Journal of Translational Medicine

RESEARCH

Open Access

Exercise reveals precocious-subclinical cardiovascular and muscular dysfunction in transfusion dependent beta-thalassemia (TDT) patients without cardiac iron overload

Michele Braggio[1], Jacopo Ceolan[3], Arianna Minoia[3], Simone Villaboni[3], Filippo Mazzi[3], Enrica Federti[3], Maria Teresa Valenti[2], Lucia De Franceschi[3], Luca Dalle Carbonare[3*] and Mattia Cominacini[3]

Abstract

Background Transfusion-dependent β-thalassemia (TDT) is characterized by reduced exercise capacity due to chronic hemolytic anemia, iron overload cardiomyopathy, and potential skeletal muscle alterations. Early cardiovascular and muscular dysfunction in the absence of cardiac iron overload remains poorly characterized.

Methods In this exploratory study, 9 TDT patients without cardiac iron overload and with normal body mass index (BMI) were matched with 9 healthy controls. Cardiopulmonary exercise testing combined with lung ultrasound (LUS-CPET) was used to assess exercise-induced lung congestion and diastolic dysfunction. Serum biomarkers of endothelial activation (ICAM-1) and muscle–metabolic function (FGF21, miR-577) were evaluated. Resting echocardiography, handgrip strength, and body composition by bioelectrical impedance analysis (BIA) were also assessed.

Results At rest, cardiac output and diastolic function were comparable between groups. However, during LUS-CPET, TDT patients exhibited significantly lower VO_2max, O_2 pulse, and chronotropic index, along with increased B-lines, consistent with exercise-induced diastolic dysfunction. TDT patients also showed reduced lean body mass and handgrip strength. FGF21 levels were elevated at rest and post-exercise, whereas miR-577 levels were reduced. Post-exercise ICAM-1 levels were significantly higher in TDT patients compared to controls.

Conclusions LUS-CPET may represent a useful non-invasive approach to identify early exercise-induced diastolic dysfunction in TDT patients without cardiac iron overload. Altered FGF21 and miR-577 profiles suggest the presence of metabolic dysregulation associated with early skeletal muscle deconditioning.

*Correspondence:
Luca Dalle Carbonare
luca.dallecarbonare@univr.it
[1]Department of Diagnostics and Public Health, University of Verona, Verona, Italy
[2]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy
[3]Department of Engineering for Innovation Medicine, University of Verona, Verona, Italy and Azienda Ospedaliera Integrata Verona, Verona, Italy

© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Introduction

Transfusion dependent thalassemia (TDT) is a worldwide distributed hereditary red cell disorder. The main clinical manifestations are chronic hemolytic anemia and ineffective erythropoiesis both link to oxidation and abnormal iron homeostasis [1, 2]. Chronic transfusion regimen requires a personalized iron chelation therapy to effectively reduce iron burden [3]. The cytotoxic organ iron accumulation might contribute to organ damage, resulting in liver and heart disease. Patients' quality of life is deeply affected by fatigue and muscle weakness which generally appears before heart iron-overload and might be related the cumulative effect of different factors such as muscle waste-associated decrease muscle strength, osteoporosis, vitamin D deficiency and chronic anemia [4–6].

Up to now there is a lack of knowledge on muscular performance and early cardiovascular disease in TDT patients beyond cardiac iron overload, with particular regard to how nutritional status and skeletal muscle phenotype contribute to exercise intolerance. Of note, Pennell et al. reported that TDT patients develop an adaptive cardiac physiology, which can lead to increasing cardiac output to maintain oxygen delivery, in absence of cardiac iron loading [7]. Indeed, recent evidence indicates that pathological heart remodeling and myocardial dysfunction might appear before heart iron overload in both mouse model and patients with TDT [8, 9]. Myocardial fibrosis and iron-induced electrical conduction disturbances can lead to arrhythmias [10]. and altered chronotropic response to exercise [11, 12]. Previous studies have shown that TDT patients have reduced cardiovascular fitness and exercise capacity in terms of maximum oxygen consumption (VO_2max), which has been related to anemia, iron overload cardiomyopathy, and possible muscular deconditioning, however, the extent and clinical characterization of early muscle-nutritional impairment (e.g., lean mass, strength and physical activity) and its relationship with exercise-induced cardiovascular abnormalities in TDT remains insufficiently defined [13–15]. Sarcopenia and low body mass index are also reported [16–19].

Identifying early functional changes before structural cardiomyopathy develops remains a clinical challenge. Recent evidence highlights the prognostic capability of lung ultrasound combined with exercise testing in heart failure with preserved ejection fraction (HFpEF) [20]. In addition, B-lines is emerging as excellent surrogate for pulmonary capillary wedge pressure (PCWP) and diastolic dysfunction with HFpEF triggered by exercise testing [21]. This might be corroborated by soluble markers of pro-inflammatory and shear stress vascular response to exercise such as intercellular adhesion molecule-1 (ICAM-1) [22]. Indeed, increased circulating ICAM-1 has been associated with atherosclerosis [23], myocardial infarction, heart failure and inflammatory cardiomyopathies [24]. In addition, Vinchi et al. have recently described increased markers of inflammatory vasculopathy in TDT patients, reaching values similar to those described in individuals with sickle cell disease [25]. Inflammatory vasculopathy might be a still an underrecognized factor in the pathogenesis of TDT related myocardial dysfunction. This is extremely interesting since mild chronic inflammation has been also related to cardiometabolic disease [26]. Although ICAM-1 is not a unique marker of inflammation, its up-regulation mirrors chronic vascular stress that may compromise skeletal-muscle perfusion and contribute to functional deconditioning and possible senescence [27–29]. Of note, increased ICAM-1 expression is also linked to physical frailty and sarcopenia [30].

Fibroblast growth factor 21 (FGF21) is another stress biomarker involved in muscle metabolism and homeostasis, as well as pathological cardiac remodeling [31]. This is strictly dependent on FGF21 function: elevated FG21 accompanies metabolic derangements affecting energy substrate metabolism. In addition, FGF21 might increase in response to exercise, allowing to explore muscle dysfunction [32]. FGF21 level might be affected by micro-RNA miR-577, which downregulates FGF21 expression targeting 3'UTR, proved by the reduction in protein kinase B levels [33].

Here, we explored whether LUS-CPET combined with a multidisciplinary approach might be a novel tool to evaluate early cardiovascular disease and muscular dysfunction in patients with TDT without cardiac iron overload.

Methods

Study design and statistical analysis

The study conducted was a cross-sectional observational study. β-thalassemic transfusion-dependent (TDT) patients were recruited, undergoing regular follow-up in an outpatient or Day Hospital setting at the Unit of General Medicine in Verona (Italy). Patients were evaluated approximately 10 days after the last transfusion. All TDT patients routinely undergo resting echocardiography and cardiac and upper abdominal magnetic resonance imaging (MRI) with specific sequences for iron accumulation detection. For the study, the values of the latest echocardiogram and the latest cardiac and upper abdominal MRI were considered. Inclusion criteria were as follows: stable TDT outpatients undergoing regular transfusions every 14–21 days, receiving 2–3 units of packed red blood cells (PRBCs) per transfusion; normal body mass index (BMI 18.5 - 24.9 kg/m^2). Patients with heart iron overload (T2* values < 20 ms) or reduced ejection fraction were excluded, as well as those unable to perform CPET,

or falling under absolute contraindications for the test [34]. Patients with changes in therapy within the previous 12 months were excluded. Only adult subjects were enrolled. A total of 9 TDT patients were evaluated (5 M, 4 F). Nine HC (5 M, 4 F) were recruited, matched for gender, age, and BMI with the patients.

Cardiopulmonary Exercise Test (CPET)

All subjects underwent a cycle ergometer (E100, COSMED; Milan, Italy) cardiopulmonary exercise test (CPET) following the latest American Thoracic Society guidelines [34]. All CPET exams were conducted and interpreted by a single experienced operator (MB). Details are reported in Supplementary materials.

Transthoracic Lung Ultrasound (LUS)

LUS was performed following the method described by the Italian Society of Echocardiography (SIEC) [35]. LUS assessments were performed by the same experienced operator, thereby minimizing inter-observer variability (MC). LUS findings were quantitatively integrated with CPET by assessing the change in B-lines from rest to peak exercise. Details are reported in Supplementary materials.

Anthropometric measurements and Bioelectrical Impedance Analysis (BIA)

In all subjects, weight and height were measured. Main anthropometric circumferences were taken by an experienced operator (MB): waist circumference was measured at the midpoint between the lowest rib and the iliac crest; hip circumference was measured at the level of the greater trochanters at the widest portion of the buttocks; thigh circumference was assessed at the midpoint between the inguinal crease and the proximal border of the patella. All measurements were obtained in duplicate using a non-elastic tape, with the subject standing in a relaxed position, and the mean value was used for analysis [36]. BIA (InBody 120, USA, Cerritos, CA, USA) measured percentage of body fat (FM) and lean body mass (LBM), along with the absolute values of both parameters. Fat-free mass index (FFMI) was calculated as fat-free mass (kg) divided by height squared (m^2), derived from BIA. Two analyses were taken each time for each subject and the mean value was reported. All subjects were asked to avoid strenuous exercise the day before BIA. Measurements were obtained with subjects in a resting state, in light clothing, and after bladder emptying.

International Physical Activity Questionnaire (IPAQ)

The International Physical Activity Questionnaire (IPAQ) in its brief version was administered to assess the levels of physical activity undertaken by the subjects and estimate weekly metabolic equivalents of task (METs) [37].

Pre- and post-exercise soluble markers

Serum samples were collected before and at 30 minutes after LUS-CPET. Enzyme-linked immunosorbent assay (ELISA) was used to measure ICAM-1, as a marker of vascular activation, and FGF21, a marker of metabolic and myocellular stress.

miRNA extraction and reverse transcription

Serum miRNA577 and 199 were extracted using the miRNeasy® protect mini kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Details are reported in Supplementary materials.

Gene expression analysis-real-time PCR

The Real-Time PCR was performed using the TaqMan Universal PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) and TaqMan pre-designed probes for each gene (Table 1s). Next, The MicroAmp Optical 96-Well Reaction Plate (applied biosystems, Thermo Fisher Scientific, Waltham, MA, USA) was used to load the 19 µL of PCR mix into the wells that were designed. Next, to get a final volume of 20 µL, 1 µL of cDNA was added. Details are reported in Supplementary materials.

Statistics

Statistical analysis was performed using Jamovi software version 2.3.28.0 (Sydney, Australia). The analysis of quantitative variables was conducted using Student's t-test for variables with homogeneous variance. When the variance was not homogeneous, the Mann-Whitney test was used. Correlation analyses were performed using the Spearman test. The Kruskal-Wallis one-way ANOVA test was employed for nominal variables for events with a sample size > 5. A formal a priori power calculation was not performed due to the exploratory nature of the study. For all analyses, significance was set at $p < 0.05$.

Results

At rest, TDT patients without cardiac iron overload display no compensatory hyperdynamic state in response to chronic anemia

Table 1 shows the characteristics of studied TDT patients and healthy controls (HC), who were gender, age and BMI-matched. TDT patients were regularly transfused on average every 19 ± 2.2 days with 2.1 ± 0.2 units of PRBCs. Patients were evaluated at 8.7 ± 1.3 days after the last transfusion and the day of evaluation Hb was 10.2 ± 0.9 g/dL. All TDT patients were on iron chelation therapy with deferasirox (mean stable dosage 750 mg/day) or deferiprone (mean stable dosage 1,250 mg/day). Mean ferritin was 498.2 ± 321.5, indicating an optimal iron chelation state. Of note 2 patients were on Luspatercept (mean stable dosage 1 mg/kg every 3 weeks) and 2 patients were under beta-blockers therapy due to

Table 1 Demographic, clinical and laboratory characteristics of study subjects

	TDT	HC	p
Gender (M/F)	5/4	5/4	-
Age (years)	40.4 ± 7.9	37.9 ± 12.2	0.54
BMI (kg/m^2)	22.4 ± 2.9	22.9 ± 3.9	0.76
Hb (g/dL)	10.2 ± 0.9	12.9 ± 0.8	**<0.001**
RDW (%)	16.1 ± 1.9	12.5 ± 0.8	**<0.001**
Plts (10^3/mm^3)	515.3 ± 255.3	293.7 ± 95.2	**0.022**
WBC (10^3/mm^3)	10.15 ± 2.88	7.61 ± 2.33	**0.032**
Creatinine (mg/dL)	0.78 ± 0.16	0.81 ± 0.22	0.26
Ferritin (ng/mL)	498.2 ± 321.5	195.7 ± 76.2	**0.021**
ALT (U/L)	18.4 ± 8.3	16.9 ± 5.6	0.40
Transfusion interval (days)	19.0 ± 2.2	-	-
PRBC (units)	2.1 ± 0.2	-	-
Deferasirox	9/9	-	-
Luspatercept	2/9	-	-
Deferiprone	1/9	-	-
Myocardial T2* (ms)	33.8 ± 2.1	-	-
Hepatic T2* (ms)	10.9 ± 6.7	-	-
Pancreatic T2* (ms)	15.6 ± 9.2	-	-
Splenectomy	7/9	-	-
Hypogonadism	2/9	-	-
Levothyroxine	3/9	-	-
Acetylsalicylic Acid (100mg)	6/9	-	-
Cholecalciferol	9/9	-	-

Note: significant p-values are printed in bold. Hb: Hemoglobin; RDW: Red Cell Distribution Width; Plt: Platelets; WBC: White Blood Cells; ALT: Alanine Aminotransferase; PRBC: packed red blood cells; T2*: magnetic resonance effective transverse relaxation time

symptomatic sinusoidal tachycardia (bisoprolol 1.25 mg daily). Concerning endocrinopathies related to TDT, 2 patients received replacement therapy for hypogonadotropic hypogonadism; 3 patients received levothyroxine for hypothyroidism. No patients had diabetes. Six patients were on 100 mg acetylsalicylic acid for thrombocytosis [38]. All patients regularly took cholecalciferol (mean stable dosage 100,000 UI monthly) for osteoporosis in combination with either zoledronic acid 4 mg yearly or denosumab 60 mg every 6 months. All patients were asymptomatic at rest under their usual therapy. In MRI for iron overload conducted within 12 months of evaluation, no myocardial iron accumulation, defined by T2* values < 20 ms, was observed in any patient in the T2*-weighted sequences (range: 36.7–31.2 ms; 1). Four TDT patients had increased liver iron concentration hepatic T2* (10.9 ± 6.7 ms). Pancreatic accumulation (15.6 ± 9.2 ms) was described in 8 TDT patients. Splenectomy was reported in 7 patients. Data of previous MRI are shown in supplementary materials.Resting echocardiographic assessment at rest revealed no significant differences between patients with TDT and HC in terms of cardiac chamber dimensions, systolic and diastolic function. Notably, despite the presence of anemia, TDT patients did not exhibit signs of increased compensatory cardiac output or cardiac index, suggesting the absence of a compensatory hyperdynamic state. Although the mean values of the indices examined fell within normal ranges (see Supplementary Table 2) compared to HC, some TDT patients exhibited pathological values in the following parameters: 3 out of 9 patients showed dilated left atrium, and 2 out of 9 had increased indexed mass. Regarding flow waves, 4 subjects showed altered E/A ratio, and as many as 8 patients had a pathological mean E/e' ratio. The data on left ventricular global longitudinal strain (LV-GLS) also showed 4 patients with pathological values and 4 within normal limits, while the data on right ventricle free wall longitudinal strain (RV-FWLS) revealed 3 patients exceeding pathological limits.

Thus, in TDT patients free from iron overload, the lack of compensatory hyperdynamic state in response to chronic anemia might underlie a possible inability of hearts to appropriately increase their performance in response to exercise.

In TDT patients, lung ultrasound cardiopulmonary exercise testing reveals muscle hypotrophy and heart diastolic dysfunction with a negative impact on strength and maximum oxygen consumption

Combined lung ultrasound cardiopulmonary exercise testing (LUS-CPET) showed significant reduced aerobic fitness levels in TDT patients, with measured VO_2max 23.3 ± 6.2 mL/min/kg compared to 38.5 ± 6.0 mL/min/kg in HC ($p < 0.001$). Data on bioelectrical impedance analysis revealed significative lower muscular and lean body mass (LBM, 44.1 ± 5.8 kg vs 54.4 ± 6.6 kg, $p = 0.003$) among TDT patients, both in absolute terms and relative to the free-fat mass index (FFMI, 16.9 ± 1.7 kg/m^2 vs 18.5 ± 1.5 kg/m^2, $p = 0.023$). Of note, BMI was within normal range (Table 1) and there were no significant differences in waist circumference and absolute fat mass between the two groups. Muscle strength was slightly but significantly reduced in TDT patients, with handgrip 36.6 ± 6.8 kg compared to 44.0 ± 8.0 kg in HC ($p = 0.049$). To evaluate the presence of possible reduced lean mass, thigh and waist circumferences were measured [39, 40]. Thigh circumference and WTR (1.77 ± 0.02 VS 1.54 ± 0.02, $p < 0.001$) were markedly lower in TDT patients, showing reduced muscular appendicular mass [41]. In addition to evaluating body muscle mass, both parameters are emerging in screening power for metabolic and cardiovascular risk being more powerful than BMI [42]. Indeed, International Physical Activity Questionnaire (IPAQ) revealed that TDT patients consume fewer metabolic equivalents of task (METs) per week compared to controls (983.2 ± 698.6 vs 5524.7 ± 1676.7; $p = 0.003$), thus more likely falling into sedentary or moderate activity classes, with no patients classified as physically active. This agrees with previous reports in other

heart diseases, showing an adaptative strategy on lifestyles of patients based on their functional limitations [43].

Resting (HRrest) and maximum heart rate (HRmax) did not differ significantly between the two groups; however, HR was significantly lower at the anaerobic threshold in TDT subjects compared to HC. Systolic blood pressure response to exercise was similar in TDT compared to HC (166.7 ± 18.5 mmHg vs 180.0 ± 15.6 mmHg, $p = 0.118$), ensuring adequate peripheral perfusion. However, maximal oxygen pulse was significantly lower in TDT patients, showing a reduction in cardiac efficiency during exertion. On the other hand, chronotropic index (ChI, 0.56 ± 0.11 vs 1.17 ± 0.20 $p < 0.001$) was markedly reduced in TDT patients, indicating a chronotropic incompetence and possibly diastolic dysfunction. As expected, the slope of oxygen consumption relative to workload during LUS-CPET (VO2/WR slope) did not differ significantly between the two groups.

Of note, baseline B lines did not differ between the two groups (3.4 ± 3.6 vs 1.3 ± 2.6, $p = 0.176$), whereas exercise testing exacerbated pulmonary congestion in TDT patients (10.7 ± 7.0 vs 3.3 ± 4.2, $p = 0.016$), an indirect sign of diastolic dysfunction [44]. Notably, LUS-CPET induced electrocardiographic abnormalities in TDT patients. In fact, exercise extrasystoles were observed in 4 out of 9 patients, with 2 displaying premature R-on-T ventricular complexes (PVCs) and 1 experiencing non-sustained ventricular paroxysmal tachycardia at peak exercise, accompanied by spontaneously resolving palpitations. One TDT patient exhibited a complete right bundle branch block (RBBB), and another 1 out of 9 showed significant ST-segment depression in the lateral leads without anginal symptoms. In addition, one patient had a self-limited episode of exercise-induced asymptomatic atrial fibrillation. Interestingly, at resting ECG Bazett corrected QT interval (QTc) was longer in TDT patients: 405.9 ± 24.7 ms compared to 380.7 ± 18.1 ms in HC ($p = 0.025$). However, the difference in resting QRS complex duration was not statistically significant between TDT and controls (81.1 ± 11.5 ms vs 78.2 ± 10.1 ms; $p = 0.580$). Similarly to PPO, the measured oxygen consumption values were significantly lower at maximum exertion (VO_2max) both in absolute terms compared to HC and in relation to the percentage of predicted values [45]. Sub-maximal VO_2 values, at the anaerobic ventilatory threshold (AT), were also lower compared to control subjects; however, this difference was not confirmed relative to predicted VO_2max. Resting and maximal minute ventilation (VE) and maximal tidal volume (Vt) were significantly lower in TDT patients (Table 2s). However, there was no altered response in terms of respiratory rate (RR) at rest and during exertion (Table 2s). Reduced VE and Vt did not seem to affect oxygen saturation measured by pulse oximetry (SpO_2), as it was not different between the two groups at rest (SpO_2rest 97.8 ± 0.7 vs 98.1 ± 0.6) and at peak exertion (SpO_2max 98.2 ± 0.8 vs 97.9 ± 0.9, $p > 0.05$). Similarly, breathing reserve at peak exertion (BR, %), the slope of ventilation in relation to carbon dioxide production (VE/VCO2) and its relative intercept on the VE axis did not differ between patients with TDT and HC, did not differ statistically between the two groups.

Collectively, our data indicate that in TDT patients without heart iron overload LUS-CPET reveals heart diastolic dysfunction and reduced peripheral muscles fitness.

Cardiopulmonary exercise test elicitates functional and molecular cardiovascular stress markers (Fig. 1)

To explore these functional alterations, we evaluated soluble markers of vascular endothelial activation such as ICAM-1 and of metabolic muscular stress such as FGF21 at baseline and after LUS-CPET [22, 46–48]. At rest, ICAM-1 levels were similar in TDT patients compared to controls (178.11 ± 26.55 vs 162.68 ± 13.33, $p = 0.216$). ICAM-1 increased more in TDT patients than in HC in response to LUS-CPET (195.90 ± 26.60 vs 161.94 ± 13.96, $p = 0.003$). At rest FGF21 was significantly increased in TDT (232.41 ± 137.5) compared to HC (105.10 ± 39.13, $p = 0.036$). Post LUS-CPET, FGF21 levels were markedly higher in TDT when compared to HC (764.81 ± 573.90 vs 128.24 ± 48.98, $p < 0.001$). In TDT patients, FGF21 levels correlated negatively with lean body mass and VO_2max ($\rho = -0.840$, $p = 0.005$ and $\rho = -0.679$, $p = 0.044$, respectively). To further explore soluble markers functionally link to metabolic and myocellular stress triggered by FGF21, we evaluated miR577 [49–51]. We first found that FGF21 3′UTR is a direct target of miR-577 with a negative effect (Fig. 1S). As predicted by our in silico analyses, in TDT patients, we observed a reduction in serum miR-577 levels in response to exercise when compared to rest condition (median TDT_miR577_pre: 0.9x [0.88–2.81]; median TDT_miR577_post: 0.1x [0.03–1.01]) ($p < 0.05$). Serum miR-577 did not change significantly after exercise in healthy controls (HC) (median HC_miR577_pre: 1.xx [0.88–2.56]; median HC_miR577_post: 0.9x [0.01–2.42]) ($p > 0.05$). We also evaluated miR-199b, a marker of systolic dysfunction [52, 53], whose levels were not affected by exercise in either HC or TDT patients.

Discussion

Here, we showed that, at rest, asymptomatic TDT patients free from heart iron-overload and normal diastolic dysfunction display no compensatory hyperdynamic state in response to chronic anemia, suggesting a possible impairment of hearts in increasing their performance in response to exercise. Indeed, we found that

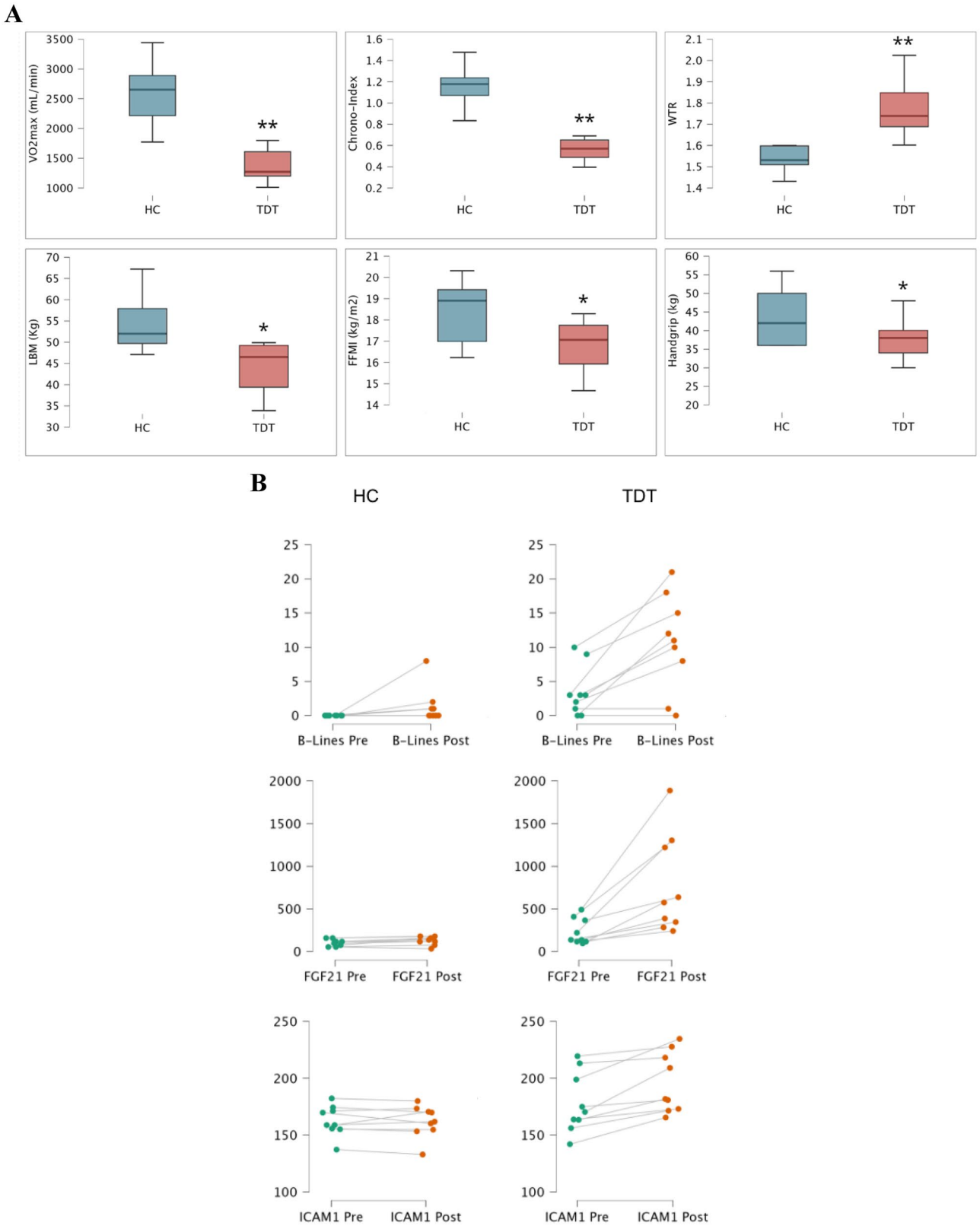


Fig. 1 **A**) Lower fitness levels and worse body composition in TDT patients. VO_2max: maximal oxygen consumption. Chrono-index: chronotropic index; WTR: waist to tight ratio; LBM: lean body mass; FFMI: fat-free mass index. HC: healthy controls; TDT: transfusion dependent thalassemic patients. Note: $*p < 0.05$, $**p < 0.001$. **B**) Exercise reveals cardiovascular and muscular damage in TDT. Intercellular adhesion molecule 1 (ICAM-1) and Fibroblast growth factor 21 (FGF21) in healthy controls (HC) and transfusion dependent thalassemic patients (TDT), at rest (pre) and after maximal exercise testing (Post)

exercise testing exacerbated pulmonary congestion (B lines) used as an indirect sign of diastolic dysfunction in TDT patients. This agrees with reduction in the VO_2max in TDT patients when compared to HC subjects. Despite reduced resting and maximal VE in TDT, no major ventilatory or gas diffusion limitations were observed, as indicated by normal values of breathing reserve, VE/VCO_2 slope, and oxygen saturation at peak exercise, consistent with previous findings [54]. To our knowledge, this is the first study to evaluate a homogeneous group of TDT patients without present or history of heart iron-overload, using cardiopulmonary exercise testing (CPET). In previous reports, some or all the enrolled patients had iron-overload cardiomyopathy with reduced BMI [6, 13, 54]. In our study, TDT patients displayed normal BMI, but we found abnormal altered waist to thigh ratio, which reflects body muscle mass. We confirmed that TDT patients have significantly lower oxygen consumption values both at maximal (VO_2max) and submaximal (AT) efforts during CPET when compared to HC. This finding might be primarily related to reduced oxygen delivery (i.e. anemia) in TDT patients, but also to the altered body composition, with reduced lower lean mass and thigh circumference than HC. This makes waist to thigh ratio a simple important marker to be used for body muscle mass evaluation in TDT patients. As described in other chronic disorders, patients tend to adapt their lifestyle to their functional limitation, generally moving towards a sedentary life [43]. Indeed, TDT patients with altered waist to thigh ratio reported lower levels of weekly physical activity at the IPAQ questionnaire, similarly to other thalassemic cohorts [55], showing sedentary habits. At this stage we cannot exclude a component of non-specific myopathy previously described in TDT patients [56]. On initial observation, TDT peripheral muscle seemed able to extract oxygen at a normal rate - i.e. normal VO2/WR slope, a common finding in early diastolic disfunction [57] – a reduction of both oxygen consumption, hemoglobin concentration and power output could have normalized this result. Interestingly, VO2/WR slope may also be normal if the component of mainly non-oxidative type II muscular fibers might be affected in TDT patients, as in sarcopenic aging [58]. Reduced physical activity, as seen in our TDT patients, and energy imbalance can also switch to a starving phenotype with FGF21 acting marker of impaired autophagy and metabolism resulting in patients' reduced strength [59]. It is also not possible to exclude that the higher levels of FGF21 are related to a reduced expression of the FGF21 receptor in TDT patients. However, muscular weakness is of particular interest, as TDT patients showed reduced thigh circumference and handgrip strength, that has been reported to be strongly associated with cardiometabolic diseases in several populations [60, 61]. Thus, altered waist to thigh ratio, as seen in our TDT cohort, is a predictor of CV diseases [62]. Indeed, the marked and significant increase in post-exercise FGF21 levels in TDT patients might be support the interplay between reduced cardiac function and lower muscular mass [63], as FGF21 provides cardiac protection and enhances remodeling by reducing oxidative stress and inflammatory responses in cardiovascular diseases [64]. Of note, we found a mirrored reduction of post exercise miR-577 in patients with TDT, a result that confirm the data on FGF21, as miR-577 inhibits its translation [33]. Lastly, FGF21 secretion in adipose tissue and liver is also positively regulated by Erythropoietin (EPO), which could explain higher basal levels in TDT patients [65]. However, despite the presence of hepatic iron overload in 4 out of 9 patients, the normal baseline levels of alanine aminotransferase and the exercise-induced increase in FGF21 suggest that this finding is more likely associated with cardiovascular and muscular dysfunction rather than hepatic alterations – as suggested by negative correlations with VO_2max and lean body mass in TDT patients. The observed differences in these key parameters showed large effect sizes, supporting the biological relevance of our findings.The results regarding the chronotropic response to exercise in subjects with TDT show an altered heart rate during exertion, although there is some discrepancy in the literature [6, 66, 67]. Notably, in our TDT patients, the chronotropic response is reduced, despite the anemic profile. Reduced chronotropic index (ChI) has been reported to be altered by both electrical and ischemic heart disorders [68]. A possible cause is related to the known competition between Ca2+ and Fe2+ ions, affecting both ryanodine-sensitive channels and divalent ion channels [12], that may alter, even without MRI-detectable iron burden, the ECG and the HR response to exercise. We found that QTc was longer in TDT patients, and arrhythmias may be early indicators of cardiopathy in thalassemia [69]. In our sample, we reported exercise induced atrial fibrillation, PVCs, non-sustained ventricular paroxysmal tachycardia and PACs. On the other hand, patients with heart failure with preserved ejection fraction (HFpEF) present similar reduced pathological chronotropic response to exercise [70], with a strong correlation with exercise-induced B lines with diastolic function worsening [44]. To our knowledge, LUS was never used in TDT, while some data are present in intensive care settings in sickle cell disease [71]. We think that LUS, specifically combined with CPET may have a prognostic value in these patients, as the possible pathophysiological mechanism by which B-lines appear during exercise is directly connected to pulmonary congestion caused by a disproportionate increase in left ventricular filling pressure. We did not perform exercise echocardiography, but LUS provided valuable non-invasive insights into exercise-induced diastolic dysfunction.

Indeed, B-lines are an excellent surrogate for PCWP during exercise testing [21]. During CPET, however, we did not document signs of pulmonary hypertension, such as VE/VCO2 slope increase, desaturation, or decrease of breathing reserve: these findings are consistent with previous literature [6]. This is of particular interest as B-lines and possible latent diastolic dysfunction seem to be an early indicator of pulmonary congestion, even in the absence of ventilatory limitations to exercise [44].

Of note, FGF21 levels correlate with diastolic dysfunction in heart failure patients [72]. However, in our sample, no basal systolic cardiac output alterations or signs of cardiac hypertrophy were found. This is noteworthy, as miR-199b is dysregulated in systolic heart failure and more severe CV diseases [53, 73, 74]. We speculate that due to diastolic cardiovascular involvement, this marker was not altered even after maximal exercise.

This agrees with the significant increase in soluble ICAM-1 in TDT patients in response to exercise when compared to healthy controls. Of note, previous studies have shown that ICAM-1 increases after exercise related to both inflammatory and vascular endothelial share stress response [22, 75]. In healthy population, sedentary habits and ageing are known to contribute to increased soluble ICAM-1 with associated higher risk of CV [76].

This study has some limitations. First, the exploratory cross-sectional design limit statistical power and may preclude causal inference; however, the collected data are consistent with early cardiovascular and muscular involvement in TDT patients, as suggested by BIA and LUS-CPET findings. Given the rarity of the condition and the strict inclusion/exclusion criteria, recruitment was inherently limited. Second, despite the homogeneity of the TDT cohort and matching with healthy controls, the latter exhibited higher IPAQ scores. This is noteworthy, as TDT patients in clinical practice are often discouraged from engaging in physical activity; accordingly, future studies should explore adapted exercise programs for this population. Importantly, reduced physical activity may only partially explain the impaired functional status observed in TDT patients [13].

Third, residual confounding related to clinical heterogeneity (e.g., therapies, splenectomy status) cannot be excluded, and larger studies with multivariable analyses are warranted. However, it is noteworthy that mean ferritin levels in TDT patients indicated an optimal iron chelation status at the time of CPET, thereby limiting potential confounding related to soluble iron overload [15].

Although stress echocardiography could have provided more direct evidence, the combination of chronotropic index impairment and exercise-induced pulmonary congestion assessed by LUS-CPET represents a simple and non-invasive approach that deserves further investigation in larger cohorts. Similarly, the use of circulating biomarkers and miRNA requires further validation in TDT, due to the explorative nature of our study.

In conclusion, our study identified the waist-to-thigh ratio as an informative parameter for estimating muscle mass and identifying muscle waste in TDT patients. We further suggest that LUS-CPET may provide valuable insights into exercise-induced diastolic dysfunction with preserved ejection fraction, representing a potential early marker of TDT-related cardiomyopathy in patients without evidence of cardiac iron overload. The response of ICAM1, FGF21, and miR-577 to exercise may highlight the interplay between vascular endothelial activation and early metabolic and myocellular stress in TDT. Our data bring the attention in prescribing low-moderate intensity aerobic exercise, which promotes lower ferritin, reduced oxidative stress [77] and possibly ICAM-1 [76], combined with strength training that may reduce circulating FGF21 levels in TDT patients [78]. Future studies should focus on the possible prognostic value of these findings and the role of exercise to protect from early muscle dysfunction patients with TDT.

Abbreviations

BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
ChI	Chronotropic Index
CPET	Cardiopulmonary Exercise Testing
FFMI	Fat-Free Mass Index
FGF21	Fibroblast Growth Factor 21
HC	Healthy Controls
HFpEF	Heart Failure with Preserved Ejection Fraction
IPAQ	International Physical Activity Questionnaire
LUS	Lung Ultrasound
METs	Metabolic Equivalent of Task
miR-577	microRNA-577
PCWP	Pulmonary Capillary Wedge Pressure
PRBCs	Packed red blood cells
TDT	Transfusion-Dependent Thalassemia
VO_2	Oxygen Consumption
VO_2/WR	Oxygen Consumption per Work Rate
WTR	Waist-to-Thigh Ratio

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-026-08295-8.

Supplementary Material 1

Acknowledgements

The authors thank the participants for their contributions.

Author contributions

LDF designed the study. MB, MC, SV, AM, MTV, EF, LDC collected data, performed analyses, and data interpretation. MB, MC wrote the first draft of the manuscript, and all authors took part in critical revision of the manuscript. The final manuscript was presented to and approved by all authors. All who were eligible to be authors in this study were included as authors.

Funding

Not applicable.

Data availability
The data that support the findings of this study are available from the corresponding author on 31 reasonable request due to privacy concerns.

Declarations

Ethics approval statement
The study was approved by the local Ethics Committee (Protocol Code FGRP_ITA3). All patients and controls provided informed consent for the study. Informed consent was obtained from all individual participants included in the study. All procedures were conducted in accordance with the principles of the Declaration of Helsinki.

Consent for publication
Not applicable.

Patient consent statement
Informed consent was obtained from all individual participants included in the study.

Permission to reproduce material from other sources
No previously published material was reproduced in this article. Permission to reproduce material was not required.

Authors disclosure
The authors have nothing to disclose.

Competing interests
The authors declare that they have no competing interests.

Received: 29 October 2025 / Accepted: 10 May 2026
Published online: 06 June 2026

References

1. Fibach E, Rachmilewitz EA. Iron overload in hematological disorders. La Presse Médicale. 2017;46(12):e296–305. https://doi.org/10.1016/j.lpm.2017.10.007.
2. Fibach E, Dana M. Oxidative stress in β-thalassemia. Mol Diagn Ther. 2019;23(2):245–61. https://doi.org/10.1007/S40291-018-0373-5.
3. Pinto VM, Mazzi F, De Franceschi L. Novel therapeutic approaches in thalassemias, sickle cell disease, and other red cell disorders. Blood. 2024;144(8):853–66. https://doi.org/10.1182/blood.2023022193.
4. Piga A. Impact of bone disease and pain in thalassemia. Hematology. 2017;2017(1):272–77. https://doi.org/10.1182/asheducation-2017.1.272.
5. De Franceschi L, Brugnara C, Beuzard Y. Dietary magnesium supplementation ameliorates anemia in a mouse model of β-thalassemia. Blood. 1997;90(3):1283–90. https://doi.org/10.1182/blood.v90.3.1283.
6. Nanas S, Vasileiadis I, Dimopoulos S, Sakellariou D, Kapsimalakou S, Papazachou O, et al. New insights into the exercise intolerance of β-thalassemia major patients. Scand Med Sci Sports. 2009;19(1):96–102. https://doi.org/10.1111/j.1600-0838.2008.00778.x.
7. Pennell DJ, Udelson JE, Arai AE, Bozkurt B, Cohen AR, Galanello R, et al. Cardiovascular function and treatment in β-thalassemia major: a consensus statement from the American heart Association. Circulation. 2013;128(3):281–308. https://doi.org/10.1161/CIR.0B013E31829B2BE6.
8. Fernandes JL, Loggetto SR, Veríssimo MPA, Fertrin KY, Baldanzi GR, Fioravante LAB, et al. A randomized trial of amlodipine in addition to standard chelation therapy in patients with thalassemia major. Blood. 2016;128(12):1555–61. https://doi.org/10.1182/blood-2016-06-721183.
9. Federti E, Vinchi F, Iatcenko I, Ghigo A, Matte A, Toya SCM, et al. Duality of Nrf2 in iron-overload cardiomyopathy. Haematologica. 2023;108(5):1335–48. https://doi.org/10.3324/haematol.2022.281995.
10. Russo V, Rago A, Pannone B, Mayer MC, Spasiano A, Calabro R, et al. Atrial fibrillation and beta thalassemia major: the predictive role of the 12-lead electrocardiogram analysis. Indian Pacing Electrophysiol J. 2014;14(3):121–32. https://doi.org/10.1016/S0972-6292(16)30753-7.
11. Kolnagou A, Michaelides Y, Kontos C, Kyriacou K, Kontoghiorghes GJ. Myocyte damage and loss of myofibers is the potential mechanism of iron overload toxicity in congestive cardiac failure in thalassemia. Complete reversal of the cardiomyopathy and normalization of iron load by deferiprone. Hemoglobin. 2008;32(1–2):17–28. https://doi.org/10.1080/03630260701726491.
12. Wijarnpreecha K, Kumfu S, Chattipakorn SC, Chattipakorn N. Cardiomyopathy associated with iron overload: how does iron enter myocytes and what are the implications for pharmacological therapy? Hemoglobin. 2015;39(1):9–17. https://doi.org/10.3109/03630269.2014.987869.
13. Piatti G, Giuditta M, Consonni D, Cassinerio E, Cappellini MD. Cardiopulmonary testing in adult patients with β-thalassemia major in comparison to healthy subjects. Ann Hematol. 2022;101(11):2445–52. https://doi.org/10.1007/s00277-022-04974-w.
14. Wahl MP, Scalzo RL, Regensteiner JG, Reusch JEB. Mechanisms of aerobic exercise impairment in diabetes: a narrative review. Front. Endocrinol. 2018;9(Apr). https://doi.org/10.3389/FENDO.2018.00181.
15. Shamsian BS, Abdar Esfahani S, Milani H, Akhlaghpoor S, Mojtahedzadeh S, Karimi A, et al. Magnetic resonance imaging in the evaluation of iron overload: a comparison of MRI, echocardiography and serum ferritin level in patients with β-thalassemia major. Clin Imag. 2012;36(5):483–88. https://doi.org/10.1016/J.CLINIMAG.2011.11.029.
16. Thavonlun S, Houngngam N, Kingpetch K, Numkarunarunrote N, Santisitthanon P, Buranasupkajorn P, et al. Association of osteoporosis and sarcopenia with fracture risk in transfusion-dependent thalassemia. Sci Rep. 2023;13(1). https://doi.org/10.1038/s41598-023-43633-6.
17. Perera NJ, Lau NS, Mathews S, Waite C, Ho PJ, Caterson ID. Overview of endocrinopathies associated with β-thalassaemia major. Intern Med J. 2010;40(10):689–96. https://doi.org/10.1111/J.1445-5994.2010.02254.X.
18. De Sanctis V, Pintor C, Gamberini MR, et al. Multicentre study on prevalence of endocrine complications in thalassaemia major. Italian working group on endocrine complications in non-endocrine diseases. Clin Endocrinol (Oxf). 1995;42(6):581–86. https://doi.org/10.1111/J.1365-2265.1995.TB02683.X.
19. Nemtsas P, Arnaoutoglou M, Perifanis V, Koutsouraki E, Orologas A. Neurological complications of beta-thalassemia. Ann Hematol. 2015;94(8):1261–65. https://doi.org/10.1007/S00277-015-2378-Z.
20. Kagami K, Obokata M, Harada T, Sorimachi H, Yuasa N, Saito Y, et al. Incremental diagnostic value of post-exercise lung congestion in heart failure with preserved ejection fraction. Eur Heart J - Cardiovasc Imag. 2023;24(5):553–61. https://doi.org/10.1093/ehjci/jead007.
21. Reddy YNV, Obokata M, Wiley B, Koepp KE, Jorgenson CC, Egbe A, et al. The haemodynamic basis of lung congestion during exercise in heart failure with preserved ejection fraction. Eur Heart J. 2019;40(45):3721–30. https://doi.org/10.1093/EURHEARTJ/EHZ713.
22. Koh Y, Park J. Cell adhesion molecules and exercise. JIR. 2018;Volume 11:297–306. https://doi.org/10.2147/JIR.S170262.
23. Poston RN, Haskard DO, Coucher JR, Gall NP, Johnson-Tidey RR. Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. Am J Pathol. 1992;140(3):665–73.
24. Noutsias M, Hohmann C, Pauschinger M, Schwimmbeck P-L, Ostermann K, Rode U, et al. sICAM-1 correlates with myocardial ICAM-1 expression in dilated cardiomyopathy. Int J Cardiol. 2003;91(2–3):153–61. https://doi.org/10.1016/S0167-5273(03)00033-0.
25. Vinchi F, Sparla R, Passos ST, Sharma R, Vance SZ, Zreid HS, et al. Vasculo-toxic and pro-inflammatory action of unbound haemoglobin, haem and iron in transfusion-dependent patients with haemolytic anaemias. Br J Haematol. 2021;193(3):637–58. https://doi.org/10.1111/bjh.17361.
26. De Rooij SR, Nijpels G, Nilsson PM, Nolan JJ, Gabriel R, Bobbioni-Harsch E, et al. Low-grade chronic inflammation in the relationship between insulin sensitivity and cardiovascular disease (RISC) population: associations with insulin resistance and cardiometabolic risk profile. Diabetes Care. 2009;32(7):1295–301. https://doi.org/10.2337/dc08-1795.
27. Santoro A, Martucci M, Conte M, Capri M, Franceschi C, Salvioli S. Inflammaging, hormesis and the rationale for anti-aging strategies. Ageing Res Rev. 2020;64:64. https://doi.org/10.1016/j.arr.2020.101142.
28. Cai D, Frantz JD, Tawa NE, Melendez PA, Oh B-C, Lidov HGW, et al. IKKβ/NF-κB activation causes severe muscle wasting in mice. Cell. 2004;119(2):285–98. https://doi.org/10.1016/j.cell.2004.09.027.
29. Tilstra JS, Robinson AR, Wang J, Gregg SQ, Clauson CL, Reay DP, et al. NF-κB inhibition delays DNA damage–induced senescence and aging in mice. J. Clin. Invest. 2012;122(7):2601–12. https://doi.org/10.1172/JCI45785.
30. Balestrieri ML, Picca A, Calvani R, et al. Circulating inflammatory, mitochondrial dysfunction, and senescence-related markers in older adults with physical Frailty and Sarcopenia: a BIOSPHERE exploratory study. Published online 2022. doi: https://doi.org/10.3390/ijms232214006.

31. Planavila A, Redondo-Angulo I, Ribas F, Garrabou G, Casademont J, Giralt M, et al. Fibroblast growth factor 21 protects the heart from oxidative stress. Cardiovasc Res. 2015;106(1):19–31. https://doi.org/10.1093/cvr/cvu263.
32. Oost LJ, Sandri M, Romanello V. The authors reply: letter on: "Fibroblast growth factor 21 controls mitophagy and muscle mass" by Oost et al. J Cachexia, Sarcopenia Muscle. 2020;11(1):338–40. https://doi.org/10.1002/jcsm.12500.
33. Chen XY, Li GM, Dong Q, Peng H. MiR-577 inhibits pancreatic β-cell function and survival by targeting fibroblast growth factor 21 (FGF-21) in pediatric diabetes. Genet Mol Res. 2015;14(4):15462–70. https://doi.org/10.4238/2015.November.30.24.
34. ATS/ACCP statement on cardiopulmonary exercise testing. Am J Respir Crit Care Med. 2003;167(2):211–77. https://doi.org/10.1164/RCCM.167.2.211.
35. Scali MC, Zagatina A, Simova I, Zhuravskaya N, Ciampi Q, Paterni M, et al. B-lines with lung ultrasound: the optimal Scan technique at rest and during stress. Ultrasound Med Biol. 2017;43(11):2558–66. https://doi.org/10.1016/j.ultrasmedbio.2017.07.007.
36. Jonathan T, Aldo S, Pieter CJ, Erik C. Reliability of standard circumferences in domain-related constitutional applications. Am J Hum Biol. 2013;25(5):637–42. https://doi.org/10.1002/ajhb.22423.
37. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exercise. 2003;35(8):1381–95. https://doi.org/10.1249/01.MSS.0000078924.61453.FB.
38. Bou-Fakhredin R, Cappellini MD, Taher AT, De Franceschi L. Hypercoagulability in hemoglobinopathies: decoding the thrombotic threat. Am J Hematol. 2025;100(1):103–15. https://doi.org/10.1002/ajh.27500.
39. Chlabicz M, Paniczko M, Jamolkowski J, Sowa P, Lapinska M, Szpakowicz M, et al. The impact of legs fat mass and legs lean fat mass on thigh circumference according cardiovascular risk: a population-based study. Eur J Prev Cardiol. 2021;28(Supplement_1). https://doi.org/10.1093/eurjpc/zwab061.252.
40. Lee DH, Keum NN, Hu FB, Orav EJ, Rimm EB, Willett WC, et al. Predicted lean body mass, fat mass, and all cause and cause specific mortality in men: prospective US cohort study. BMJ. 2018;362. https://doi.org/10.1136/bmj.k2575.
41. Tatsukawa Y, Misumi M, Kim YM, Yamada M, Ohishi W, Fujiwara S, et al. Body composition and development of diabetes: a 15-year follow-up study in a Japanese population. Eur J Clin Nutr. 2018;72(3):374–80. https://doi.org/10.1038/s41430-017-0077-7.
42. Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. Obes Rev. 2012;13(3):275–86. https://doi.org/10.1111/j.1467-789X.2011.00952.x.
43. Bayonas-Ruiz A, Muñoz-Franco FM, Ferrer V, Pérez-Caballero C, Sabater-Molina M, Tomé-Esteban MT, et al. Cardiopulmonary exercise test in patients with hypertrophic cardiomyopathy: a systematic review and meta-analysis. JCM. 2021;10(11):2312. https://doi.org/10.3390/jcm10112312.
44. Simonovic D, Coiro S, Deljanin-Ilic M, Kobayashi M, Carluccio E, Girerd N, et al. Exercise-induced B-lines in heart failure with preserved ejection fraction occur along with diastolic function worsening. ESC Heart Fail. 2021;8(6):5068–80. https://doi.org/10.1002/ehf2.13575.
45. Hansen JE, Sue DY, Wasserman K. Predicted values for clinical exercise testing. Am Rev Respir Dis. 1984;129(2_Part_2):S49–55. https://doi.org/10.1164/arrd.1984.129.2p2.s49.
46. Jung HW, Park JH, Kim DA, Jang I-Y, Park SJ, Lee JY, et al. Association between serum FGF21 level and sarcopenia in older adults. Bone. 2021;145:145. https://doi.org/10.1016/j.bone.2021.115877.
47. Roh E, Hwang SY, Yoo HJ, Baik SH, Cho B, Park YS, et al. Association of plasma FGF21 levels with muscle mass and muscle strength in a national multicentre cohort study: Korean Frailty and aging cohort study. Age Ageing. 2021;50(6):1971–78. https://doi.org/10.1093/ageing/afab178.
48. Tezze C, Romanello V, Sandri M. FGF21 as modulator of metabolism in health and disease. Front. Physiol. 2019;10(Mar). https://doi.org/10.3389/fphys.2019.00419.
49. Wang YH, Zhang XY, Han YQ, Yan F, Wu R. MicroRNA-577 inhibits cardiomyocyte apoptosis induced by myocardial infarction via targeting PARP1. Eur Rev Med Pharmacol Sci. 2019;23(21). https://doi.org/10.26355/eurrev_201911_19451.
50. Long YF, Chow SKH, Cui C, Wong RMY, Zhang N, Qin L, et al. Does exercise influence skeletal muscle by modulating mitochondrial functions via regulating MicroRNAs? A systematic review. Ageing Res Rev. 2023;91:91. https://doi.org/10.1016/j.arr.2023.102048.
51. Ogasawara R, Akimoto T, Umeno T, Sawada S, Hamaoka T, Fujita S. MicroRNA expression profiling in skeletal muscle reveals different regulatory patterns in high and low responders to resistance training. Physiol Genomics. 2016;48(4):320–24. https://doi.org/10.1152/physiolgenomics.00124.2015.
52. Duygu B, Poels EM, Juni R, Bitsch N, Ottaviani L, Olieslagers S, et al. miR-199b-5p is a regulator of left ventricular remodeling following myocardial infarction. Noncoding RNA Res. 2017;2(1):18–26. https://doi.org/10.1016/j.ncrna.2016.12.002.
53. Li Z, Liu L, Hou N, Song Y, An X, Zhang Y, et al. miR-199-sponge transgenic mice develop physiological cardiac hypertrophy. Cardiovasc Res. 2016;110(2):258–67. https://doi.org/10.1093/cvr/cvw052.
54. Cracowski C, Wuyam B, Klein V, Lévy P. Lung function and exercise capacity in thalassaemia major. Eur Respir J. 1998;12(5):1130–36. https://doi.org/10.1183/09031936.98.12051130.
55. Garipy C, Lal A, Fung E. Reduced physical activity in adult and pediatric patients with thalassemia. Blood. 2010;116(21):5174–5174. https://doi.org/10.1182/blood.v116.21.5174.5174.
56. Shapira Y, Glick B, Finsterbush A, Goldfarb A, Rosenmann E. Myopathological findings in thalassemia major. Eur Neurol. 2008;30(6):324–27. https://doi.org/10.1159/000117365.
57. Kaiser T, Datta D. Can diastolic dysfunction Be identified on cardiopulmonary exercise testing? Chest. 2017;152(4):A976. https://doi.org/10.1016/j.chest.2017.08.1011.
58. Nilwik R, Snijders T, Leenders M, Groen BBL, van Kranenburg J, Verdijk LB, et al. The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size. Exp Gerontology. 2013;48(5):492–98. https://doi.org/10.1016/j.exger.2013.02.012.
59. Oost LJ, Kustermann M, Armani A, Blaauw B, Romanello V. Fibroblast growth factor 21 controls mitophagy and muscle mass. J Cachexia, Sarcopenia Muscle. 2019;10(3):630–42. https://doi.org/10.1002/jcsm.12409.
60. Tatangelo T, Muollo V, Ghiotto L, Schena F, Rossi AP. Exploring the association between handgrip, lower limb muscle strength, and physical function in older adults: a narrative review. Exp Gerontology. 2022;167:167. https://doi.org/10.1016/j.exger.2022.111902.
61. Lawman HG, Troiano RP, Perna FM, Wang CY, Fryar CD, Ogden CL. Associations of relative handgrip strength and cardiovascular disease biomarkers in U.S. Adults, 2011–2012. Am J Prev Med. 2016;50(6):677–83. https://doi.org/10.1016/j.amepre.2015.10.022.
62. Li C, Ford ES, Mokdad AH, Giles WH. Abstract 3674: association of waist-to-thigh ratio with history of cardiovascular diseases among US adults: findings from the national health and nutrition examination survey 1999–2004. Circulation. 2007;116(suppl_16). https://doi.org/10.1161/circ.116.suppl_16.ii_834.
63. Refsgaard Holm M, Christensen H, Rasmussen J, Johansen ML, Schou M, Faber J, et al. Fibroblast growth factor 21 in patients with cardiac cachexia: a possible role of chronic inflammation. ESC Heart Fail. 2019;6(5):983–91. https://doi.org/10.1002/ehf2.12502.
64. Salminen A, Kaarniranta K, Kauppinen A. Integrated stress response stimulates FGF21 expression: systemic enhancer of longevity. Cellular Signalling. 2017;40:10–21. https://doi.org/10.1016/j.cellsig.2017.08.009.
65. Noguchi CT. Erythropoietin regulates metabolic response in mice via receptor expression in adipose tissue, brain, and bone. Exp Hematol. 2020;92:32–42. https://doi.org/10.1016/j.exphem.2020.09.190.
66. Ribeil JA, Arlet JB, Dussiot M, Cruz Moura I, Courtois G, Hermine O, et al. Ineffective erythropoiesis in β -thalassemia. ScientificWorldjournal. 2013;2013(1):11. https://doi.org/10.1155/2013/394295.
67. Sohn EY, Kato R, Noetzli LJ, Gera A, Coates T, Harmatz P, et al. Exercise performance in thalassemia major: correlation with cardiac iron burden. Am J Hematol. 2013;88(3):193–97. https://doi.org/10.1002/AJH.23370.
68. Brubaker PH, Kitzman DW. Chronotropic incompetence. Circulation. 2011;123(9):1010–20. https://doi.org/10.1161/circulationaha.110.940577.
69. Malagù M, Marchini F, Fiorio A, Sirugo P, Clò S, Mari E, et al. Atrial fibrillation in β-thalassemia: overview of mechanism, significance and clinical management. Biology. Published online 2022. doi: 2022;11(1):148. https://doi.org/10.3390/biology11010148.
70. Nayor M, Houstis NE, Namasivayam M, Rouvina J, Hardin C, Shah RV, et al. Impaired exercise tolerance in heart failure with preserved ejection fraction: quantification of Multiorgan System reserve capacity. JACC Heart Fail. 2020;8(8):605–17. https://doi.org/10.1016/j.jchf.2020.03.008.
71. Razazi K, Deux JF, De Prost N, Boissier F, Cuquemelle E, Galactéros F, et al. Bedside lung ultrasound during acute chest syndrome in sickle cell disease. Med (U States). 2016;95(7):e2553. https://doi.org/10.1097/MD.0000000000002553.

72. Chou RH, Huang PH, Hsu CY, Chang C-C, Leu H-B, Huang C-C, et al. Circulating fibroblast growth factor 21 is associated with diastolic dysfunction in heart failure patients with preserved ejection fraction. Sci Rep. 2016;6(1):6. https://doi.org/10.1038/srep33953.
73. Koop AMC, Duygu B, Ottaviani L, Poels E, Van De Kolk KW, Du Marchie Sarvaas GJ, et al. 4929Contribution of miR-199b to right ventricular remodelling due to pressure overload. Eur Heart J. 2018;39(suppl_1):ehy566.4929. https://doi.org/10.1093/eurheartj/ehy566.4929.
74. Xiao J, Liang D, Zhang Y, Liu Y, Zhang H, Liu Y, et al. MicroRNA expression signature in atrial fibrillation with mitral stenosis. Physiol Genomics. 2011;43(11):655–64. https://doi.org/10.1152/physiolgenomics.00139.2010.
75. Faes C, Balayssac-Siransy E, Connes P, Hivert L, Danho C, Bogui P, et al. Moderate endurance exercise in patients with sickle cell anaemia: effects on oxidative stress and endothelial activation. Br J Haematol. 2014;164(1):124–30. https://doi.org/10.1111/bjh.12594.
76. Many GM, Jenkins NT, Witkowski S, Damsker JM, Hagberg J. The effects of aerobic training and age on plasma sICAM-1. Int J Sports Med. 2012;34(3):253–57. https://doi.org/10.1055/s-0032-1316317.
77. Dehkordi A, Hasani T, Fekri K, Deris F, Etemadifar S. Effects of aquatic exercise on dimensions of quality of life and blood indicators in patients with beta-thalassemia major. Int J Prev Med. 2020;11(1). https://doi.org/10.4103/ijpvm.ijpvm_290_19.
78. Shabkhiz F, Khalafi M, Rosenkranz S, Karimi P, Moghadami K. Resistance training attenuates circulating FGF-21 and myostatin and improves insulin resistance in elderly men with and without type 2 diabetes mellitus: a randomised controlled clinical trial. Eur J Sport Sci. 2021;21(4):636–45. https://doi.org/10.1080/17461391.2020.1762755.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.