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RESEARCH ARTICLE

Autonomic, functional, skeletal muscle, and cardiac abnormalities are associated with increased ergoreflex sensitivity in mitochondrial disease

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Aims Mitochondrial disease (MD) is a genetic disorder affecting skeletal muscles, with possible myocardial disease. The ergoreflex, sensitive to skeletal muscle work, regulates ventilatory and autonomic responses to exercise. We hypothesized the presence of an increased ergoreflex sensitivity in MD patients, its association with abnormal ventilatory and autonomic responses, and possibly with subclinical cardiac involvement.

Methods and results Twenty-five MD patients (aged 46 ± 3 years, 32% male) with skeletal myopathy but without known cardiac disease, underwent a thorough evaluation including BNPs, galectin-3, soluble suppression of tumorigenesis 2 (sST2), high sensitivity troponin T/I, catecholamines, ECG, 24-h ECG recording, cardiopulmonary exercise testing, echocardiography, cardiac/muscle magnetic resonance (C/MMR), and ergoreflex assessment. Thirteen age- and sex-matched healthy controls were chosen. Among these myopathic patients, subclinical cardiac damage was detected in up to 80%, with 44% showing fibrosis at CMR. Ergoreflex sensitivity was markedly higher in patients than in controls (64% vs. 37%, $P < 0.001$), and correlated with muscle fat to water ratio and extracellular volume at MMR (both $P < 0.05$). Among patients, ergoreflex sensitivity was higher in those with cardiac involvement ($P = 0.034$). Patients showed a lower peak oxygen consumption (VO_2/kg) than controls ($P < 0.001$), as well as ventilatory inefficiency ($P = 0.024$). Ergoreflex sensitivity correlated with reduced workload and peak VO_2/kg (both $P < 0.001$), and several indicators of autonomic imbalance ($P < 0.05$). Plasma norepinephrine was the unique predictor of myocardial fibrosis at univariate analysis ($P < 0.05$).

Conclusions Skeletal myopathy in MD is characterized by enhanced ergoreflex sensitivity, which is associated with a higher incidence of cardiac involvement, exercise intolerance, and sympathetic activation.

Keywords Mitochondrial disease • Myopathy • Exercise • Dyspnoea • Autonomic balance

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Introduction

Mitochondrial disease (MD) is a neuromuscular disorder with an estimated prevalence of 1 in 4300 adults.[1] Mitochondrial dysfunction most frequently affects the organs with high energy requirements, such as the skeletal muscle and the heart.[2] Skeletal myopathy is a main feature of MD, probably accounting for exercise intolerance, dyspnoea on effort, and muscle fatigue, although the exact mechanisms of these symptoms have not been clarified yet.[3] Cardiac involvement is another frequent finding, with 30% of adult patients displaying ECG and/or echocardiographic abnormalities,[4] while cardiac magnetic resonance (CMR) discloses late gadolinium enhancement (LGE), denoting myocardial fibrosis, in 33% of patients.[5] Cardiac involvement has been associated with adverse prognosis,[4] underscoring the need for therapies able to slow down the progression of cardiac damage.

The ergoreflex is a neuromuscular reflex increasing ventilation and sympathetic outflow during exercise.[6] Ergoreflex sensitivity has been thoroughly investigated in chronic heart failure (CHF), where increased ergoreflex sensitivity is associated with secondary sarcopenic myopathy and contributes to disease evolution, by inducing adrenergic activation, as well as exercise intolerance, dyspnoea, and fatigue.[6,7] Skeletal myopathy and exercise intolerance are also prominent features of MD,[8] and increased sympathetic outflow has also been reported.[9]

In the present study, we aimed to assess ergoreflex sensitivity in MD patients with skeletal myopathy and without history of heart disease, as well as its relationships with subclinical cardiac involvement, exertional dyspnoea, and autonomic imbalance.

Methods

Study population

Between January 2014 and October 2015, a total of 25 patients with ascertained MD and without cardiac involvement were referred for cardiovascular assessment to the Fondazione Toscana Gabriele Monasterio, Pisa, Italy, by the Neurology Clinic, University of Pisa, Italy. They had previously undergone clinical evaluation by expert neurologists (D.O. and M.M.), search for pathogenetic mutations, ischaemic forearm test, muscle biopsy, electromyogram, and brain magnetic resonance imaging.[10,11] Informed consent was obtained from each patient; the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institutional research committee.

The measurement of ergoreflex sensitivity and cardiological evaluation were performed during a maximum time interval of 3 days, taking care to avoid potential interferences between examinations. Thirteen healthy controls were matched to patients with respect to age and gender, and underwent a subgroup of exams: proton muscle spectroscopy, ergo- and baroreflex assessment, cardiopulmonary exercise testing (CPET), 24-h ECG Holter recording, and plasma norepinephrine (NE) and epinephrine (E) assays.

Electrocardiogram, echocardiogram, and cardiac magnetic resonance

Standard 12-lead ECG and transthoracic echocardiography (TTE) were performed. Three-dimensional TTE images were obtained using a Philips IE33 Ultrasound machine (Philips Medical Systems, Palo Alto, CA, USA). Wall thickness, chamber volumes, and systolic/diastolic function were assessed according to current guidelines.[12] Speckle tracking analysis was performed using the TomTec software (4D LV-Analysis©, TomTec Imaging Systems, version 4.6, 2012); the results were interpreted according to a recent meta-analysis.[13]

Gadolinium-enhanced CMR (1.5 T total body scan, General Electric Healthcare, Milwaukee, WI, USA) was performed in all patients without contraindications. Standard protocols were used to evaluate the volumes and function of the cardiac chambers.[14] The extent of LGE was automatically calculated on short-axis images, by adopting a signal intensity threshold of >6 standard deviations (SDs) above the mean signal intensity of the remote normal myocardium.[15] Standard CMR examination was completed by T1 mapping analysis, which allows quantification of myocardial extracellular volume.[15] During the same session, an acquisition was performed in the proximal muscles of an upper limb in order to assess muscle extracellular volume (T1 mapping),[15] and proton muscle spectroscopy (*biceps brachii* muscle of the non-dominant arm). Custom-written software was used for these analyses.

Cardiopulmonary exercise testing and analysis of respiratory function

Patients with no disabling motion limitation underwent a symptom-limited CPET on a bicycle ergometer using a protocol gradually increasing pedal resistances so as to reach a maximum power output within 10 ± 2 min (Vmax, Sensormedics, Yorba Linda, CA, USA). Oxygen consumption (VO_2), carbon dioxide output (VCO_2), and minute ventilation (VE) were measured through breath-to-breath gas analysis (Vmax, Sensormedics, Conshohocken, PA, USA). The following parameters were derived: peak respiratory quotient, peak VO_2/kg, VO_2/work rate slope, peak oxygen pulse (ratio between VO_2 and heart rate at peak exercise), anaerobic threshold, maximum workload, and minute ventilation/carbon dioxide production (VE/VCO_2) slope. Trends in heart rate and blood pressure during exercise were considered; the chronotropic response index (CRI) was also calculated.[16]

A pulmonary cause of reduced exercise tolerance was searched for by arterial blood gas analysis, and pulmonary function testing with measurement of lung diffusion capacity and maximal inspiratory and expiratory pressures.

Ergoreflex assessment

A protocol validated in CHF was used to assess ergoreceptor sensitivity.[6] Two exercises were performed with the non-dominant arm, in a random order and separated by a 30-min rest: (i) a control bout that involved gripping the handle of a dynamometer at 50% of the pre-determined maximal strength at the rate of 40 pulls/min until exhaustion; and (ii) the same exercise followed by, from 10 s before the end of exercise, 3 min of circulatory occlusion by forearm cuff inflation to >30 mmHg above systolic arterial pressure. This protocol has been demonstrated to fix the metabolic state of the muscles, thus evoking a sustained ergoreceptor response.[6] Handgrip tests were performed on a conventional dynamometer (Lode dynamometer). Ventilation was recorded continuously through a gas analyser (Vmax, Sensormedics); signals were digitalized (National Instruments, USA, sampling frequency 500 Hz), and analysed through a custom-written software. Ergoreceptor sensitivity was quantified as the percentage of the ventilatory response to exercise maintained by circulatory

occlusion during the third minute, compared with the third minute of basal recovery.[6]

Chemo- and baroreflex assessment

Chemosensitivity was assessed using the rebreathing technique.[17] After a 5-min baseline recording during spontaneous breathing, normocapnic–hypoxic and normoxic–hypercapnic trials were randomly performed. The hypoxic ventilatory response was expressed by the linear regression slope between VE and SaO_2 during a hypoxic–normocapnic trial. The hypercapnic ventilatory response was expressed by the linear regression slope between VE and end-tidal pressure of CO_2, during the hypercapnic–normoxic trial. Baroreflex has been examined in all patients with a non-invasive technique. The ECG signal and the arterial pressure were registered continuously for 10 min, with the patient supine and resting. The ratio between the SD of the RR interval and the SD of the systolic arterial pressure quantified baroreflex sensitivity.[18]

Electrocardiographic Holter recording

Twenty-four hour ECG Holter recording was obtained by a three-lead (pre-cordial, posterior, and inferior leads) digital system (Elamedical, France). The following parameters were calculated: 24-h average values of normal RR intervals (RR), standard deviation of all RR (SD), standard deviation of 5-min mean values of RR (SDANN), square root of the mean of the sum of the squares of differences between adjacent RRs (rMSSD), and the number of adjacent RRs differing by >50 ms, as a percentage of the total number of RRs (pNN50), as indexes of autonomic function.[19]

Biohumoral assessment

Several biomarkers were dosed after nocturnal fasting and following 20 min of supine position: blood cell count, thyroid hormones, creatinine, transaminases, NT-proBNP, high-sensitivity cardiac troponin T (hs-cTnT) and I (hs-cTnI), NE, E, active renin, aldosterone, galectin-3 (Gal-3), soluble suppression of tumorigenesis (sST2), myoglobin, lactic dehydrogenase (LDH), and creatine phosphokinase (CPK). The details are provided in previous publications.[20,21]

Statistical analysis

Statistical analysis was performed using IMB SPSS Statistics (version 22, 2013). Variables with normal distribution were presented as mean ± SD, and those with skewed distribution as median and interquartile range (IR). Mean differences among groups were evaluated through the unpaired Student *t*-test or Mann–Whitney U-test. Discrete variables were compared by the χ^2 test with Yates correction or the Fisher exact test. The Spearman rank correlation was used for correlations. Several known predictors of myocardial fibrosis (plasma renin activity, aldosterone, Gal-3, sST2, hs-TnI, hs-TnT, NE, and NT-proBNP) were screened using logistic regression analysis. A *P*-value <0.05 was considered significant.

Results

Skeletal myopathy

Study population characteristics are summarized in *Table 1*. All patients had received a genetic diagnosis of MD. Skeletal myopathy was denoted by limitation of motion in 20 patients (80%), and by subclinical signs (abnormal lactic acid response to forearm ischaemic test, myopathic pattern at electromyogram, and histological and immunoenzymatic clues of mitochondrial myopathy) in the other 5 patients (20%). T1 mapping analysis revealed increased muscle extracellular volume in 18 (72%) patients (ECV: median 21%, IR 20–22% in 7 males, reference values 14 ± 3%; median 19%, IR 16–20% in 11 females, reference values 18 ± 4%). The presence of skeletal myopathy was further confirmed by proton muscle spectroscopy: the fat to water ratio, which increases in cases of muscle damage with fibro-fatty replacement, was significantly higher in patients ($n = 8$) than in controls ($n = 8$) (median 0.18, IR 0.17–0.25 vs. median 0.08, IR 0.02–0.12; $P = 0.003$).

Despite the clinical and instrumental evidence of myopathy, conventional biomarkers associated with muscular involvement were rarely increased, with myoglobin being above the upper reference value (≤110 ng/mL) in only 2 patients, out of 25 (8%), LDH (≤500 IU/L) in 6/25 (24%), and CPK (≤125 IU/L) in 8/25 (32%).

Cardiac involvement: electrocardiographic and imaging findings

Sixteen patients out of 25 (64%) displayed a cardiac involvement, when considering global findings at ECG, standard TTE, and CMR (Supplementary material online, *Table S1*). The most frequent findings were sinus tachycardia ($n = 6$; 25%), ventricular repolarization abnormalities ($n = 5$; 20%), concentric LV hypertrophy [3 patients (12%), without hypertension], and LGE indicating myocardial fibrosis (8/18 patients; 44%) (Supplementary material online, *Figure S1*). T1 mapping analysis confirmed the expansion of myocardial extracellular spaces (Supplementary material online, *Table S1*).[15] Although biventricular systolic function was within limits in all patients at conventional TTE examination, patients showed altered global longitudinal strain (median −19.5%, IR −21.5/−17.3% vs. median −22.2%, IR −13.9/−19.4%, $P = 0.034$) and longitudinal strain in two-chamber view (median −19.3, IR −21.1/−15.5% vs. median −21.8, IR −25.3/−20.0%, $P = 0.018$) as compared with controls, revealing an initial impairment of cardiac function. When adding strain analysis at TTE to basal cardiac screening (ECG, standard TTE, and CMR), 20 patients (80%) displayed some form of cardiac involvement.

Cardiac involvement: biohumoral data

The hs-TnT levels were above the upper reference level (14 ng/L) in 10/25 patients (40%), while hs-TnI was constantly within limits (15.6 ng/L in women, 34.2 ng/L in men). Plasma NE was higher than the cut-off (500 ng/L) in 9/25 patients (36%), while plasma renin activity and aldosterone were within limits in all patients, and NT-proBNP was increased (i.e. >125 ng/L) in 2/25 (8%). Gal-3 levels were higher than the cut-off (17.2 ng/mL) in 4/25 (16%), and sST2 was increased (i.e. >18.8 ng/mL) in 11/25 (44%). Median and IR values of all these biomarkers are reported in the Supplementary material online, *Table S2*.

Table 1 Study population characteristics

	Patients ($n = 25$)	Controls ($n = 13$)	*P*-value
Age (years)	46 ± 3	41 ± 4	0.104
Male sex (*n*, %)	8 (32)	5 (38)	-
BMI (kg/m^2)	23.5 (20.4–25.6)	23.2 (21.2–24.2)	0.693
Creatinine (mg/dL)	0.60 (0.47–0.73)	–	–
ALT (IU/L)	22 (14–38)	–	–
TSH (mIU/L)	2.22 (1.49–2.75)	–	–
Hb (g/L)	134 (128–160)	–	–
mtDNA mutation (*n*, %)	19 (76)	–	–
	Single deletions, $n = 6$; A3243G, $n = 3$; A1555G, $n = 2$; other point mutations, $n = 8$		
nDNA mutation (*n*, %)	6 (24)	–	–
	Genes Twinkle, POLG, or AARS, each $n = 2$		
CPEO (*n*, %)	7 (28)	–	–
MELAS (*n*, %)	1 (4)	–	–
KSS (*n*, %)	1 (4)	–	–
Encephalomyopathy (*n*, %)	8 (32)	–	–
Isolated skeletal myopathy (*n*, %)	4 (16)	–	–
Diabetes (*n*, %)	4 (16)	–	–
Sensorineural hearing loss (*n*, %)	3 (12)	–	–
Smoking (*n*, %)	6 (24)	2 (15)	–
Hypercholesterolaemia (*n*, %)	7 (28)	1 (8)	–
Systemic hypertension (*n*, %)	5 (20)	0 (0)	–

Eleven patients (44%) had been diagnosed with a mitochondrial syndrome: chronic progressive external ophtalmoplegia (CPEO), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), or Kearns–Sayre syndrome (KSS). Five patients (20%) were not co-operative due to encephalomyopathy. No patient displayed anaemia, thyroid, kidney, or liver disease.
Age is reported as mean ± SD. ALT, BMI, creatinine, Hb, and TSH are reported as median and interquartile range.
ALT, alanine aminotransferase; BMI, body mass index; Hb, haemoglobin; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; TSH, thyroid-stimulating hormone.

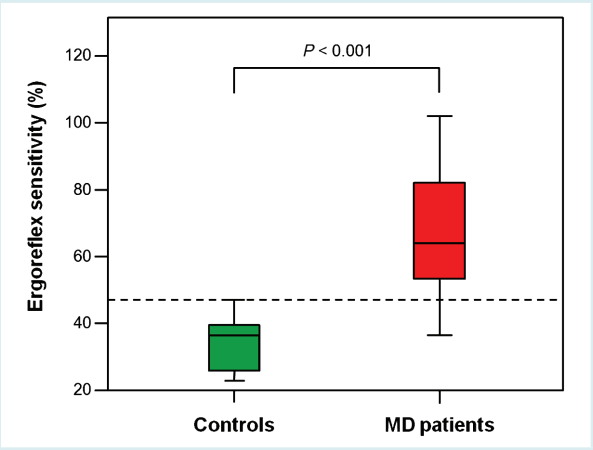

Figure 1 Ergoreflex assessment. Note the markedly different trends in ventilation of a patient and a control matched with respect to sex, age, and level of physical activity. MD, mitochondrial disease.

Reduced exercise capacity and autonomic imbalance

Cardiopulmonary exercise testing, performed in a subgroup of 20 patients, was remarkably abnormal in MD patients (Supplementary material online, *Table S3*). Indeed, peak workload, peak VO_2/kg, anaerobic threshold, and peak O_2 pulse were significantly lower, while VE/VCO_2 slope was significantly higher in patients than in controls, in spite of normal haemoglobin values, and preserved cardiac output and pulmonary function (as shown above). A slight reduction in maximal expiratory pressure was noted, suggesting an initial involvement of respiratory muscles (Supplementary material online, *Table S3*).

Seventeen patients (85%) displayed chronotropic incompetence at CPET, attested by a lower CRI. Compared with controls, patients had lower SD and SDANN values at ECG Holter recording, and lower baroreflex sensitivity (Supplementary material online, *Table S4*), pointing to a sympatho-vagal imbalance with adrenergic predominance.

Increased ergoreflex sensitivity

The measurement of ergoreflex sensitivity was easily performed in all patients except for 5 patients with cognitive impairment and severe limitation of motion (*Figure 1*). No patient complained of discomfort or pain during the manoeuvre. Ergoreflex sensitivity was markedly higher in patients ($n = 20$) than in controls ($n = 13$): median 64% ventilatory response to post-exercise circulatory occlusion vs. 37% ($P < 0.001$). The value corresponding to the upper 95th percentile of controls (47%) was chosen as a cut-off for normal ergoreflex sensitivity. Ergoreflex sensitivity appeared to be enhanced in 16 patients (89%) (*Figure 2*).

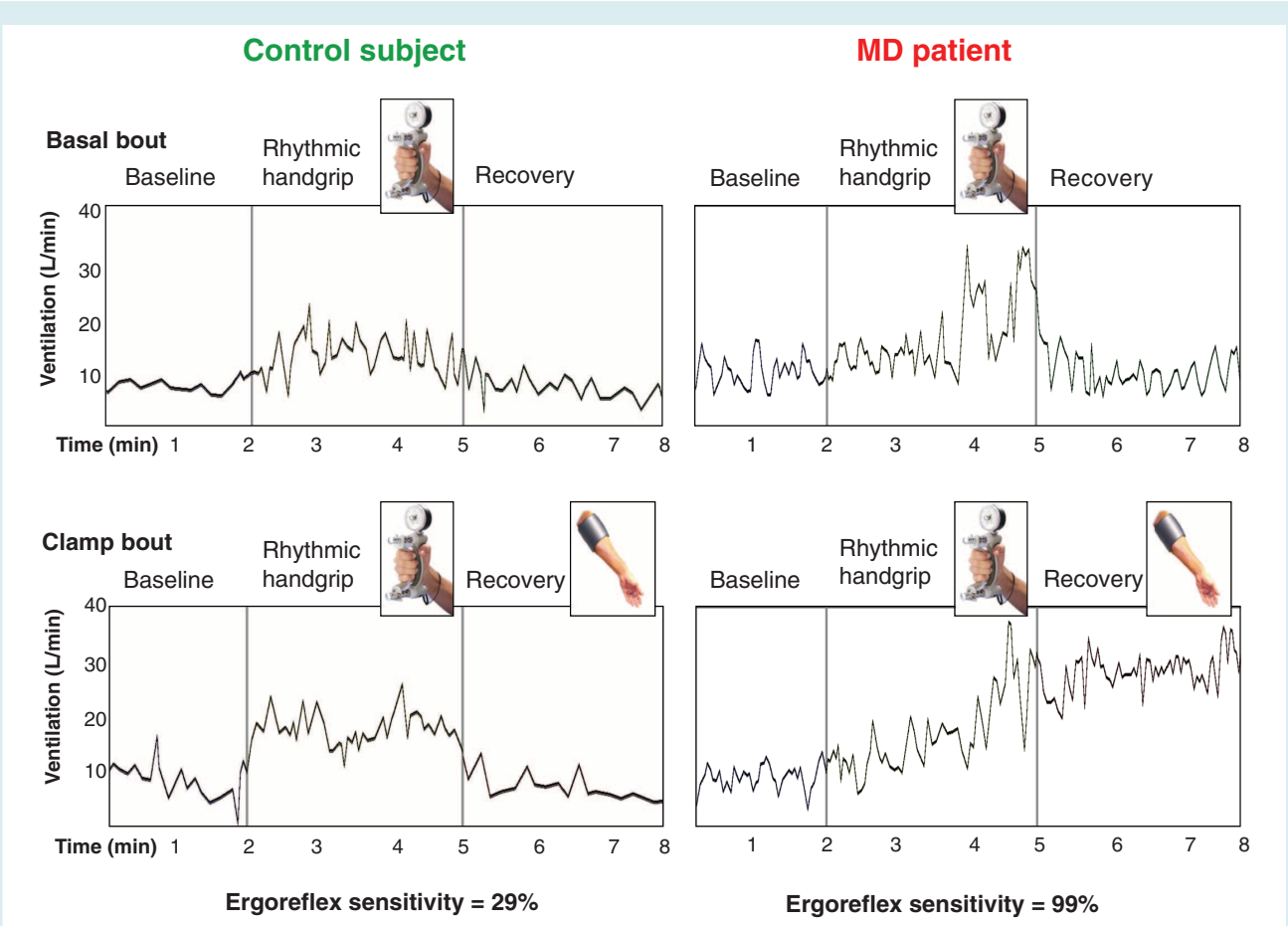

Figure 2 Increased ergoreflex sensitivity in patients with mitochondrial disease (MD). The mean ventilatory response to post-exercise circulatory occlusion is 37% [interquartile range (IR) 26–41%] in controls and 64% (IR 53–82%) in MD patients ($P = 0.001$). As a cut-off, the 95th percentile of the control group (47%) was chosen. No control subject had increased ergoreflex sensitivity, compared with 16/18 patients (89%).

Skeletal myopathy, ergoreflex activation, and cardiac involvement

In the whole study population, ergoreflex sensitivity correlated with muscle ECV ($P = 0.017$), and the fat to water ratio ($P = 0.006$) (*Figure 3*).

Ergoreflex sensitivity was significantly higher in patients with abnormal findings at ECG, TTE, or CMR, than in those without (median 79%, IR 63–86% vs. median 57%, IR 47–64; $P = 0.034$); when adding strain analysis at TTE, the difference in ergoreflex sensitivity was confirmed ($P = 0.026$). A strong positive correlation was observed between ergoreflex sensitivity and quantitative LGE (Rho = 0.869, $P = 0.048$), in patients with both ergoreflex assessment and LGE at CMR.

Ergoreflex activation, exercise limitation, and autonomic imbalance

Ergoreflex sensitivity also displayed strong inverse correlations with peak workload, peak VO_2/kg, and peak O_2 pulse, as well as a direct correlation with the VE/VCO_2 slope (*Figure 4*). In addition, ergoreflex sensitivity was inversely correlated with SD, SDANN, and CRI, and directly with plasma NE (*Figure 5*).

Sympathetic overactivity and myocardial fibrosis

In addition to its correlation with ergoreflex sensitivity, NE was associated with the presence of LGE at CMR: 860.5 ng/L (536.0–968.8) vs. 312.5 ng/L (182.0–445.5) ($P = 0.034$). Among several biomarkers relating to myocardial fibrosis (which, as stated above, were plasma renin activity, aldosterone, Gal-3, sST2, hs-TnI, hs-TnT, NE, and NT-proBNP), NE was found to be the only predictor of LGE at univariate analysis (expB 5.739, $P < 0.05$). NE showed a good discriminative power in the prediction of LGE, as confirmed by the area under the curve (AUC) at receiver operating characteristic (ROC) analysis (0.800, best cut-off 692 ng/L, sensitivity 90%, and specificity 75%).

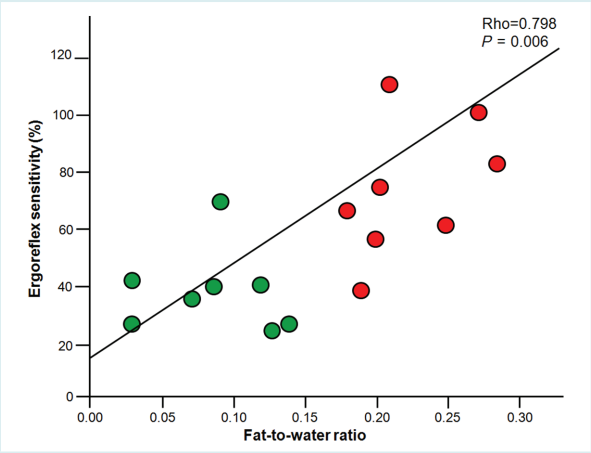

Figure 3 Muscle damage and ergoreflex sensitivity. The fat to water ratio at proton muscle spectroscopy correlated with ergoreflex sensitivity. Note the strength of the correlation despite the limited number of controls ($n = 8$, green dots) and patients ($n = 8$, red dots).

Discussion

No study so far has explored the clinical value of ergoreflex in MD patients. Herein we report for the first time that ergoreflex sensitivity is markedly enhanced in MD patients with skeletal myopathy and without history of overt heart involvement. Increased ergoreflex correlates with subclinical cardiac involvement, as well with indicators of muscle damage, exercise intolerance, and autonomic imbalance with sympathetic predominance.

In our population, skeletal myopathy had been demonstrated by thorough neurological assessment in all MD patients. In a subgroup of patients, skeletal myopathy was confirmed and quantified by muscle magnetic resonance techniques, indicating an increase in muscle ECV and fat to water ratio, two indices that to our knowledge have never been measured in patients with MD.

Ergoreflex sensitivity was two-fold higher in patients than in controls, with 89% of MD patients showing an ergoreflex sensitivity above the chosen cut-off point (95% of the control group). Ergoreflex sensitivity correlated with two indicators of muscle damage at magnetic resonance evaluation, thus establishing a link between skeletal myopathy and ergoreflex activation.

The prevalence of subclinical cardiac damage was much higher (80%) than previously reported (30%)[4] when both gadolinium-enhanced CMR and a second-level ultrasound examination technique (strain measurement) were added to ECG and TTE. These findings underscore the additional value of CMR, which has been considered as an optional exam, beyond ECG and TTE, in the screening algorithm proposed by Bates *et al.*[2] A role for strain evaluation, never proposed before, can also be taken into consideration. In contrast, biomarkers of cardiac damage, which were not included in the Bates algorithm,[2] did not increase the sensitivity of our screening protocol. Indeed, NT-proBNP was almost constantly negative and no patient showed increased cardiac-specific hs-TnI levels, while hs-TnT was often positive, probably reflecting foetal gene re-expression of troponin T in damaged muscles, as proposed in other forms of myopathy.[22,23]

Ergoreflex sensitivity was significantly higher in patients with subclinical cardiac involvement. This result outlines a potential novel pathogenetic factor that, activated at the level of diseased skeletal muscle, may promote sympathetic activation, possibly contributing to cardiac damage.

Chronotropic incompetence, depressed heart rate variability, and increased basal NE levels indicated a sympatho-vagal imbalance with sympathetic predominance, adding to previous observations in small populations of MD patients.[9,24] Ergoreflex sensitivity correlated with plasma NE concentration (Rho = 0.336, $P = 0.006$), several indices of decreased heart rate variability, and impaired heart rate response to exercise (*Figure 5*). Interestingly, NE emerged as the only predictor of LGE among several biomarkers potentially associated with myocardial fibrosis. An extensive body of research on CHF pathophysiology has outlined a cardiotoxic effect of chronic and unopposed sympathetic stimulation.[25] Therefore, it can be postulated that increased ergoreflex sensitivity may predispose to adrenergic overactivation, contributing to cardiac disease in MD patients. Of note, sympatho-vagal imbalance with adrenergic predominance is an established marker of clinical severity and poor outcome, not only in CHF,[26] but also in neuromuscular disorders.[27,28]

When correlating ergoreflex sensitivity to the performance at CPET, the results suggested that increased ergoreflex sensitivity could limit the tolerance to exercise, by causing an early onset of dyspnoea, and possibly a greater sensitivity to muscle fatigue. Of note, all these hypotheses had been previously demonstrated in CHF.[6]

In the 'muscle hypothesis' of CHF, an initial reduction in LV function activates catabolic and reduces anabolic factors that cause skeletal myopathy. This, in turn, sensitizes muscle ergoreceptors, which leads to exercise intolerance and sympathetic activation. The combined effects of a persistent catabolic state and inactivity further worsen skeletal muscle structure and function, and may eventually lead to a progressive effect on remodelling of the left ventricle. In patients with MD and skeletal myopathy, the muscle is significantly and primarily damaged.[6] We interpret our findings by postulating that a primary muscle disorder increases ergoreflex sensitivity, causing a chronic sympatho-vagal imbalance that can have deleterious consequences on the heart. The degree of myocardial damage in our cohort is too small to sustain the ergoreflex activation, as in heart failure, but this mechanism could play a role in patients with overt heart failure.

Ergoreflex assessment can be easily performed in all co-operative patients (80% in our population). It could implement the diagnostic algorithm of MD, since ergoreflex sensitivity is markedly enhanced in MD patients compared with controls. Ergoreflex evaluation might even aid in risk stratification, since ergoreflex sensitivity correlates with several parameters of exercise performance and autonomic function.

Our results also support a therapeutic strategy with exercise training. Indeed, exercise training is emerging as an important therapeutic option for CHF patients, targeting ergoreflex overactivity;[29]

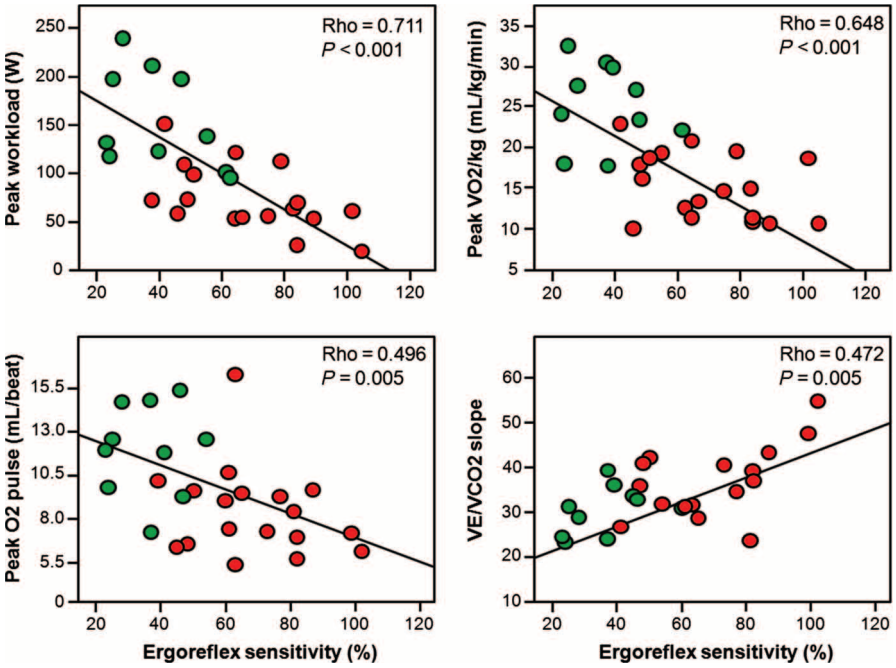

Figure 4 Ergoreflex sensitivity and performance at cardiopulmonary exercise testing. When considering both controls (green dots) and patients (red dots), ergoreflex sensitivity displayed positive correlations with peak workload, peak oxygen consumption/kg (VO_2/kg), and peak oxygen (O_2) pulse, and an inverse correlation with the minute ventilation/carbon dioxide output (VE/VCO_2) slope.

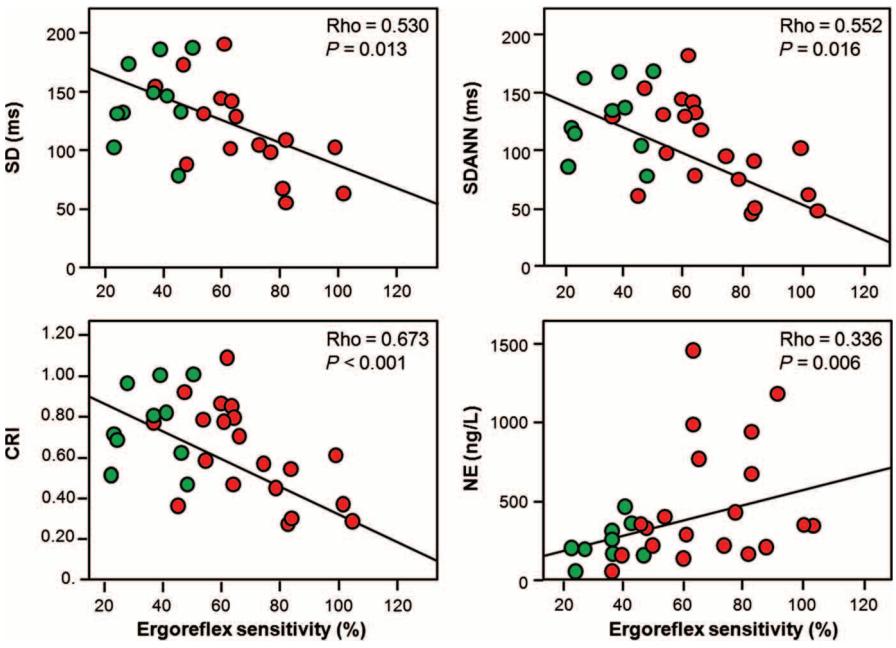

Figure 5 Ergoreflex sensitivity and autonomic function. In the whole study population (namely, controls, green; and patients, red), ergoreflex sensitivity was positively correlated with norepinephrine (NE) levels, and inversely correlated with the standard deviation of all RR intervals (SD) and standard deviation of 5-min mean values of RR intervals (SDANN) at Holter monitoring. An inverse correlation was found between ergoreflex sensitivity and chronotropic response index (CRI) at cardiopulmonary exercise testing; 2/10 controls (20%) and 17/20 patients (85%) displayed chronotropic incompetence, as defined by a CRI <0.80.

it has also been evaluated in the setting of MD, with some promising results in terms of quality of life and exercise tolerance.[9,30] A therapeutic paradigm including either exercise training or beta-blockade deserves some consideration in MD patients, especially in those presenting with ergoreflex and/or sympathetic overactivity, without significant limitation of motion or contraindication to beta-blockers. Finally, a possible treatment might be electrical muscle stimulation, recently proposed in the setting of CHF.[31] This may be of particular value in MD patients unable to follow a physical training programme.

Finally, the role of biomarkers deserves some consideration. So far, biomarkers have received little attention in the setting of MD, although they play a role in: (i) the diagnosis of MD (namely lactic acid trend during forearm ischaemic test); (ii) the evaluation of baseline status (haematocrit, and renal, liver, and thyroid function); (iii) the assessment of neurohormonal status; and (iv) the search for cardiac damage. In the present study, we focused on the last two points, reporting that biomarkers have a limited role in the early detection of cardiac disease (see above), hs-TnT levels seem to mirror skeletal muscle more than cardiac damage, resting plasma NE increases in the context of chronic sympathetic activation, and NE is a predictor of LGE at CMR, suggesting a cause–effect relationship between sustained adrenergic activation and subclinical, ongoing cardiac damage. The utility of hs-TnT as a biomarker of muscle damage, the need to measure hs-TnI to assess cardiac necrosis, and the link between NE and myocardial fibrosis should all be assessed in future studies.

With regard to study limitations, the small number of patients did not allow subgroup analyses considering the type of mutation, clinical or instrumental indicators of muscle disease, or other potentially relevant characteristics. Nevertheless, our population was larger than in many other studies on this rare disorder. Moreover, the possible parallelism between MD- and CHF-related myopathy should be confirmed and also analysed with regard to abnormal breathing pattern,[32,33] role of iron deficiency/supplementation,[34] and effects of feedback modulation.[35]

In conclusion, skeletal myopathy in MD is characterized by enhanced ergoreflex sensitivity, that is associated with a higher incidence of subclinical cardiac involvement, exercise intolerance, and sympathetic activation. Our results provide a conceptual framework for future studies considering the diagnostic and prognostic role of ergoreflex sensitivity, and its role as a therapeutic target in patients with MD.

Supplementary Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Patterns of cardiac involvement in mitochondrial disease.

Table S1. Main findings at electrocardiogram (ECG), transthoracic echocardiogram (TTE), and cardiac magnetic resonance (CMR).

Table S2. Biohumoral evaluation.

Table S3. Cardiopulmonary exercise testing and evaluation of the respiratory function.

Table S4. Chemoreflex and baroreflex assessment and 24-h ECG recording.

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