ESC European Society of Cardiology

European Journal of Preventive Cardiology (2022) **29**, 445–451
doi:10.1093/eurjpc/zwaa134

FULL RESEARCH PAPER
Exercise testing and training

Safety and feasibility of upper limb cardiopulmonary exercise test in Friedreich ataxia

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Received 20 August 2020; revised 19 October 2020; editorial decision 10 November 2020; accepted 12 November 2020; online publish-ahead-of-print 9 December 2020

Aims To explore the feasibility of upper limbs cardiopulmonary exercise test (CPET) in Friedreich ataxia (FRDA) patients and to compare the results with sex, age, and body mass index (BMI) matched cohort of healthy controls (HC).

Methods and results Cardiopulmonary exercise test was performed using an upper limbs cycle ergometer on fasting subjects. Peak oxygen uptake (peak VO_2) was recorded as the mean value of VO_2 during a 20 s period at the maximal effort of the test at an appropriate respiratory exchange rate. The ventilatory anaerobic threshold (AT) was detected by the use of the V-slope method. We performed echocardiography with an ultrasound system equipped with a 2.5 MHz multifrequency transducer for complete M-mode, two-dimensional, Doppler, and Tissue Doppler Imaging analyses. We studied 55 FRDA and 54 healthy matched controls (HC). Peak VO_2 showed a significant 31% reduction in FRDA patients compared to HC (15.2 ± 5.7 vs. 22.0 ± 6.1 mL/kg/min; $P < 0.001$). Peak workload was reduced by 41% in FRDA (42.9 ± 12.5 vs. 73.1 ± 21.2 W; $P < 0.001$). In FRDA patients, peak VO_2 is inversely correlated with the Scale for Assessment and Rating of Ataxia score, disease duration, and 9HPT performance, and directly correlated with activities of daily living. The AT occurred at 48% of peak workload time in FRDA patients and at 85% in HC ($P < 0.001$).

Conclusions Upper limb CPET is useful in the assessment of exercise tolerance and a possible tool to determine the functional severity of the mitochondrial oxidative defect in patients with FRDA. The cardiopulmonary exercise test is an ideal functional endpoint for Phases II and III trials through a simple, non-invasive, and safe exercise test.

Keywords Friedreich ataxia • Cardiopulmonary exercise test • Mitochondrial • Endpoint • Trial

Introduction

Friedreich ataxia (FRDA) is an autosomal recessive ataxia[1] caused by a trinucleotide guanine-adenine-adenine (GAA) expansion in the first intron of the *FXN* gene,[2] which encodes for a 210 amino acid mitochondrial protein called frataxin. Even if its function remains not fully elucidated, a growing body of evidence has shown a link to iron–sulphur cluster (ISC) and haeme biogenesis, iron-binding/storage, and iron chaperone activity.[3,4] Frataxin levels and *FXN*/mRNA are severely reduced in FRDA[5] and are causative of the disease. The clinical symptoms begin in childhood or adolescence and are characterized by progressive ataxia with corticospinal tract and peripheral nerve

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involvement. Beyond neurological involvement, cardiovascular impairment represents the major health issue of these patients. Heart failure, secondary to FRDA cardiomyopathy, is the predominant cause of death. Type II diabetes, scoliosis, foot deformities, optic atrophy, and deafness are other relatively frequent symptoms.[6] To date, there is no known disease-modifying therapy to slow FRDA disease progression or provide symptomatic treatment.

Different studies underline the alteration of mitochondrial function as the neurodegenerative pathological mechanism of FRDA. Frataxin binds Fe^{2+} and contributes to its controlled oxidation to Fe^{3+} and to its incorporation into ISC in the mitochondrial matrix.[7] ISCs are cofactors for proteins involved in numerous important functions, such as respiration (complexes I–III), β-oxidation of lipids, lipoid acid synthesis, Krebs cycle (aconitase), and iron homeostasis.[8] Frataxin deficiency causes a reduction of ISC formation resulting in an impairment of mitochondrial respiratory chain function, increased production of radical oxygen species (ROS),[9] and mitochondrial biogenesis defect.[10]

Impairment of tissue energy metabolism *in vivo* was highlighted by phosphorus magnetic resonance spectroscopy studies that showed a reduced rate of mitochondrial Adenosine triphosphate (ATP) synthesis in FRDA patients.[11] Furthermore, non-invasive continuous near-infrared muscle spectroscopy, that assesses the delivery and utilization of oxygen in response to exercise, showed inadequate muscular oxygen consumption in FRDA patients.[12] However, there are no validated diagnostic instruments that allow for an *in vivo* evaluation of mitochondrial function in FRDA patients, or to assess its link to cardiovascular performance, particularly before cardiovascular symptoms begin.

The cardiopulmonary exercise test (CPET) is the gold standard for the assessment of oxygen uptake (VO_2), peak workload, and for non-invasive evaluation of the anaerobic threshold (AT). Specifically, VO_2 max reflects the maximal ability for the human body to deliver, obtain, and consume oxygen during maximal exercise,[13] with VO_2 at peak exercise conventionally measured as the VO_2 averaged over a 20–30 s period at maximal effort, with the attainment of an appropriate respiratory exchange rate (RER).[14] The AT is the time of transition from aerobic to anaerobic metabolism and it reflects the metabolic condition above which blood lactate increases and pH starts to decrease, generating the isocapnic compensatory buffering through bicarbonate. This metabolic state leads to an increase of carbon dioxide (VCO_2) release, as compared with VO_2, due to the additional CO_2 produced by lactic acid buffering. Cardiopulmonary exercise test provides valuable insights into the integrated functioning of the ventilatory, cardiovascular, and skeletal muscle systems. During exercise, the heart and lungs support the increased metabolic requirements of skeletal muscle, primarily by delivering oxygen and removing carbon dioxide. In physiological condition, VO_2 is limited by the ability of the cardiorespiratory system to deliver oxygen to the exercising muscles.[15] When optimal O_2 delivery is ensured by the correct operation of this system (i.e. no cardiac or pulmonary diseases), it has been described that the ability to produce energy, consume O_2, and generate CO_2 mainly depends on mitochondrial fitness.[14,16] Therefore, a fascinating hypothesis investigated by the present report is that if CPET is performed when no clear cardiovascular or pulmonary impairment occurs (i.e. initial and intermediate forms of FRDA, in which mitochondrial proliferation, loss of contractile proteins, development of myocardial fibrosis due to frataxin deficit have not yet caused alteration of heart function but only at muscular levels), CPET can be considered as a possible novel and non-invasive measurement of mitochondrial fitness.[14,16] However, considering that FRDA patients are not able to perform CPET with a classic cycle ergometer because of the early lower limbs ataxia and weakness (i.e. they would not be capable of reaching the AT and maintaining an appropriate target frequency of pedalling), we investigated whether the upper limb CPET could represent a valuable alternative tool in FRDA.

Aims of the present study were to explore the safety and the feasibility of upper limbs CPET in FRDA patients, to compare the results with a sex-, age-, and body mass index (BMI)-matched cohort of healthy subjects (HC) purposefully enrolled as a control population, and to explore whether CPET could represent a possible non-invasive tool to indirectly measure mitochondrial fitness in FRDA patients.

Methods

Study population

We compared 55 FRDA patients to 54 age-, sex-, and BMI- matched HC. HCs were defined as subjects without an established diagnosis of any diseases. Before CPET, a fast-echocardiographic exam was performed, to exclude systolic or diastolic dysfunction, as well as valvular diseases. HCs were not athletes and not involved in training programs. Inclusion and exclusion criteria for FRDA are as follows molecular diagnosis of FRDA with a homozygous expansion in the first intron of the *FXN* gene; age ≥12 years; body weight >30, <90 kg; Scale for Assessment and Rating of Ataxia (SARA) score < 30; and able to perform a CPET. Exclusion criteria were contraindications to CPET; any clinically relevant cardiac, pulmonary, hepatic, or renal disease; any clinically relevant electrocardiogram (ECG) abnormalities; any abnormal and clinically relevant laboratory exams at screening visit; anaemia with haemoglobin < 10 g/dL; positive history for thrombosis; drug-resistant arterial hypertension; drug-resistant epilepsy.

Cardiopulmonary exercise test

We performed the CPET using an upper limbs cycle ergometer (Ergoselect 400, Ergoline GmbH, Blitz, Germany) on fasting subjects. A standardized ramp protocol of 10 W/min was started and continued until limiting symptoms, chest pain, signs of ischaemia, or arrhythmias developed, or other indications for exercise termination appeared. Subjects were instructed to keep cranking at a constant rate (50–60 rpm) during the test. Subjects were advised that they were free to stop whenever they wished but were encouraged to continue for as long as possible.

Respiratory gas exchange measurements were obtained breath by breath by a commercially available system (Vmax 29 C; Sensormedics, Yorba Linda, CA, USA). VO_2 at peak exercise was measured as the VO_2 averaged over a 20–30 s period at maximal effort, with the attainment of an appropriate respiratory exchange rate[14,17] (RER). The ventilatory AT was detected by the use of the V-slope method. The ventilation per minute (VE) vs. VCO_2 relationship (ventilatory efficiency) was measured by plotting ventilation against VCO_2 obtained every 10 s of exercise (VE/VCO_2 slope). The VE/VCO_2 slope was calculated as a linear regression function, excluding the non-linear part of the relationship after the onset of acidotic drive to ventilation.

Peak exercise oxygen pulse was calculated by dividing VO_2 by the maximum heart rate (HR) during exercise and was expressed in millilitres per beat. Heart rate was recorded during all the tests, including the AT and peak.

The mechanical work efficiency during exercise was estimated as the ratio of total power output to total O_2 required to develop it, after calculating the respective caloric equivalents as follows: 1 litre VO_2/min = 4.96 calories, assuming a respiratory exchange ratio of 0.95, and 1 W/min = 0.014 calories. Mechanical work efficiency reflects the ability to convert metabolic energy into external mechanical work.

Echocardiography

We used an ultrasound system equipped with a 2.5 MHz multifrequency transducer (Aplio, Toshiba, Japan) for complete M-mode, two-dimensional, Doppler, and tissue Doppler imaging echocardiographic analyses. M-mode and two-dimensional recordings were made with the patients in the lateral recumbent position.

Quantitative motor tests

A standardized neurological examination included the SARA. The SARA scale is a scale consisting of eight items (gait, stance, sitting, speech disturbance, finger chase, nose–finger test, fast alternating hand movements, heel-shin slide). The scale score range was 0–40, with 40 being the worst condition. The scale was validated in FRDA.[18] More quantitative motor tests included the 9-hole pegboard test (9HPT). The 9HPT is used to measure finger dexterity in patients, is administered by asking the patients to take and then remove, the pegs from a container, one by one, and place them into the holes on the board, as quickly as possible. For the 9HPT, the mean of the scores from two trials with each hand was calculated independently.

EQ-5D

The EQ-5D is a standardized tool to measure the quality of life. It can be used in a variety of medical conditions. It gives a simple indication of health status. It can be self-administered and takes only a few minutes.[19]

Activities of daily living and instrumental ADL

Activities of daily living (ADL)/instrumental ADL (IADL) scales are used as an indicator of a person's functional status. The inability to perform ADL/IADL results in the dependence of other individuals and/or mechanical devices and may lead to unsafe conditions and poor quality of life. ADL is scored from 0 to 6, IADL is scored from 0 to 8, lower scores representing more severe disability.

Statistical analysis

We tested normal distribution was tested with the Kolmogorov–Smirnov test. The continuous variable analysis was conducted using the unpaired *t*-test, categorical variables were compared using a χ^2 test. *P*-values of less than 0.05 were considered statistically significant. We analysed the effect of time, factors, and covariates on different variables with a generalized linear model for repeated measures. The statistical relationship between variables was calculated using the Pearson correlation coefficient. We used Bonferroni correction for multiple comparisons and the final significance shown in tables and text incorporates this correction. Data are expressed as mean ± SD or median [interquartile range (IQR)] when appropriate. Statistical analysis was performed using SPSS version 26 (IBM, New York, NY, USA) running on a MAC OSX 10.15.5 (Apple, Cupertino, CA, USA).

Ethics

The protocol was approved by the local ethics committee (No. 256/11). The study was performed in accordance with the Declaration of Helsinki European guidelines CPMP/ICH/135/95 and Italian law D.M.15/07/1997. All patients and HC gave written informed consent prior to any clinical trial activity.

Results

Age, gender, weight, height, and BMI did not differ between FRDA and HC (*Table 1*). The clinical characteristics of FRDA patients, including echocardiographic measurements, are reported in *Table 2*. None of the subjects displayed impairment in the basic spirometry parameters [i.e. forced expiratory volume in 1 s (FEV1) and forced vital capacity], with no differences between the two groups (data do not show).

With regard to CPET parameters, VO_2 peak showed a significant 31% reduction in FRDA patients compared to HC performance

Table 1. Baseline demographics

Variables	FRDA *n* = 55	HC *n* = 54	*P*-value
Age	35.3 ± 13.8	32.1 ± 10.5	0.186
Gender (M/F)	29/27	27/27	0.851
Weight	64.5 ± 15.8	68.5 ± 14.3	0.173
Height	166.5 ± 10.0	170.0 ± 9.0	0.065
BMI	23.1 ± 4.6	23.5 ± 3.5	0.557
Haemoglobin (mg/dL)	13.3 ± 1.2	13.8 ± 1.5	0.32

BMI, body mass index; FRDA, Friedreich ataxia; HC, healthy controls.

Table 2. Clinical demographics of FRDA patients

Variable	Mean ± SD
Disease duration (years)	16.0 ± 9.0
GAA1	621 ± 297
GAA2	842 ± 291
SARA	19.2 ± 7.1
ADL	5.1 ± 1.3
EQ-5D-VAS	59.6 ± 18.2
9HPT (s)	84.2 ± 49.4
Interventricular septum—diastolic	9.8 ± 1.3
Interventricular septum—systolic	12.8 ± 1.3
Left ventricular mass/body surface	83.5 ± 16.1
Ejection fraction	62.0 ± 0.06
E/A	1.46 ± 0.50
Isovolumic relaxation time (IVRT) (ms)	90.40 ± 18.25
E/E′	7.08 ± 1.96

9-HPT, 9-hole pegboard test; ADL, activities of daily living; EQ-5D-VAS, visual analog scale of the European quality of life scale; FRDA, Friedreich ataxia; I-ADL, instrumental activities of daily living; SARA, scale for the rating and assessment of ataxias; SD, standard deviation.

(15.2 ± 5.7 for FRDA and 22.0 ± 6.1 mL/kg/min for HC; $P < 0.001$; *Figure 1A*). Peak workload was also significantly reduced by 41% in FRDA (42.9 ± 12.5 for FRDA and 73.1 ± 21.2 W for HC; $P < 0.001$). In FRDA patients, VO_2 peak inversely correlated with the SARA score, disease duration, and 9HPT performance, and directly correlated with ADL (*Table 3*). VO_2 peak did not correlate with BMI and interventricular septum thickness. The AT occurred at 48% of peak workload time in FRDA patients and at 85% in HC (*Figure 1B*). Time to peak workload was 7.2 ± 2.6 for FRDA vs. 9.1 ± 2.1 for HC; $P < 0.001$; time to AT was 3.5 ± 0.8 for FRDA vs. 7.7 ± 1.9 min for HC; $P < 0.001$; *Figure 1C*. As compared with controls, FRDA patients had 36% less oxygen consumption at the AT (10.7 ± 3.4 for FRDA vs. 16.6 ± 4.7 mL/kg/min for HC; $P < 0.001$).

The individual data points of patients and controls are shown in *Figure 1D*. Oxygen consumption at the AT did not correlate with workload (R = -0.161; $P = 0.387$) in FRDA, but it did in HC

Table 3. Correlations between peak VO_2 and clinical measures in FRDA

Variable	*R*	*P*-value
BMI	-0.333	0.084
Interventricular septum thickness	-0.281	0.234
Ejection fraction	-0.091	0.514
SARA	-0.434	0.006
Disease duration	-0.397	0.018
9HPT	-0.437	0.006
ADL	0.536	<0.001

9-HPT, 9-hole pegboard test; ADL, activities of daily living; MI, body mass index; FRDA, Friedreich ataxia; I-ADL, instrumental activities of daily living; SARA, scale for the rating and assessment of ataxias; VO_2, oxygen consumption.

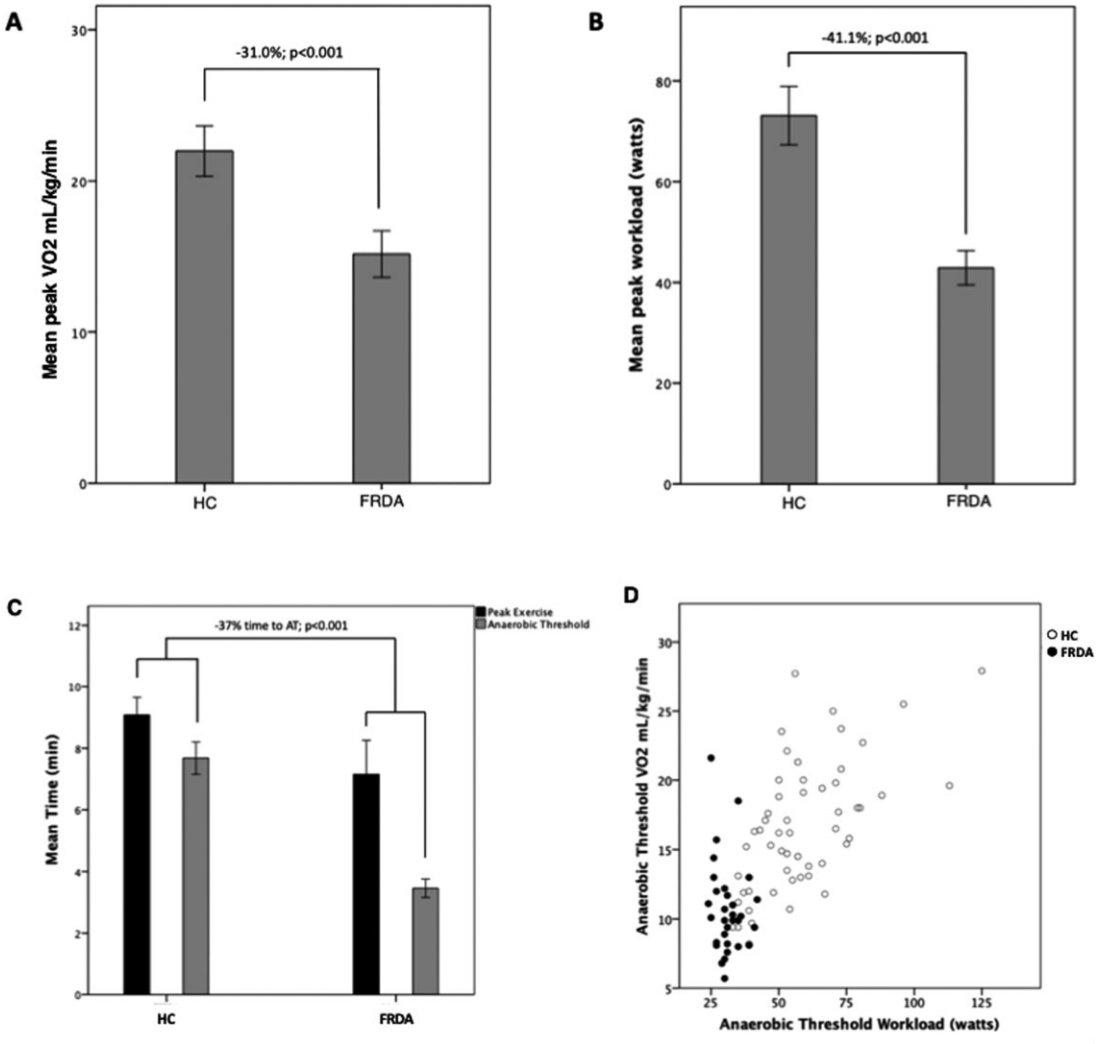

Figure 1. Whiskers are ± 2* standard error mean. (*A*) Mean peak VO_2 in FRDA and HC. (*B*) Mean peak workload in FRDA and HC. (*C*) Mean time to the anaerobic threshold in FRDA and HC. (*D*) Relationship between workload and VO_2 at the anaerobic threshold. AT, anaerobic threshold; FRDA, Friedreich ataxia; HC, healthy controls; PeakVO_2, peak oxygen consumption.

($R = 0.610$; $P < 0.001$; *Figure 1D*). Mechanical efficiency (%) at the AT was 21.9 ± 11.3 for FRDA and 19.6 ± 4.1 for HC ($P = 0.294$), and 18.2 ± 6.8 for FRDA and 17.1 ± 3.5 for HC ($P = 0.325$) at peak exercise. VE/VCO_2 slope was higher in FRDA than in HC (33.0 ± 5.4 for FRDA and 27.1 ± 4.9 for HC; $P < 0.001$). When adjusted for age and gender,[20] 87.3% of FRDA patients had VE/VCO_2 slope values in the pathological range.

Baseline HR (79.9 ± 17.8 for FRDA vs. 83.4 ± 14.8 for HC; $P = 0.275$) and peak HR (140.1 ± 19.0 for FRDA vs. 140.2 ± 24.9 for HC; $P = 0.984$) did not differ between groups. Peak exercise oxygen pulse was 25% lower in FRDA patients as compared to HC (8.0 ± 2.4 for FRDA vs. 10.8 ± 3.4 for HC; $P < 0.001$). HC and FRDA main CPET data are shown in *Table 4*. No gender differences were detected.

Discussion

Several are the findings of the present report. Firstly, CPET is a non-invasive, safe, and potentially repeatable tool in FRDA patients. All patients were able to correctly perform CPET and reached the AT, suggesting that CPET with an upper limb ergometer allows efficient testing of patients with a SARA <30.[21] Secondly, FRDA patients have some differences when compared to HC, despite displaying similar cardiovascular and respiratory function. Therefore, these alterations are virtually related to muscular metabolism impairment, in FRDA strictly linked to mitochondrial alteration. Indeed, at peak exercise, FRDA patients showed 30% less oxygen consumption than matched HC and a reduced maximum effort. Noteworthy, they rapidly reached the AT that occurred in less than half the time required in HC (3.5 vs. 7.7 min: $P < 0.001$). These observations lead to two additional important considerations.

First, the rapid AT achievement suggests that FRDA patients spent more than half of the entire exercise time using their anaerobic metabolism, while HC, as expected, used prevalently an aerobic metabolism. This suggests that FRDA patients carry out most of their daily activities using anaerobic metabolism, which is a very inefficient process for ATP generation, considering that it yields only 2 ATP per glucose moiety compared with at least 30 ATP of oxidative phosphorylation.[22] The clinical correlate could be the frequently reported symptom of fatigue, which was found in several studies involving FRDA patients.[23–25]

Second, the switch to anaerobic metabolism occurred at a low level of effort and around the same time in FRDA, independent from workload, whereas in HC the time to AT was spread-out and clearly correlated with the workload. A possible explanation is that in FRDA muscular cells fail to supply even small increases in ATP demand using oxidative phosphorylation (OXPHOS) and activate anaerobic glycolysis abruptly. Indeed, previous studies showed that an increase in mitochondrial activity produces only a modest increase in VO_2 (20–40%),[26] whereas an increase in mitochondrial number and good mitochondria network dynamics is more effective. Notably, the mitochondrial copy number is reduced in FRDA cells and in animal models of the disease.[10,27,28] This in line with earlier magnetic resonance spectroscopy studies that showed a marked deficit of muscle mitochondrial ATP production during physical exercise in FRDA patients,[11] and with the drastic reduction in the mitochondrial respiratory chain complexes I, II, and III in FRDA.[29] On the other hand, the values of mechanical efficiency in our patients were similar to those of control subjects. This indicates that mitochondrial dysfunction prevents the provision of adequate energy supply in FRDA, but its conversion into external mechanical work is unaffected.

An additional interesting result was that AT has been reached at a relatively high percentage of effort in HC, probably determined by the type of exercise and the muscles involved. Further studies are needed to verify this finding.

The echocardiographic indices of systolic function (ejection fraction), ventricular relaxation (*E/A* and IVRT), and ventricular filling pressure (*E/E′*) were on average comparable to the values observed in normal subjects. Baseline and peak HR in FRDA patients were not different from controls, which excludes the presence of chronotropic incompetence. Even if peak exercise oxygen pulse was lower in FRDA patients than in controls, we did not observe the early plateau that is typically seen in systolic dysfunction. Indeed, typical CPET findings for heart failure patients are a lower peak heart rate associated with the achievement of a low plateau of the peak oxygen pulse.[30,31] Notably, basic spirometry parameters were in the normal range, allowing us to exclude that the impaired exercise ability and oxygen consumption of our FRDA patients was a mere consequence of cardiovascular and/or pulmonary impairment. Taken together, all our findings support the hypothesis that the limiting factor of VO_2 and exercise capacity was mitochondrial dysfunction.

Table 4. HC and FRDA CPET data

	HC mean ± SD	HC median (IQR)	FRDA mean ± SD	FRDA median (IQR)	*P*-value
Peak VO_2	21.99 ± 6.1	20.5 (10.5)	15.16 ± 5.7	16.8 (4.5)	<0.001
Peak workload	73.1 ± 21.2	71 (32.3)	42.9 ± 12.6	43 (18)	<0.001
Peak HR	140 ± 25	143 (35)	140 ± 19	142 (21)	0.984
VE/VCO2 slope	27.1 ± 4.9	26.7 (6.3)	33 ± 5.4	31.5 (6.8)	<0.001
Peak exercise oxygen pulse	10.7 ± 3.4	11 (3.5)	8 ± 2.4	7.6 (2.8)	<0.001
AT oxygen pulse	9 ± 2.8	9 (3)	6.6 ± 2.1	5.9 (3.3)	<0.001
Time to peak workload	9.1 ± 2.1	9.1 (4.04)	7.2 ± 2.6	5.6 (3)	<0.001
Oxygen consumption at the AT	16.6 ± 4.7	25 (6.6)	10.7 ± 3.4	10.1 (3.8)	<0.001

AT, anaerobic threshold; FRDA, Friedreich ataxia; HC, healthy controls; HR, heart rate; IQR, interquartile ranges; VE/VCO_2 slope, ventilation per minute/carbon dioxide production; VO_2, oxygen consumption.

Finally, when adjusted for age and gender, the VE/VCO_2 slope was increased in FRDA compared to HC, probably because of a compensatory hyperventilation secondary to metabolic acidosis. Indeed, the failure of aerobic respiration in FRDA patients and the early activation of anaerobic glycolysis leads to the rise of lactate production and pH reduction. Intensified alveolar ventilation results in a lowered arterial CO_2 and an increased VE. Because FRDA patients do not display echocardiographic signs of ventilation–perfusion mismatch (e.g. heart failure or pulmonary arterial hypertension), this observation further supports the presence of OXPHOS inefficiency and the need to produce energy by exploiting early anaerobic metabolism.

As limitations, the present study did not include invasive haemodynamic assessment,[32,33] nor any echocardiographic re-evaluation over time. Considering that data comparing upper and lower limb CPET has shown differences between the two methods,[34] our results cannot be directly compared with data deriving from classical cycle ergometer test. However, the upper limbs CPET is the only way to perform a CPET in FRDA, since these patients would not be capable of reaching the anaerobic threshold and maintaining the target frequency of pedalling. As a further strength of the present report, we compare FRDA performance with an age-, sex-, and BMI-matched population of healthy subjects, allowing us to have reliable information from FRDA patients.

In conclusion, the present study shows the feasibility and the safety of upper limb CPET as a non-invasive method useful in the assessment of cardiovascular performance and exercise tolerance in FRDA. Further, our results suggest that CPET can be used to have indirect information on the severity of the mitochondrial oxidative defect in patients with FRDA. However, further studies are needed to clarify this point.

Acknowledgements

We are grateful to all patients and their families for taking part to the trial. We are grateful to Jennifer Farmer of the Friedreich's Ataxia Research Alliance (FARA) and to Paolo Zengara of the Italian Association Ataxic Syndromes—Associazione Italiana per la lotta alle Sindromi Atassiche—(AISA) for their assistance in recruiting patients through the entire study.

Funding

This study was supported from the Keith Michael Andrus Award from the Friedreich' Ataxia Research Alliance (FARA) to Francesco Saccà and Alessandro Filla, from a grant from the Italian Association Ataxic Syndromes- Associazione Italiana per la lotta alle Sindromi Atassiche (AISA) to Francesco Saccà, and an unrestricted grant from AISA sez. Campania to Francesco Saccà. Andrea Salzano receives research grant support from CardioPath, Department of Advanced Biomedical Sciences, Federico II University, Naples, Italy, and UniNA & Compagnia di San Paolo in the frame of the STAR program.

Conflict of interest: F.S. received public speaking honoraria from Biogen, Mylan, Novartis, Roche, Sanofi, Teva; served on advisory boards for Almirall, Argenx, Avexis, Biogen, Forward Pharma, Merk, Novartis, Pomona, Roche, Sanofi. All other authors report no conflict of interest.

Data availability

The data used to conduct the research are available upon reasonable request. Requests should be addressed to the corresponding author, that will be in charge of accepting or denying the request. Restrictions may occur due to the European General Data Protection Regulation.

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