maximal oxygen uptake (VO_2max), measured during CPET, is regarded as the reference measure of aerobic capacity. Higher cardiorespiratory fitness is consistently associated with lower risks of cardiovascular and all-cause mortality [7].

The relationship between objectively assessed aerobic capacity and resting circulating redox biomarkers in apparently healthy adults remains incompletely characterized. A recent study in healthy young men found a positive association between VO_2max and total antioxidant capacity but no significant associations with superoxide dismutase or catalase activities or with a marker of lipid peroxidation [8].

These findings suggest that different components of antioxidant defense may show distinct associations with aerobic capacity.

Growth differentiation factor 15 (GDF-15) is a pleiotropic, stress-responsive cytokine whose circulating levels increase in response to cellular stress, including oxidative stress and mitochondrial dysfunction, and are associated with cardiometabolic abnormalities and adverse cardiovascular outcomes [9]. Although an inverse association between cardiorespiratory fitness and resting circulating GDF-15 has previously been reported [10], the relationship between GDF-15 and circulating redox biomarkers in apparently healthy adults remains poorly characterized.

The primary aim of this study was to evaluate the association between aerobic capacity, objectively assessed by CPET, and the profile of resting circulating redox biomarkers in apparently healthy adults. As a secondary, exploratory objective, we examined the associations of self-reported leisure-time physical activity with aerobic capacity, body composition, selected clinical and biochemical parameters, circulating redox biomarkers, and GDF-15.

Methods

Study population

This study was a cross-sectional analysis of data collected within the Polish Longitudinal University Study (Białystok PLUS), a population-based study designed to investigate determinants of chronic non-communicable diseases among Białystok residents aged 20–79 years at the time of sampling. Participants were selected from the municipal population register using stratified random sampling by sex and 5-year age groups to reflect the demographic structure of the city.

The present analysis included individuals examined between 6 May and 5 July 2024. Of the 171 individuals assessed during this period, 88 met the predefined age criterion of 20–50 years. Eighteen participants were subsequently excluded because of incomplete data ($n = 4$), failure to meet the predefined maximal-effort criteria required for the operational determination of VO_2max during cardiopulmonary exercise testing (CPET; $n = 8$), use of nonsteroidal anti-inflammatory drugs within the week preceding the examination ($n = 2$), long-term use of glucocorticosteroids ($n = 2$), a diagnosis of thalassemia ($n = 1$), or a diagnosis of chronic obstructive pulmonary disease ($n = 1$). The final analytical sample comprised 70 adults aged 20–50 years. The age range of 20–50 years was prespecified to focus on adults in early and middle adulthood, in whom the prevalence of established chronic disease is generally lower than in older populations. Based on the standardized questionnaire, none of the participants reported a history of cardiovascular disease, kidney disease, diabetes, or cancer. The flow chart of participant selection is shown in Fig. 1.

Questionnaire, physical activity, and clinical assessments

All study procedures were performed at the Białystok PLUS Research Center in Medical University of Białystok. Sociodemographic, lifestyle, and clinical data were collected using a standardized questionnaire developed for the Białystok PLUS study.

Leisure-time physical activity was assessed based on participants' self-reported time spent engaging in physical activity during a typical week. The following response categories were used: no physical activity, ≤ 1 h/week, 2–5 h/week, 6–9 h/week, 10–14 h/week, and ≥ 15 h/week. For the analyses, participants were classified into two groups according to their reported leisure-time physical activity: <2 h/week and ≥ 2 h/week. This threshold was determined by the structure of the questionnaire response categories and was not intended to correspond to current physical activity guideline recommendations.

Anthropometric parameters were assessed using standardized procedures. Height was measured using a calibrated SECA 264 stadiometer, whereas waist and hip circumferences were measured using a non-elastic SECA 201 measuring tape (SECA, Hamburg, Germany). Total body mass, lean mass, fat mass, and percentage body fat (%FM) were assessed using dual-energy X-ray absorptiometry (Lunar iDXA, GE Healthcare, Chicago, IL, USA). The lean mass-to-fat mass ratio (LM/FM) was calculated by dividing lean mass by fat mass. The waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference, while body mass index (BMI) was calculated as body mass in kilograms divided by height in meters squared.

Handgrip strength was measured five times using a digital handgrip dynamometer (SAEHAN DHD-1, Saehan Corporation, Masanhoewongu, Gyeongsangnamdo, Changwonsi, Republic of Korea), with consecutive measurements alternating between hands. Both the mean value across all five measurements and the highest value recorded were included in the analyses.

Blood collection and routine biochemical analyses

Venous blood samples were collected in the morning after at least 8 hours of fasting. Participants were advised to avoid strenuous physical exercise, alcohol consumption, caffeine-containing beverages, and dietary supplements for 24–48 hours before blood collection. Blood was collected into serum-separation tubes containing a clot activator for serum preparation, EDTA tubes for complete blood count and glycated hemoglobin (HbA1c) measurements, and EDTA tubes containing sodium fluoride for plasma glucose determination.

Routine biochemical analyses, including total cholesterol (T-Chol), HDL cholesterol (HDL-Chol), triglycerides (TG), fasting glucose (FG), and HbA1c, were performed on fresh samples on the day of collection. Serum and EDTA plasma were separated, aliquoted, and stored at -80°C in the Medical University Biobank until analysis. Biochemical analyses were performed in a medical laboratory using standardized methods. FG, T-Chol, HDL-Chol, and TG were measured using a Cobas c111 analyzer (Roche Diagnostics, Meylan, France). Non-HDL cholesterol (non-HDL-Chol) was calculated by subtracting HDL cholesterol (HDL-Chol) from total cholesterol (T-Chol). Glycated hemoglobin (HbA1c) was

determined using high-performance liquid chromatography (HPLC) on a D-10 analyzer (Bio-Rad, Hercules, CA, USA).

Redox biomarkers and GDF-15 measurements

Redox biomarkers were measured in samples stored in the Medical University Biobank following collection. All samples underwent a single freeze–thaw cycle and were thawed immediately before analysis. No visibly hemolyzed samples were included in the analyses. In accordance with the kit manufacturers' instructions, all redox biomarker measurements were performed in duplicate within one month of blood sample collection. Plasma total antioxidant status (TAS) was determined using the ImAnOx (TAS/TAC) kit, whereas plasma total oxidant status (TOS) was assessed using the PerOx (TOS/TOC) kit (Immundiagnostik AG, Bensheim, Germany). The oxidative stress index (OSI) was calculated as the ratio of TOS to TAS. Plasma catalase (CAT) activity was measured using the Catalase Assay Kit (No. 707002), superoxide dismutase (SOD) activity using the Superoxide Dismutase Assay Kit (No. 706002), and glutathione peroxidase (GPx) activity using the Glutathione Peroxidase Assay Kit (No. 703102) (Cayman Chemical Company, Ann Arbor, MI, USA). TAS, TOS, CAT, SOD, and GPx were measured spectrophotometrically using a microplate reader (Infinite M200 Pro, Tecan, Männedorf, Switzerland).

The relative abundance of GDF-15 was measured in plasma using the Olink Target 96 Cardiovascular III panel (Olink Proteomics, Uppsala, Sweden), based on proximity extension assay technology with qPCR readout on the Olink Signature Q100 platform. Results were reported as Normalized Protein eXpression (NPX) values on an arbitrary $\log_2$ scale.

Cardiopulmonary exercise testing

Cardiopulmonary exercise testing was performed after blood collection. The test was conducted using a Vyntus CPX metabolic cart (Vyaire Medical GmbH, Höchberg, Germany) and a VIAsprint 150P electronically braked cycle ergometer (ergoline GmbH, Bitz, Germany). Participants underwent a symptom-limited incremental exercise test, with predefined termination criteria including angina, clinically significant arrhythmia, or systolic blood pressure > 240 mmHg, and received standardized verbal encouragement to achieve maximal effort. Among participants included in the final analytical sample, all exercise tests were terminated upon reaching maximal exhaustion, and none were stopped prematurely because of the predefined clinical safety criteria. Respiratory gas-exchange variables were measured continuously. VO_2max was operationally defined as the mean oxygen uptake over the final eight breaths of the exercise phase [11], provided that the predefined additional criterion of maximal effort, namely a peak respiratory exchange ratio (RER) > 1.10, was met [12, 13].

Statistical analysis

Continuous variables are presented as medians with interquartile ranges (IQRs), whereas categorical variables are presented as numbers and percentages. Baseline characteristics were summarized for the entire study group and according to leisure physical activity category (< 2 h/week vs. ≥2 h/week). Continuous variables were compared using the Mann–Whitney U test, while categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate.

Associations between VO₂max/kg and selected clinical and biochemical variables were evaluated using linear regression models. In the primary analysis, the dependent variable and continuous predictors were standardized using z-scores; therefore, results for continuous variables are reported as standardized β coefficients with 95% confidence intervals (CIs). Sex was entered as a binary covariate, whereas smoking status was modeled as a categorical variable with non-smoker as the reference category.

Three regression approaches were applied. Crude models included age, male sex, or a single biomarker entered separately. Model 1 included each biomarker with adjustment for age and sex. Model 2 included each biomarker with adjustment for age, sex, smoking status, and lean mass-to-fat mass ratio. Robust standard errors were calculated using the HC3 covariance estimator. For each model, the β coefficient, 95% CI, P value, sample size, and R² were reported. Analyses were based on complete cases available for each model.

Correlations between selected continuous variables were assessed using Pearson correlation coefficients based on pairwise complete observations. To control for multiple comparisons in the correlation analyses, the Benjamini–Hochberg false discovery rate procedure was applied, and adjusted P values were reported as q values. A two-sided P value < 0.05 was considered statistically significant. Statistical analyses were performed using Python (v3.12.12) with the following libraries: pandas (v2.3.3), NumPy (v2.2.6), SciPy (v1.16.3), statsmodels (v0.14.6), and matplotlib (v3.10.0).

Results

The study included 70 apparently healthy adults, comprising 25 men and 45 women, aged 20–50 years. Among the participants, 61% were married or cohabiting, 93% were employed, and 63% had attained higher education. Thirty-four participants (49%) reported engaging in leisure-time physical activity for at least 2 h/week.

Participant characteristics for the overall sample and according to leisure-time physical activity category are presented in Table 1. No statistically significant between-group differences were observed in age, sex, current smoking status, fasting plasma glucose, HbA1c, triglycerides, total cholesterol, non-HDL cholesterol, BMI, mean or maximum handgrip strength, CAT activity, and TOS. Compared with participants reporting < 2 h/week of leisure-time physical activity, those reporting ≥ 2 h/week had significantly higher VO₂max/kg, VO₂max normalized to lean mass, LM/FM ratio, TAS, and GPx and SOD activities, as well as significantly lower WHR, %FM, OSI, and relative GDF-15 abundance (Fig. 2).

Table 1
General characteristics overall and by leisure-time physical activity group

Variables	Overall	PA < 2h/ week	PA ≥ 2h/ week	P
Age (years)	37.0 (31.0, 41.8)	37.5 (31.8, 41.0)	37.0 (30.0, 42.0)	0.672
Sex (women)	45 (64.3)	24 (66.7)	21 (61.8)	0.859
Currently smokers	13 (18.6)	9 (25.0)	4 (11.8)	0.221
VO ₂ max/kg (ml/kg/min)	23.9 (21.4, 27.7)	22.9 (20.1, 25.7)	25.9 (22.1, 32.3)	0.005
VO ₂ max/kg LM (ml/kg LM/min)	38.9 (35.0, 42.7)	37.2 (34.4, 41.7)	40.2 (36.4, 44.6)	0.039
FG (mg/dl)	95 (89–100)	92 (88.7–98.9)	95.9 (88.9–102)	0.208
HbA1C (%)	5.2 (5.0-5.4)	5.2 (5-5.3)	5.2 (5-5.5)	0.397
TG (mg/dl)	81 (64–116)	74 (60–109)	87 (65–121)	0.323
T-Chol (mg/dl)	189 (165–211)	194 (166–213)	187 (164–201)	0.340
non-HDL (mg/dl)	119 (95–149)	121 (92–151)	119 (98–145)	0.730
BMI (kg/m ²)	24.4 (22.4, 27.9)	25.1 (22.8, 29.9)	23.7 (21.9, 25.6)	0.067
WHR	0.90 (0.87, 0.97)	0.93 (0.88, 1.01)	0.88 (0.86, 0.90)	0.001
% FM	35.6 (29.9, 40.5)	37.8 (32.1, 41.4)	33.6 (23.8, 38.7)	0.022
LM/FM	1.83 (1.46, 2.44)	1.65 (1.40, 2.21)	2.12 (1.63, 3.32)	0.015

Bold indicates P < 0.05.

Data are presented as number (percentage) or median (interquartile range). PA – physical activity, VO₂ max/kg – maximal oxygen consumption relative to body total mass, VO₂ max/kg LM – maximal oxygen consumption relative to body lean mass, FG – fasting glucose, HbA1C – glycated hemoglobin, TG – triglyceride, T-Chol – total cholesterol, non-HDL – non high-density lipoprotein, BMI – body mass index, WHR - waist / hip ratio, % FM – percentage of fat mass, LM/FM – lean mass/fat mass, TAS – total antioxidative status, GPx – glutathione peroxidase, SOD – superoxide dismutase, Cat – catalase, TOS – total oxidative status, OSI – oxidative stress index.

Variables	Overall	PA < 2h/ week	PA ≥ 2h/ week	P
Handgrip strength Maximum (kg)	38.1 (29.5, 51.4)	38.2 (28.1, 47.9)	37.8 (33.5, 51.4)	0.369
Mean (kg)	32.0 (26.5, 42.1)	31.6 (23.9, 41.7)	32.6 (29.9, 42.3)	0.202
TAS (μmol/l)	244 (217, 308)	233 (213, 258)	276 (227, 317)	0.010
GPx (nmol/min/ml)	144 (90, 215)	124 (86, 176)	176 (102, 234)	0.010
SOD (U/ml)	3.1 (2.4, 4.7)	2.8 (2.3, 3.5)	3.5 (2.6, 4.7)	0.004
Cat (nmol/min/ml)	114 (84, 129)	112 (83, 118)	117 (92, 134)	0.066
TOS (μmol/l)	84 (73, 97)	86 (74, 99)	81 (68, 94)	0.196
OSI (TOS/TAS)	0.33 (0.26, 0.43)	0.37 (0.29, 0.44)	0.28 (0.23, 0.38)	0.022
GDF-15	2.0 (1.8, 2.3)	2.1 (1.9, 2.4)	1.9 (1.7, 2.1)	0.037
Bold indicates P < 0.05.				
Data are presented as number (percentage) or median (interquartile range). PA – physical activity, VO ₂ max/kg – maximal oxygen consumption relative to body total mass, VO ₂ max/kg LM – maximal oxygen consumption relative to body lean mass, FG – fasting glucose, HbA1C – glycated hemoglobin, TG – triglyceride, T-Chol – total cholesterol, non-HDL – non high-density lipoprotein, BMI – body mass index, WHR - waist / hip ratio, % FM – percentage of fat mass, LM/FM – lean mass/fat mass, TAS – total antioxidative status, GPx – glutathione peroxidase, SOD – superoxide dismutase, Cat – catalase, TOS – total oxidative status, OSI – oxidative stress index.				

Figure 3 presents the correlation matrix for the analyzed variables. VO₂max/kg was positively correlated with the LM/FM ratio, handgrip strength, and antioxidant biomarkers. Negative correlations were observed between VO₂max/kg and age, TOS, OSI, and adiposity-related measures.

The results of the linear regression analyses examining the associations between VO₂max/kg and selected clinical and biochemical variables are presented in Table 2 and Figs. 4–6. In univariable analyses, VO₂max/kg was positively associated with male sex, the LM/FM ratio, TAS, and GPx, SOD, and CAT activities, and negatively associated with age, TOS, and OSI. After adjustment for age and sex in Model 1, positive associations remained for the LM/FM ratio, TAS, and GPx, SOD, and CAT activities, whereas TOS and OSI remained negatively associated with VO₂max/kg. After further adjustment for age, sex, smoking status, and the LM/FM ratio in Model 2, TAS and GPx, SOD, and CAT activities remained positively associated with VO₂max/kg, whereas OSI remained negatively associated with VO₂max/kg.

Table 2
Linear regression models between VO₂max/kg and other variables

Variables	Model crude			Model 1 (for age and sex)			Model 2 (for age, sex, smoking, LM/FM)		
	β (95%CI)	P	R ²	β (95%CI)	P	R ²	β (95%CI)	P	R ²
Age	-0.305 (-0.552, -0.058)	0.015	0.093	-	-	-	-	-	-
Male sex	0.910 (0.394, 1.425)	< 0.001	0.193	-	-	-	-	-	-
Smoking	0.080 (-0.251, 0.410)	0.637	0.004	0.049 (-0.225, 0.322)	0.727	0.271	-	-	-
LM/FM	0.696 (0.412, 0.980)	< 0.001	0.484	0.610 (0.214, 1.007)	0.003	0.540	-	-	-
TAS	0.824 (0.683, 0.964)	< 0.001	0.679	0.736 (0.592, 0.881)	< 0.001	0.743	0.607 (0.443, 0.771)	< 0.001	0.830
GPx	0.749 (0.579, 0.919)	< 0.001	0.561	0.665 (0.504, 0.825)	< 0.001	0.667	0.548 (0.387, 0.708)	< 0.001	0.805
SOD	0.760 (0.597, 0.923)	< 0.001	0.578	0.667 (0.509, 0.825)	< 0.001	0.664	0.540 (0.375, 0.706)	< 0.001	0.789
CAT	0.659 (0.471, 0.847)	< 0.001	0.434	0.572 (0.407, 0.737)	< 0.001	0.580	0.451 (0.280, 0.622)	< 0.001	0.734
TOS	-0.306 (-0.519, -0.093)	0.005	0.094	-0.343 (-0.539, -0.147)	< 0.001	0.380	-0.132 (-0.308, 0.045)	0.143	0.570
OSI	-0.592 (-0.805, -0.380)	< 0.001	0.351	-0.557 (-0.752, -0.361)	< 0.001	0.563	-0.385 (-0.597, -0.174)	< 0.001	0.666
GDF-15	-0.149 (-0.307, 0.009)	0.064	0.022	-0.102 (-0.298, 0.093)	0.305	0.278	-0.119 (-0.345, 0.107)	0.300	0.569

Abbreviations – see Table 1.

Discussion

Regular physical activity and higher cardiorespiratory fitness are consistently associated with a lower risk of cardiovascular morbidity and mortality [7, 14, 15]. Exercise-induced adaptations involve multiple physiological pathways, including changes in vascular function, metabolism, inflammation, mitochondrial activity, and redox signaling [16]. However, the contribution of circulating redox status to the health benefits associated with physical activity and cardiorespiratory fitness remains incompletely understood.

In this cross-sectional study of apparently healthy adults aged 20–50 years participating in the Białystok PLUS study, higher aerobic capacity, assessed as body-mass-normalized VO_2max during CPET, was associated with a more favorable circulating redox biomarker profile. Higher $\text{VO}_2\text{max/kg}$ was positively associated with TAS and the activities of GPx, SOD, and CAT and inversely associated with OSI. Importantly, these associations persisted after adjustment for age, sex, smoking status, and the LM/FM ratio, suggesting that the relationships between aerobic capacity and the redox profile were evident beyond the measured differences in demographic characteristics, smoking status, and body composition. Given the cross-sectional design, however, the direction of these relationships cannot be determined.

The inverse association between $\text{VO}_2\text{max/kg}$ and OSI was retained in the fully adjusted model, whereas the association with TOS was attenuated and no longer statistically significant. OSI is a derived ratio of TOS to TAS and is intended to reflect the balance between circulating oxidant burden and antioxidant capacity [17]. In the present study, its relationship with aerobic capacity may partly reflect the strong positive association between $\text{VO}_2\text{max/kg}$ and TAS. Accordingly, TAS, TOS, and OSI should be interpreted as complementary components of the circulating redox profile rather than as fully independent indicators. Similarly, the correlations among TAS and the activities of GPx, SOD, and CAT suggest that these measures may capture related aspects of antioxidant defense. Taken together, the consistent direction of these associations supports the presence of a coordinated relationship between aerobic capacity and circulating antioxidant status.

Previous studies examining the relationship between aerobic capacity and redox biomarkers in healthy populations have produced heterogeneous findings. Sayar et al. [8] reported a weak positive association between VO_2max and total antioxidant capacity in healthy young men but found no significant relationships with SOD or CAT activities or with a marker of lipid peroxidation. Differences between that study and the present analysis may be related to participant age, sex distribution, habitual physical activity, aerobic fitness, biomarker selection, or laboratory methodology. Galván-Álvarez et al. [18] reported an association between higher aerobic fitness and greater basal expression of antioxidant-related proteins in skeletal muscle, particularly SOD2, in a heterogeneous adult population. Although measurements obtained from skeletal muscle cannot be directly compared with circulating enzyme activities, these findings support the concept that aerobic fitness may be linked to the regulation of endogenous antioxidant systems. Associations between aerobic capacity and redox-related biomarkers have also been reported in older adults [19] and in clinical populations, including patients with chronic obstructive pulmonary disease and heart failure [20, 21]. Despite differences in study populations and

redox assessment methods, these observations are broadly consistent with a relationship between aerobic capacity and oxidative–antioxidant homeostasis.

Participants reporting at least 2 h/week of leisure-time physical activity had higher $\text{VO}_2\text{max/kg}$, VO_2max normalized to lean mass, TAS, GPx activity, and SOD activity and lower OSI than participants reporting less than 2 h/week. They also had a lower WHR and body fat percentage and a higher LM/FM ratio. These findings are consistent with the expected relationships between habitual leisure-time physical activity, aerobic fitness, and body composition and further suggest that a more active lifestyle may be accompanied by a more favorable circulating redox profile. The physical activity measure was self-reported and did not provide detailed information on exercise intensity, total physical activity, or sedentary behavior. In addition, the 2-h/week threshold reflected the structure of the questionnaire response categories and should therefore be interpreted as an analytical grouping rather than as a specific activity dose corresponding to current physical activity guidelines.

Body composition may also contribute to the observed relationships with redox biomarkers. Excess adipose tissue is associated with chronic low-grade inflammation, mitochondrial dysfunction, and increased oxidative stress [22]. Conversely, individuals with higher aerobic fitness frequently have a lower proportion of body fat and a higher proportion of metabolically active lean tissue. The differences in WHR, percentage fat mass, and LM/FM ratio between the physical activity groups may therefore represent one of the pathways linking habitual physical activity with redox status. At the same time, body composition may act both as a confounding factor and as an intermediate component of this relationship. The persistence of several associations after adjustment for the LM/FM ratio indicates that the observed relationships between aerobic capacity and redox biomarkers were not solely attributable to differences in body composition. Because $\text{VO}_2\text{max/kg}$ is expressed relative to total body mass, however, some relationships with adiposity-related variables may also be influenced by ratio normalization [23].

Evidence from intervention studies indicates that the effects of exercise on oxidative stress depend on exercise intensity, duration, frequency, training status, and the timing of biological sample collection. Acute exercise can transiently increase reactive oxygen species production, whereas repeated exercise-induced redox signaling may stimulate endogenous antioxidant adaptations [5, 6]. Meta-analyses by Ye et al. [24], Wang et al. [25], and Xie et al. [26] suggest that regular exercise may modify enzymatic and non-enzymatic antioxidant defenses, although the magnitude and direction of these effects vary across populations, exercise protocols, and biomarkers. The present findings are compatible with this adaptive framework and suggest that higher aerobic capacity and greater habitual physical activity may be accompanied by a more favorable resting redox profile. Longitudinal and intervention studies will be valuable in clarifying the relative contributions of habitual exercise, intrinsic fitness, body composition, and other lifestyle-related factors to these associations.

Participants reporting at least 2 h/week of leisure-time physical activity also had lower relative GDF-15 abundance expressed on the Olink NPX $\log_2$ scale. Although GDF-15 was not independently associated

with $\text{VO}_2\text{max/kg}$ after multivariable adjustment in the present study, Enarsson et al. [10] reported an inverse association between cardiorespiratory fitness and circulating GDF-15 in a larger population-based sample of 50-year-old adults, which persisted after adjustment for cardiovascular risk factors and body composition. Differences in sample size, age distribution, normalization of aerobic capacity, and covariate structure may contribute to the differences between studies. GDF-15 is a stress-responsive protein associated with oxidative and mitochondrial stress, inflammation, cardiometabolic abnormalities, and adverse cardiovascular outcomes [9, 27, 28]. The lower relative GDF-15 abundance observed among participants reporting more leisure-time physical activity is therefore consistent with a potentially lower chronic metabolic or cellular stress burden. Because this group comparison was exploratory, further studies are warranted to clarify the relationships among physical activity, aerobic capacity, GDF-15, and circulating redox biomarkers.

The present study extends previous observations by combining objective CPET-based assessment of aerobic capacity, DXA-derived body composition, and simultaneous measurement of several circulating oxidative and antioxidant biomarkers in adults without clinically manifest cardiovascular disease. The findings show a consistent association between higher aerobic capacity and a more favorable circulating redox biomarker profile and show that participants reporting greater leisure-time physical activity also exhibited more favorable redox and body-composition characteristics. These observations provide additional insight into redox-related pathways that may contribute to the established relationship between aerobic fitness, physical activity, and cardiovascular health.

Study strengths and limitations

This study combined the objective assessment of aerobic capacity by cardiopulmonary exercise testing with detailed DXA-based body composition analysis and the simultaneous measurement of multiple oxidative and antioxidant biomarkers. Participants were drawn from the population-based Białystok PLUS study and examined using standardized procedures. Several limitations should nevertheless be acknowledged. The cross-sectional design precludes causal inference, and the relatively small sample may limit the generalizability of the findings. Leisure-time physical activity was self-reported and may therefore have been affected by recall bias or misclassification. In addition, VO_2max was defined using secondary maximal-effort criteria rather than confirmation of a VO_2 plateau.

Conclusion

Our results indicate that higher aerobic capacity and at least 2 h/week of self-reported leisure-time physical activity were associated with a more favorable systemic redox balance, potentially benefiting cardiovascular health. However, because of the cross-sectional design, causality, the underlying mechanisms, and clinical significance cannot be established. Longitudinal and intervention studies are needed to determine whether improving aerobic capacity or increasing physical activity enhances redox homeostasis and whether these changes translate into improved cardiovascular health.

Declarations

Conflict of interest declaration

The authors declare no conflict of interest.

Authors' contributions

Kinga Zujko-Kowalska: writing – original draft, conceptualization, methodology, investigation, formal analysis; **Małgorzata Elżbieta Zujko:** methodology, investigation, formal analysis; **Wojciech Łaguna:** software; **Marlena Dubatówka:** investigation; **Jacek Jamiołkowski:** methodology; **Paweł Sowa:** methodology; **Marcin Kondraciuk:** investigation; **Anna Szpakowicz:** methodology; **Łukasz Małek:** writing – review and editing, supervision; **Karol Kamiński:** writing – review & editing, data curation, project administration, supervision.

Acknowledgements

Not applicable.

Funding

This research was funded by Medical University of Białystok, grant number B.SUB.25.285. This publication is part of the project “Characteristics of the development of multimorbidity and its impact on functioning in society – distant observation of Białystok PLUS”, funded from the state budget by the Medical Research Agency ([https:// www.abm.gov.pl/](https://www.abm.gov.pl/)) under the grant agreement dated April 15, 2024, Project No. 2023/ABM/03/000024-00.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical approval and consent to participate

The study was conducted in accordance with the ethical principles of the World Medical Association Declaration of Helsinki and was approved by the Ethics Committee of the Medical University of Białystok on March 31, 2016 (approval no. R-I-002/108/2016). All participants were informed about the study protocol and objectives and provided written informed consent prior to participation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

1. Shaito, A. et al. Oxidative stress-induced endothelial dysfunction in cardiovascular diseases. *Front. Biosci. (Landmark Ed)*. **27** (3), 105 (2022).
2. Tsirebolos, G. et al. Emerging markers of inflammation and oxidative stress as potential predictors of coronary artery disease. *Int. J. Cardiol.* **376**, 127–133 (2023).
3. Bull, F. C. et al. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *Br. J. Sports Med.* **54** (24), 1451–1462 (2020).
4. Strain, T. et al. National, regional, and global trends in insufficient physical activity among adults from 2000 to 2022: a pooled analysis of 507 population-based surveys with 5·7 million participants. *Lancet Glob Health.* **12** (8), e1232–e1243 (2024).
5. Powers, S. K., Radak, Z., Ji, L. L. & Jackson, M. Reactive oxygen species promote endurance exercise-induced adaptations in skeletal muscles. *J. Sport Health Sci.* **13** (6), 780–792 (2024).
6. Lu, Y., Wiltshire, H. D., Baker, J. S. & Wang, Q. Effects of high intensity exercise on oxidative stress and antioxidant status in untrained humans: A systematic review. *Biology (Basel)*. **10** (12), 1272 (2021).
7. Ross, R. et al. Stroke Council. Importance of Assessing Cardiorespiratory Fitness in Clinical Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement From the American Heart Association. *Circulation* **134** (24), e653–e699 (2016).
8. Sayar, K., Bamosa, A., Al-Asoom, L., Salem, A. M. & Muaidi, Q. Association between VO₂max and antioxidant capacity in healthy young Saudi men. *J. Public. Health Res.* **14** (4), 22799036251407364 (2025).
9. di Candia, A. M., de Avila, D. X., Moreira, G. R., Villacorta, H. & Maisel, A. S. Growth differentiation factor-15, a novel systemic biomarker of oxidative stress, inflammation, and cellular aging: Potential role in cardiovascular diseases. *Am. Heart J. Plus.* **9**, 100046 (2021).
10. Enarsson, M. et al. Association between cardiorespiratory fitness and circulating proteins in 50-year-old Swedish men and women: a cross-sectional study. *Sports Med. Open.* **7** (1), 52 (2021).
11. Johnson, J. S., Carlson, J. J., VanderLaan, R. L. & Langholz, D. E. Effects of sampling interval on peak oxygen consumption in patients evaluated for heart transplantation. *Chest* **113** (3), 816–819 (1998).
12. Edvardsen, E., Hem, E. & Anderssen, S. A. End criteria for reaching maximal oxygen uptake must be strict and adjusted to sex and age: a cross-sectional study. *PLoS One.* **9** (1), e85276 (2014).
13. Poole, D. C., Wilkerson, D. P. & Jones, A. M. Validity of criteria for establishing maximal O₂ uptake during ramp exercise tests. *Eur. J. Appl. Physiol.* **102** (4), 403–410 (2008).
14. Singh, B. et al. Comparison of objectively measured and estimated cardiorespiratory fitness to predict all-cause and cardiovascular disease mortality in adults: A systematic review and meta-

- analysis of 42 studies representing 35 cohorts and 3.8 million observations. *J. Sport Health Sci.* **14**, 100986 (2025).
15. Garcia, L. et al. Non-occupational physical activity and risk of cardiovascular disease, cancer and mortality outcomes: a dose-response meta-analysis of large prospective studies. *Br. J. Sports Med.* **57** (15), 979–989 (2023).
 16. Qiu, Y. et al. Exercise sustains the hallmarks of health. *J. Sport Health Sci.* **12** (1), 8–35 (2023).
 17. Sánchez-Rodríguez, M. A. & Mendoza-Núñez, V. M. Oxidative stress indexes for diagnosis of health or disease in humans. *Oxid. Med. Cell. Longev.* **2019**, 4128152 (2019).
 18. Galvan-Alvarez, V. et al. Antioxidant enzymes and Nrf2/Keap1 in human skeletal muscle: Influence of age, sex, adiposity and aerobic fitness. *Free Radic Biol. Med.* **209** (Pt 2), 282–291 (2023).
 19. Rosado-Pérez, J. & Mendoza-Núñez, V. M. Relationship between aerobic capacity with oxidative stress and inflammation biomarkers in the blood of older Mexican urban-dwelling population. *Dose Response.* **16** (2), 1559325818773000 (2018).
 20. Genç, A. et al. Association analyses of oxidative stress, aerobic capacity, daily physical activity, and body composition parameters in patients with mild to moderate COPD. *Turk. J. Med. Sci.* **44** (6), 972–979 (2014).
 21. Lazar-Poloczek, E. et al. Ceruloplasmin as redox marker related to heart failure severity. *Int. J. Mol. Sci.* **22** (18), 10074 (2021).
 22. Bosch-Sierra, N. et al. The impact of weight loss on inflammation, oxidative stress, and mitochondrial function in subjects with obesity. *Antioxid. (Basel).* **13** (7), 870 (2024).
 23. Lee, J. & Zhang, X. Is there really a proportional relationship between VO₂max and body weight? A review article. *PLoS One.* **16** (12), e0261519 (2021).
 24. Ye, Y. et al. The effects of aerobic exercise on oxidative stress in older adults: a systematic review and meta-analysis. *Front. Physiol.* **12**, 701151 (2021).
 25. Wang, Y. et al. Effects of physical exercise on biomarkers of oxidative stress in healthy subjects: A meta-analysis of randomized controlled trials. *Open. Life Sci.* **18** (1), 20220668 (2023).
 26. Xie, Y., Gu, Y., Li, Z., Zhang, L. & Hei, Y. Effects of exercise on different antioxidant enzymes and related indicators: a systematic review and meta-analysis of randomized controlled trials. *Sci. Rep.* **15** (1), 12518 (2025).
 27. Kim, K. H. & Lee, M. S. GDF15 as a central mediator for integrated stress response and a promising therapeutic molecule for metabolic disorders and NASH. *Biochim. Biophys. Acta Gen. Subj.* **1865** (3), 129834 (2021).
 28. Conte, M. et al. GDF-15 plasma level is inversely associated with level of physical activity and correlates with markers of inflammation and muscle weakness. *Front. Immunol.* **11**, 915 (2020).

Figures

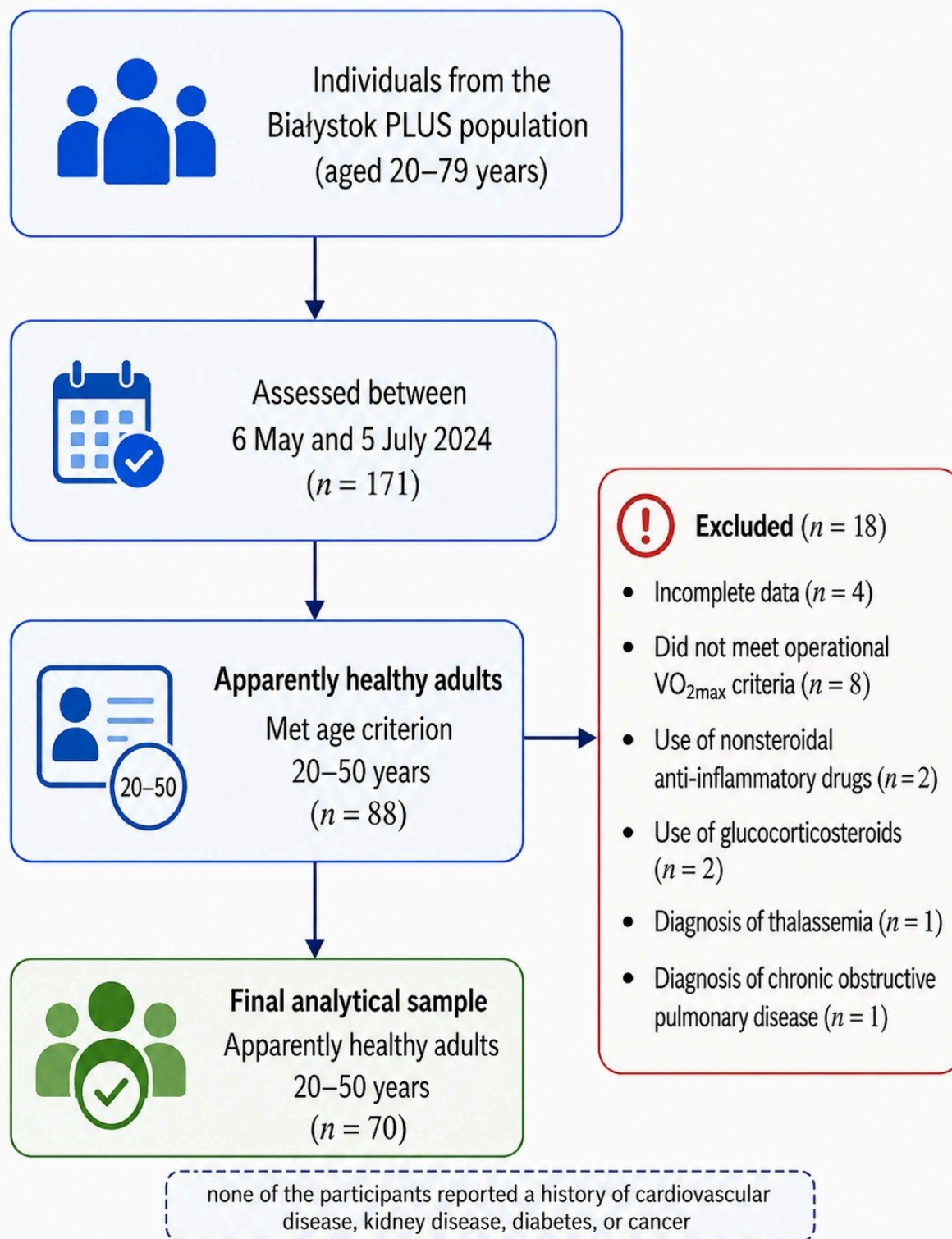


Figure 1

Flow chart of participant selection.

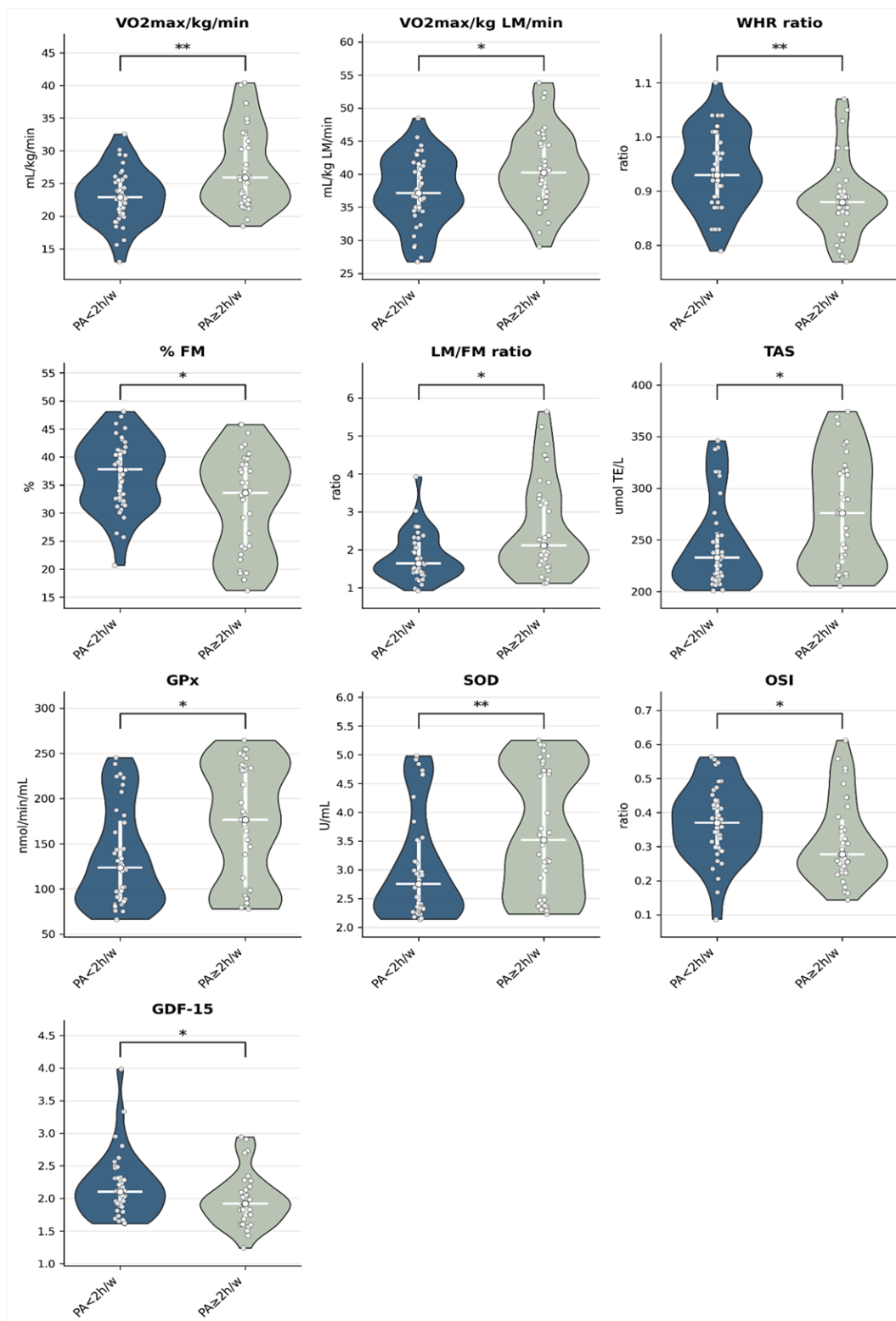
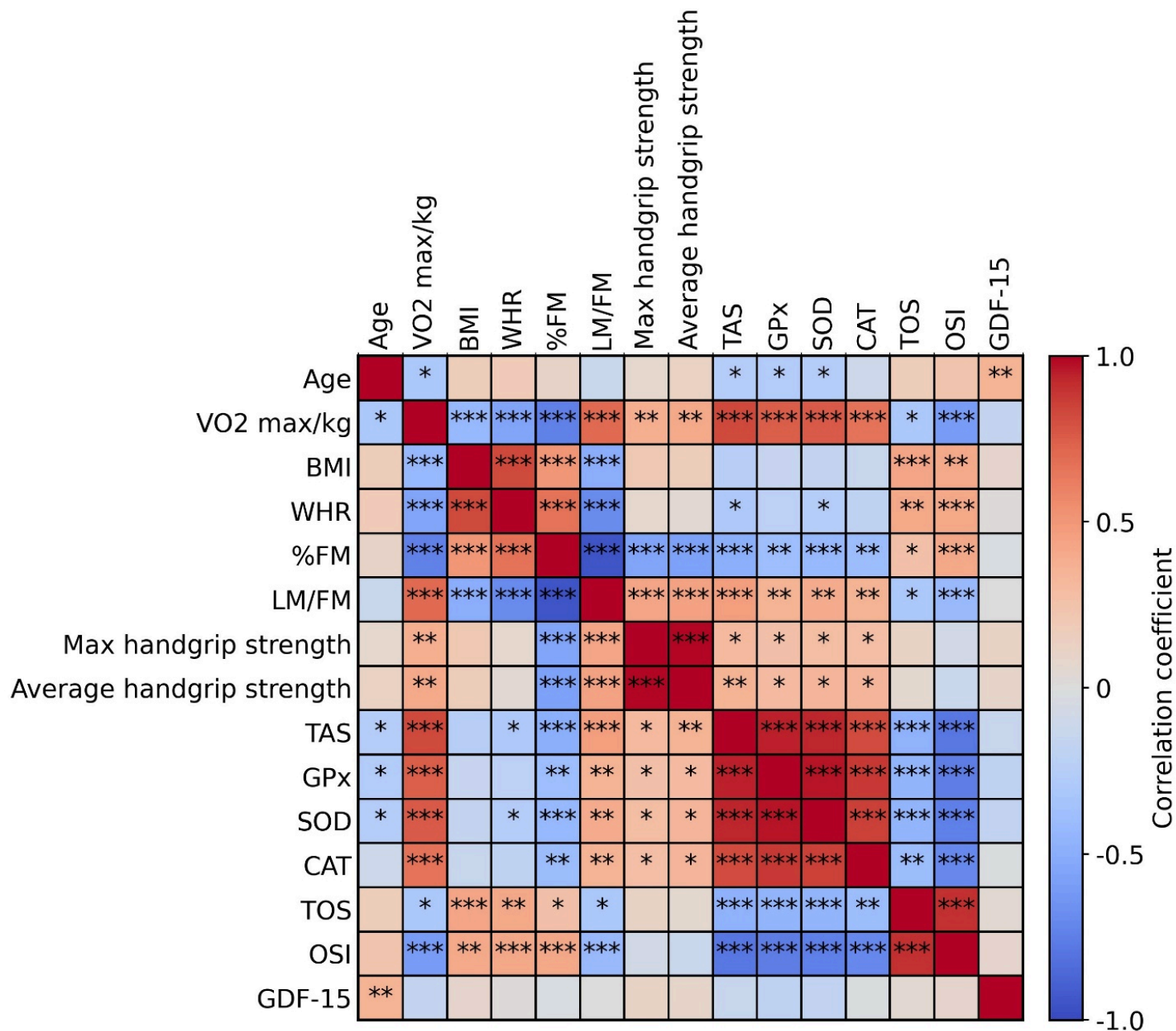


Figure 2

Significant differences in the analyzed parameters depending on leisure-time physical activity.



Symbols denote Benjamini-Hochberg FDR-adjusted P values: * $q < 0.05$, ** $q < 0.01$, *** $q < 0.001$

Figure 3

Heatmap of correlations between analyzed parameters.

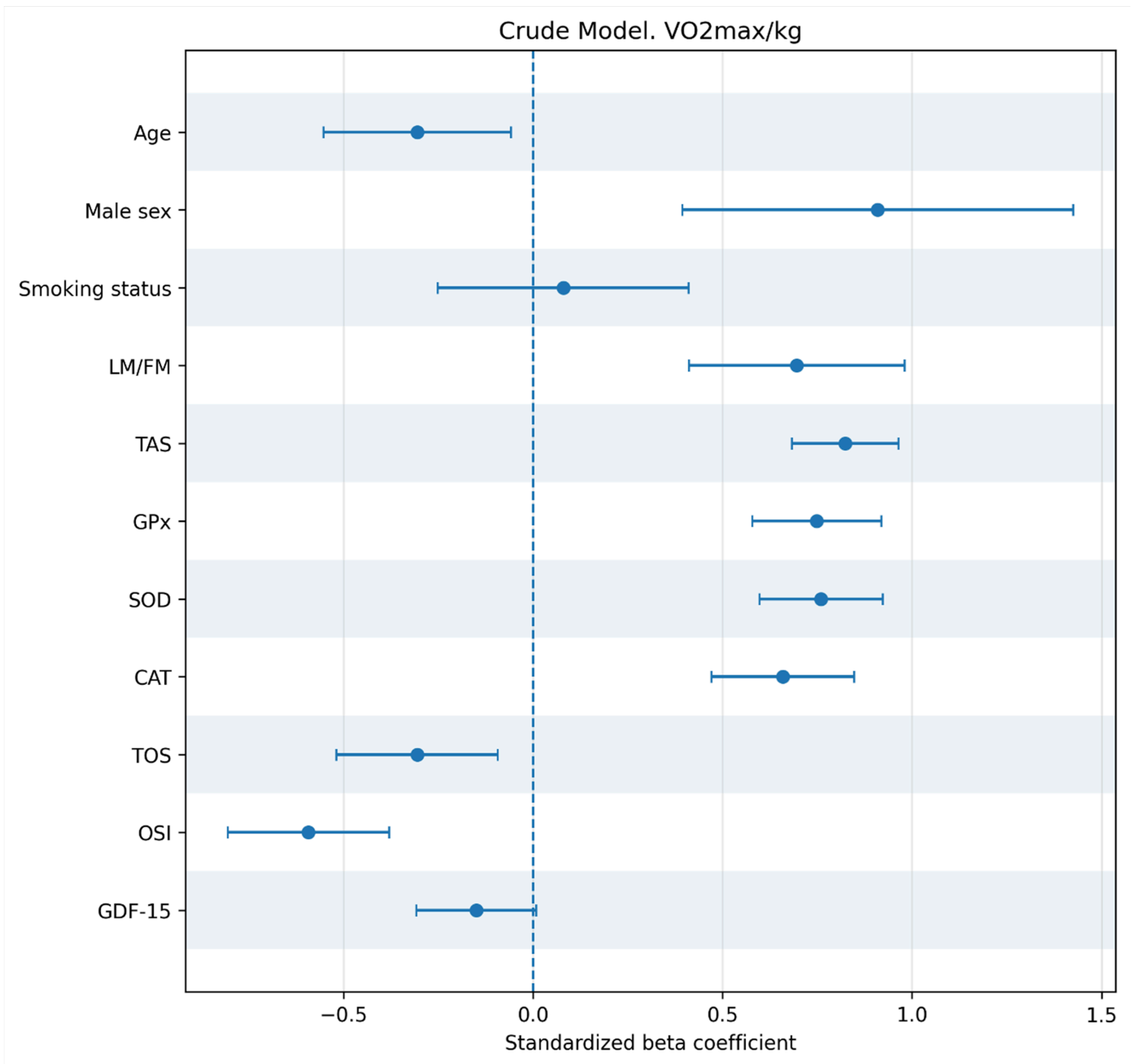


Figure 4

The results of linear regression models of the associations between VO₂max/kg and selected clinical and biochemical variables (crude model).

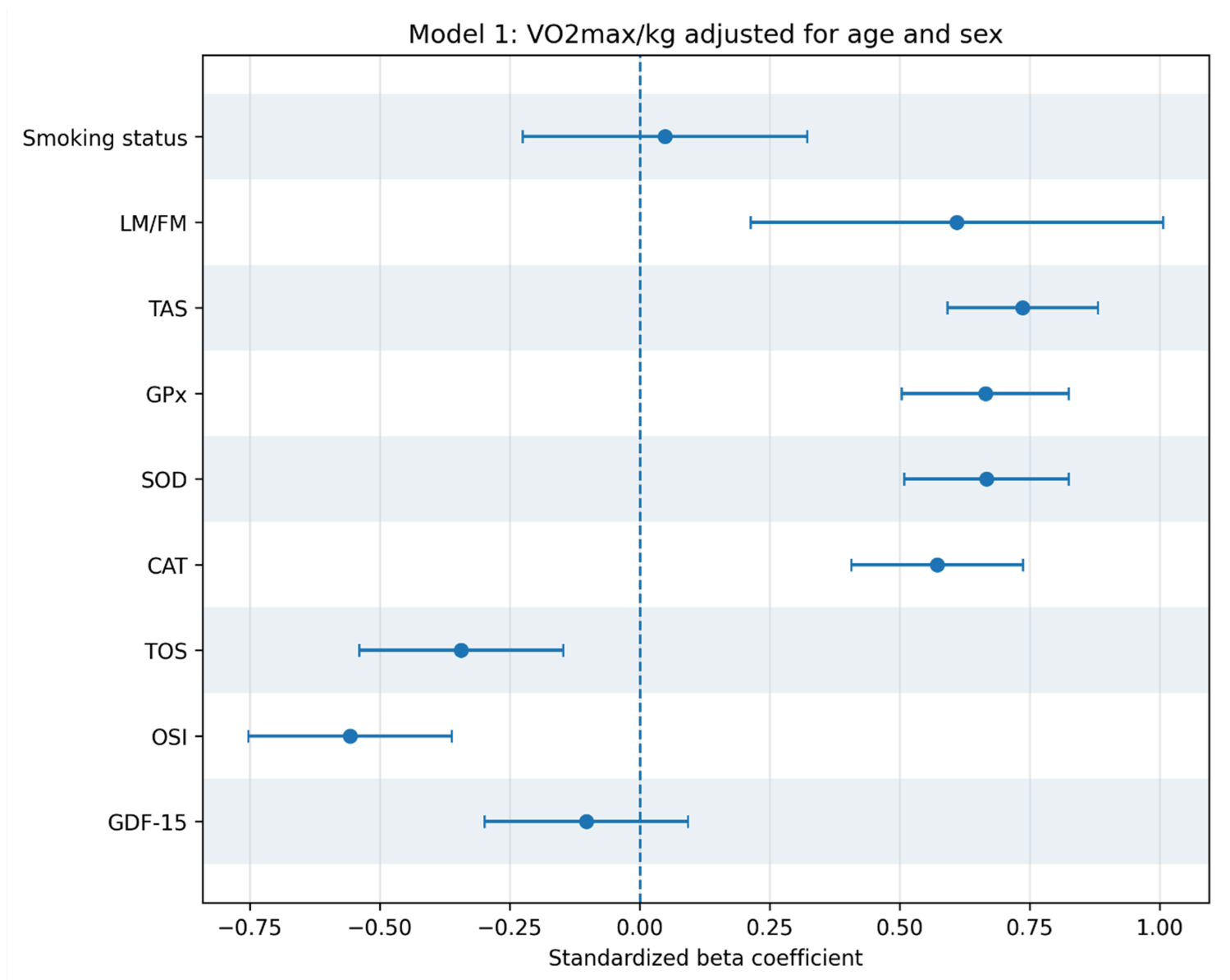


Figure 5

The results of linear regression models of the associations between VO₂max/kg and selected clinical and biochemical variables (model 1).

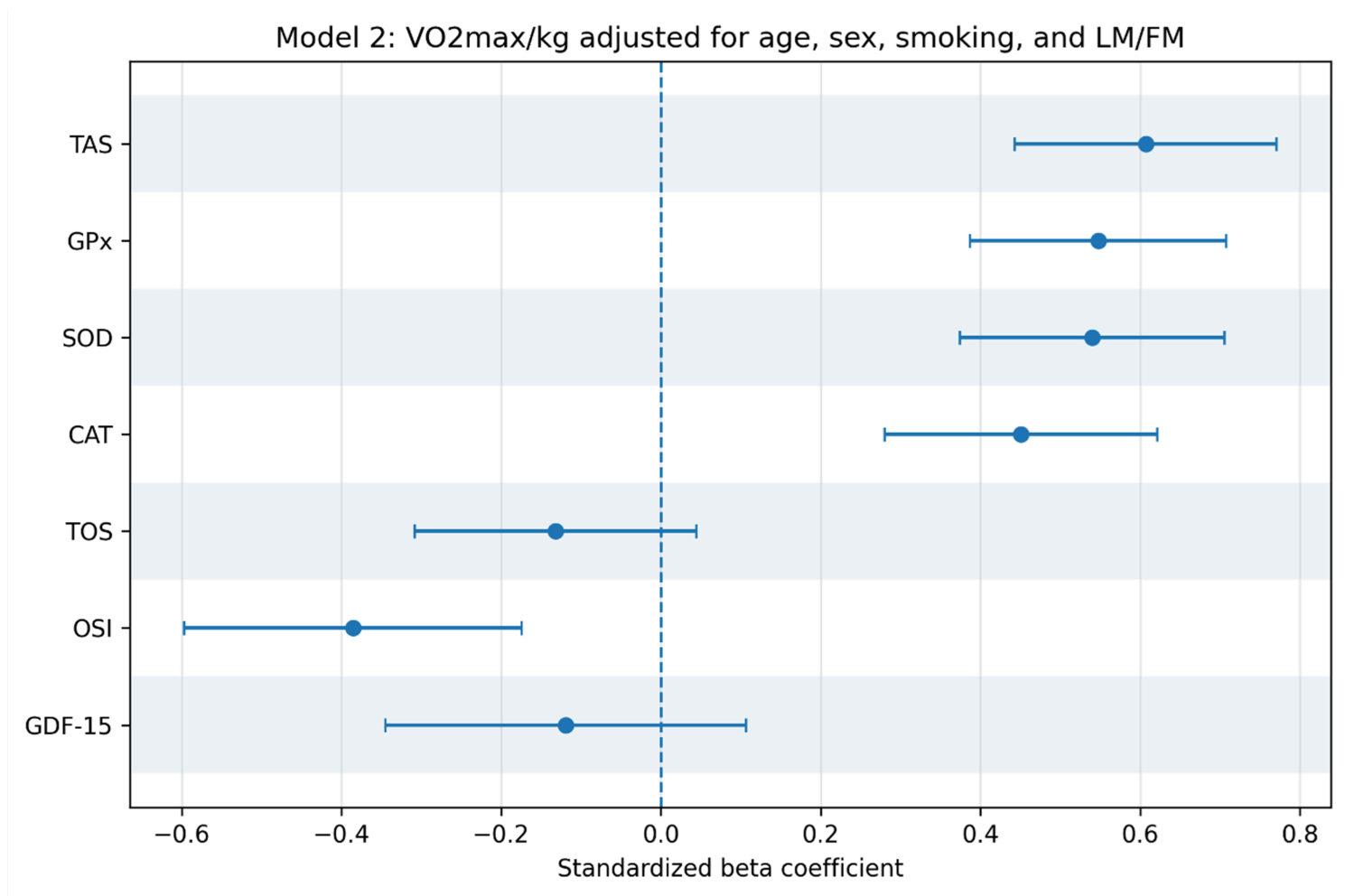


Figure 6

The results of linear regression models of the associations between VO₂max/kg and selected clinical and biochemical variables (model 2).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Graphicalabstract.tif](#)