"MUSCULAR DE-CONDITIONING AND REDUCED CARDIAC INOTROPISM DUE TO IRON DEPOSITION REDUCE EXERCISE TOLERANCE IN BETA THALASSEMIA MAJOR"

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To the Editor:

Despite intensive transfusion regimens and several options for iron chelation therapy have emerged in the past decade, beta-thalassemia major patients (TM) often display reduced capacity of exercise and suffer fatigue. Moreover, only a minority of patients undertakes regular physical activity being convinced that anemia is a limiting factor.

The exact pathophysiological mechanisms of this limited exercise capacity are still debated: a few studies exist on this topic, which mostly attribute the poor physical performance to anemia, iron-mediated cardiotoxicity and muscular deconditioning (1-3).

In order to estimate the relative contribution of each factor to exercise intolerance, we performed an incremental cardiopulmonary exercise test (CPET) on a cycle ergometer in 54 adult TM patients without echocardiographic evidence of pulmonary arterial hypertension and regularly followed-up at the Centre for Rare Diseases of the Policlinico Hospital in Milan, Italy. We used a standardized CPET criteria to attribute various patterns of physiological changes during exercise to specific causes (4). Briefly, reduced exercise capacity was defined as $V'O_2$ peak < 84% of the predicted value, achieving a respiratory exchange ratio (RER) >1.15, indicating maximal effort. Cardiovascular dysfunction (CD) was defined as O_2 pulse, that is the $V'O_2$ peak/heart rate ratio, ≤ 80% of the predicted value. Ventilatory limitation to exercise (VL) was defined as breathing reserve < 15% of the maximal voluntary ventilation; signs of abnormal gas exchange are Hb O_2 sat.< 88% and $V'E/VCO_2$ slope >34. Muscular deconditioning (MD) was defined as early-onset metabolic acidosis ($V'O_2$@AT <50% of the predicted value), low O_2 pulse, significant breathing reserve, normal maximum heart rate. Deficit of lung perfusion (DLP) was estimated in the presence of a pressure difference between alveolar and end-tidal carbon dioxide exhaled ($p_{(a\text{-}et)}CO_2$) > 0 and an increase of $V'E/V'CO_2$ slope >34, indicating a ventilation/perfusion mismatch.

Prior to CPET, respiratory function tests were also obtained and we administered to patients a modified version of International Physical Activity Questionnaire (IPAQ) (5) to evaluate the relationship between cardiopulmonary functional capacity and physical activity level (PA).

Magnetic resonance (MRI T2*) for iron evaluation in the heart and in the liver was carried out within one year before the study evaluation.

Statistical analyses were performed with Stata16 (StataCorp 2019, College Station, Texas).

TM patients enrolled in the study were regularly transfused, every 21-25 days, in order to maintain pre-transfusional hemoglobin concentration within 9-10 g/dl range; all tests were planned within 7 days from the last transfusion. All patients were receiving iron chelation therapy: deferasirox 33, deferoxamine 12 and deferiprone 1, while 8 patients were taking deferoxamine combined with deferiprone. The main characteristics of patients are shown in Figure 1 A.

3 out 54 subjects (5.5 %) had cardiac iron overload (cardiac T2* ≤20 msec) and 17 (31.5%) suffered hepatic iron overload (LIC > 4.23 mg/g dw).

At the time of enrolment no patients had symptoms or clinical signs of respiratory or cardiac disease. Echocardiographic measurements were within normal values, but males frequently showed the end diastolic diameter of left ventricle (EDDLV) and the end systolic diameter of left ventricle (ESDLV) higher than females (both p< 0.001).

Altogether, no alterations in the mean value of respiratory function parameters at rest were observed, except for lung diffusion of carbon monoxide (DLCO) whose average value was reduced (normal value >80% of predicted). Additionally, males showed higher TLC (p=0.02), FVC (p<0.001) and FEV_1 (p=0.006) values than females. No patients had FEV_1/FVC <0.70 of the predicted value, while 9 patients had a restrictive pattern (4 had a mild restriction and 5 a moderate restriction).

About CPET results, summarized in Figure 1 B, peak oxygen uptake ($V'O_2$ peak), expressing maximum exercise capacity, was decreased (i.e. < 84% of predicted) in 44 out of 54 TM patients (81.5%); O_2 pulse was lower than 80% of the predicted value in 28 out of 54 patients (51.8%); the anaerobic threshold ($V'O_2$@AT) was reduced in 15 out of 54 (27.8%). Males had higher values than females for $V'O_2$ peak (p=0.05), O_2 pulse (p<0.001) and V'E peak (p<0.001).

No TM patients showed ventilatory limitation or experienced oxygen desaturation during exercise. On the basis of the CPET criteria cited above, we attributed the limited exercise tolerance to muscular deconditioning (MD) (n=9), cardiac dysfunction (CD) (n=4), mixed cardiovascular dysfunction (CD) and MD (n= 17), deficit of lung perfusion (DLP) (n=2), or combined DLP and MD (n= 5); mixed CD and DLP (n= 7); 3 patients showed a moderate/severe reduction of exercise capacity due to combined MD, CD and DLP; no CPET alterations were found in 7 patients.

At univariate regression analyses, $V'O_2$ peak and O_2 pulse were positively correlated with cardiac T2* (r=0.26, p=0.06 and r=0.29, p=0.03, respectively) (Figure 1 C and D), i.e. negatively correlated with cardiac iron stores. No correlations were found between $V'O_2$ peak and O_2 pulse with parameters of respiratory function. There was no correlation between exercise performance and degree of anemia.

In multivariable linear regression analysis adjusted for gender, age and mean Hb a positive association between cardiac T2* and $V'O_2$ peak (+1.3 mL/min/kg per 10 msec; 95% confidence interval (CI): -0.4; +5.9) and O_2 pulse (+0.6 L/min per 10 msec; 95% CI: +0.1; +1.0) was found, while there was a negative association with V'E peak (-5.0 n/min per 10 msec; 95% CI: -10.4; +0.5).

Outcome measurements of total PA (low, moderate and vigorous) derived from the IPAQ Questionnaire, expressed as metabolic minutes equivalent per week (MET-minutes/week), showed that only 15 out of 54 TM patients (27.8%) undertook vigorous PA. We found a positive association between between MET-minutes/week with PA (p-trend<0.001). $V'O_2$ peak was 21.1 mL/min/kg (SD 5.4) in those with low physical activity and 25.6 mL/min/kg (SD 5.7) among those with moderate/vigorous PA (p=0.04).

The main finding of this study is a significant impairment in exercise capacity of adult TM patients. Several factors may contribute to this, but the two most important causes of exercise limitation in our group were muscular deconditioning and reduced cardiac inotropism due to iron deposition, found in 34 (63%) and 31 (57.4%) out of 54 TM patients, respectively.

Myocardial iron deposition has a high prevalence in beta-TM and seems to be the trigger for the development of heart disease; cardiac complications are the main cause of death in these patients. We found that $V'O_2$ peak and O_2 pulse are strongly related to cardiac iron burden T2*. O_2 pulse provides a valid means to estimate stroke volume at peak exercise; nevertheless, it also depends on the arterial-venous oxygen difference which depends on muscle function/oxidative capacity. Even if it is known that echocardiographic parameters can remain normal, even in the face of severe myocardial siderosis, is likely that the cardiac output will be unable to increase sufficiently to provide skeletal muscles with adequate perfusion, with a consequent premature onset of anaerobic metabolism.

A sedentary attitude is frequent in TM patients, as they are told since childhood to avoid exercise, which therefore leads to significant muscular deconditioning. On the contrary, muscular deconditioning is absent when patients perform regular physical activity in comparison to patients

with a more sedentary lifestyle (6). There are strong correlations between reduction in $V'O_2$ peak and reduction in muscle mass and in inspiratory muscle weakness.

Neurophysiological studies detected a peripheral motor polyneuropathy, myopathy or both, in up to 50% of beta-TM patients, and this was correlated with iron overload (7). In addition, respiratory muscles strength, assessed by measuring the maximum static inspiratory pressure (Pimax), was reduced in TM patients (3), thus showing that a reduced capacity of exercise is highly correlated to muscle strength performance.

Although a few studies have been conducted with regular physical activity programs, the general trend suggests that exercise training could improve O_2 pulse and $V'O_2$ peak; aerobic exercise may be also useful in decreasing serum ferritin levels in TM by decreasing intestinal iron absorption. Prompting patients to increase daily PA could significantly improve their quality of life (8).

Anemia, due to reduced arterial oxygen concentrations, will also affect the oxygen supply to the muscles. Circulating hemoglobin and exercise performance are strictly linked: acute correction of anemia by blood transfusion in TM patients is associated to a relevant increase of exercise performance, and in particular, $V'O_2$ peak increases of 82.5 mL/min per g/dL of Hb increase (9). Nevertheless, mild anemia is a common factor for all TM patients and could not entirely justify differences in exercise intolerance, suggesting that different pathophysiological mechanisms, alone or in combination each other, may play a role.

In conclusion, exercise intolerance in TM patients is likely multifactorial: even if further studies need to be carried out with larger sample size, longer follow-up periods and aerobic training programs, this study contributes to elucidate the underlying pathophysiological mechanisms.

LEGEND TO THE FIGURE:

LAD: left atrial diameter; *EDDLV:* end diastolic diameter of left ventricle, *ESDLV:* end systolic diameter of left ventricle; *LVEF:* left ventricular ejection fraction; *mPAP:* mean pulmonary arterial pressure; *cardiac T2*:* a magnetic resonance parameter; *LIC:* liver iron concentration; *PA:* physical activity; *$V'O_2$ peak:* peak oxygen uptake at maximum exercise*; $V'O_2$@AT:* oxygen uptake at anaerobic threshold; *O_2 pulse:* oxygen uptake divided by heart rate; *HR max:* maximum heart rate/min; *V'E peak:* peak ventilation at maximum exercise; *V'O2/work:* relationship between oxygen uptake and workload: *MVV:* maximal voluntary ventilation; *BR:* breathing reserve; *Δ Hb O_2*

sat: difference in oxygen saturation between rest and at maximum exercise; *V'E/V'O2* and *V'E/V'CO2*: ventilatory equivalents for oxygen and carbon dioxide.

AUTHOR CONTRIBUTION

All Authors contributed to manuscript drafting or critical review and final approval for submission.

CONFLICT OF INTEREST

The Authors declare no competing financial interest.

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None.

DATA AVAILABILITY STATEMENT

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

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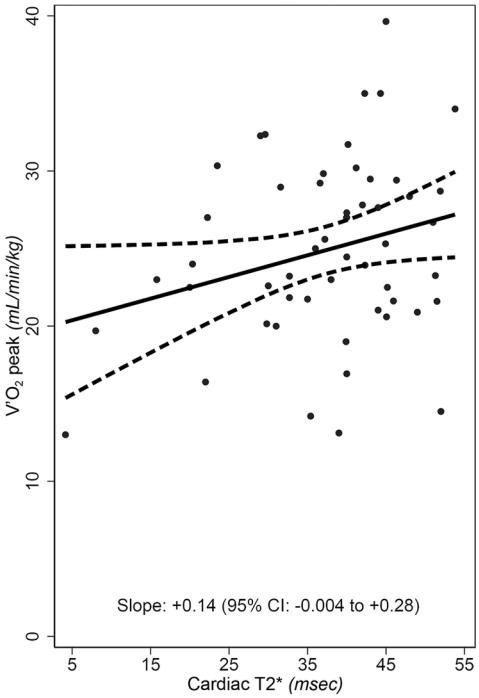
A) **Main characteristics of TM patients** *(mean ± SD)*

	Males	Females	p-value
Age *(years)*	37.1 ± 6.2 *[n = 28]*	37.8 ± 7 *[n = 26]*	*0.30*
Haemoglobin *(g/L)*	9.7 ± 0.5 *[n = 28]*	9.6 ± 0.5 *[n = 26]*	*0.70*
Ferritin *(ng/mL)*	1,030 ± 1,101 *[n = 28]*	691 ± 516 *[n = 26]*	*0.16*
LAD *(mm)*	35.2 ± 6.9 *[n = 18]*	37.8 ± 6.1 *[n = 19]*	*0.21*
EDDLV *(mL/m²)*	79 ±12.7 *[n = 24]*	100.3 ± 17.9 *[n = 21]*	*< 0.001*
ESDLV *(mL/m²)*	26.2 ± 4 *[n = 10]*	40.7 ± 11.2 *[n = 12]*	*< 0.001*
LVEF *(%)*	64 ± 3.1 *[n = 28]*	62.5 ± 3.7 *[n = 26]*	*0.09*
mPAP *(mmHg)*	26.3 ± 4.3 *[n = 26]*	27.4 ± 4.8 *[n = 27]*	*0.64*
Cardiac T2* *(msec)*	37 ± 14 *[n = 28]*	37 ± 9 *[n = 26]*	*0.44*
LIC *(mg/g dw)*	5.0 ± 5.4 *[n = 28]*	3.7 ± 3.6 *[n = 26]*	*0.52*
Total PA *(MET-min/week)*	921 ± 438 *[n = 28]*	920 ± 463 *[n = 26]*	*0.80*

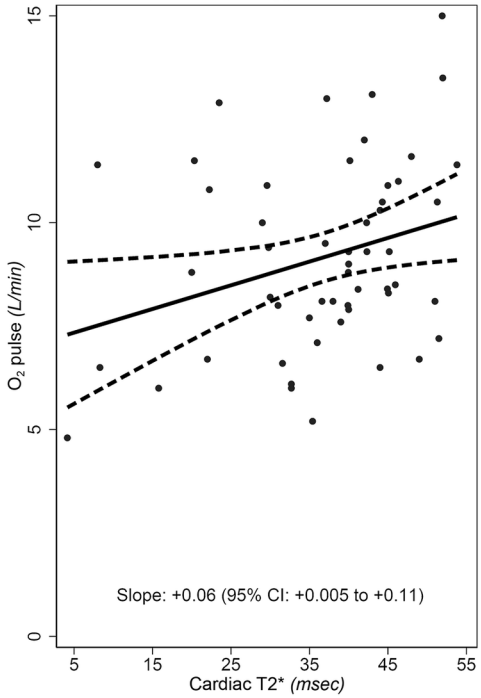
B) **CPET results** *(mean ± SD)*

	Males	Females	p-value
V'O₂ peak *(mL/min/kg)*	26.3 ± 6.3 *[n = 27]*	23.4 ± 5 *[n = 26]*	*0.05*
V'O₂ @AT *(mL/kg/min)*	14 ± 4.1 *[n = 23]*	12.5 ± 3.4 *[n = 21]*	*0.28*
O₂ pulse *(L/min)*	10.3 ± 2.3 *[n = 28]*	8 ± 1.5 *[n = 26]*	*< 0.001*
HR max *(bpm)*	161.1 ± 19.4 *[n = 28]*	158 ± 17.9 *[n = 26]*	*0.26*
V'E peak *(n/min)*	65.7 ± 14.2 *[n = 19]*	51.3 ± 11.8 *[n = 18]*	*< 0.001*
V'O₂/work *(mL/min/watt)*	12.9 ± 1.5 *[n = 28]*	12.9 ± 1.9 *[n = 25]*	*0.91*
MVV *(L/min)*	111.8 ± 22.1 *[n = 22]*	91.7 ± 20 *[n = 20]*	*0.006*
BR *(L/min)*	50.9 ± 16.7 *[n = 13]*	40.5 ± 16.6 *[n = 13]*	*0.12*
Δ Hb O₂ Sat. *(%)*	1.3 ± 1.3 *[n = 28]*	1.3 ± 1.3 *[n = 25]*	*0.82*
V'E/V'O₂ slope	35.3 ± 5.1 *[n = 28]*	35.7 ± 3.9 *[n = 26]*	*0.72*
V'E/V'CO₂ slope	30.7 ± 3.7 *[n = 28]*	31.1 ± 3.7 *[n = 26]*	*0.92*

C) **Association between V'O₂ peak, and cardiac T2***



D) **Association between O₂ pulse and cardiac T2***



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