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ABSTRACT

Purpose: We determined the effects of an innovative 8-week exercise intervention (aerobic, resistance and inspiratory muscle training) for patients with mitochondrial disease (MD).

Methods: Several endpoints were assessed in 12 patients (19–59 years, 4 female) at pre-training, post-training and after 4-week detraining: aerobic power, muscle strength/power and maximal inspiratory pressure (main endpoints), ability to perform activities of daily living (ADL), body composition, quality of life and blood myokines (secondary endpoints).

Results: The program was safe with patients' adherence being 94±5%. A significant time-effect was found for virtually all main endpoints ($P \leq 0.004$), indicating a training improvement. Similar findings ($P \leq 0.003$) were found for ADL tests, total/trunk/leg lean mass, total fat mass, femoral fracture risk and general health perception. No differences were found for blood myokines, except for an acute exertional increase in interleukin-8 at post-training/detraining ($P=0.002$) and in fatty acid binding protein 3 at detraining ($P=0.002$).

Conclusion: An intervention including novel exercises for MD patients (*e.g.*, inspiratory muscle training) produced benefits in numerous indicators of physical capacity, and induced a previously unreported shift towards a healthier body composition phenotype.

Key Words: rare diseases; mitochondrial diseases; OXPHOS; VO_{2peak}; resistance training; inspiratory muscle training.

INTRODUCTION

Mitochondrial disorders are the most prevalent types of neuromuscular disease for which no therapy is available. Despite considerable heterogeneity in disease presentation, mitochondrial disorders commonly involve skeletal muscle tissue leading to mitochondrial myopathy and poor exercise capacity (1). The latter is reflected by low levels of peak oxygen uptake (VO_{2peak}) or early onset of anaerobic metabolism (*i.e.*, the so-called 'anaerobic' or 'ventilatory threshold' (VT) (2) or by poor muscle oxygen extraction (as assessed with near-infrared spectroscopy) during graded cycle-ergometer/treadmill testing (3,4).

Several studies have demonstrated the benefits of physical training in this patient group, usually in the form of endurance (or 'aerobic') exercise performed for a relatively long period (~2–12 months), on important outcomes, mainly VO_{2peak} (5-12). This type of exercise intervention improves muscle mitochondrial biogenesis and oxidative capacity, as reflected by an increase in the activities of citrate synthase (CS) (6,7,10) and respiratory chain complexes (6,10). Considerably less work has addressed the effects of a second type of exercise that should also be an integral component of any training programme, resistance ('strength') exercise (13, 14).

Muscle-derived molecules, collectively known as 'myokines' might account for some of the multi-systemic benefits of exercise, especially anti-inflammatory and healthy metabolic adaptations such as decreased insulin resistance (15). This is an important consideration in view of the high prevalence of physical inactivity coupled to obesity in this patient group (16). No study has evaluated exercise effects on the myokine profile of these patients. Moreover, controversy exists regarding the effects of exercise interventions on quality of life (QoL), usually assessed with the Short Form-36 (SF-36) questionnaire (5,8,9,10-14,16), and scarce data are available on how exercise training impacts patients' ability to perform activities of daily living (ADL) (5,13). Myopathy can also affect ventilatory muscles in these patients, which can further aggravate their exercise intolerance (18), but no previous study has implemented specific

inspiratory muscle training (using a threshold-loading device, which can be easily utilized at home with little time investment). The effects of exercise interventions on other phenotypes related to mitochondrial disorders, muscle and bone mass, remain to be determined.

We determined the effects of a 'complete/combined' exercise intervention consisting of innovative aerobic, resistance and inspiratory muscle exercises, performed by patients with mitochondrial disease on outcomes indicative of: aerobic power, muscle strength/power and maximal inspiratory pressure (PI_{max}) (main endpoints); and ability to perform ADL, body composition, QoL and blood myokines (both under resting conditions and in response to acute exercise) (secondary endpoints). We hypothesised that our exercise intervention would lead to significant improvements, especially in main endpoints.

MATERIALS AND METHODS

Experimental design and ethics

This study was approved by the local ethics committee of the *Hospital 12 de Octubre* (Madrid, Spain; approval number: 14/359) and was performed in accordance with the Declaration of Helsinki during April 2015–January 2017. All participants provided written informed consent. Inclusion criteria were as follows: outpatient (male/female) aged 18–60 years; diagnosed with a mitochondrial disorder according to clinical, histomorphologic, respiratory chain and/or genetic evidence (19); living in the Madrid area; and having no comorbidity or acute condition contraindicating exercise. Study endpoints were assessed at three time-points: baseline ('pre-training'), after an exercise intervention ('post-training'), and after a subsequent 4-week training cessation period ('detraining'). Participants were told not to deviate from their habitual dietary habits during the study period. The researchers responsible for assessing participants were blinded to time of evaluation. Assessment/training sessions were performed in the rehabilitation

department (for aerobic exercises) or gymnasium (for strength exercises). The patient study design is summarised in **Fig. 1**.

Fourteen patients originally agreed to participate but two of them withdrew during the first 3 weeks (one due to urine infection and the other one due to personal reasons). The main clinical/diagnostic characteristics of the patients who completed the study (n=12) are shown in **Table 1**; 46% were overweight [Body Mass Index (BMI)=25–29.9 $kg \cdot m^{-2}$], 8% obese (BMI >30 $kg \cdot m^{-2}$), and 8% underweight (BMI <18.5 $kg \cdot m^{-2}$).

Exercise training

The intervention lasted 8 weeks and included three weekly sessions (Monday, Wednesday and Friday; total number of planned sessions, 24; session duration, 60–90 min). Each session was supervised by experienced fitness instructors (one per participant) and included three main components: aerobic, muscle resistance ('strength') and specific inspiratory muscle training. Make-up sessions (on a Tuesday/Thursday) were allowed if a planned session was missed. To determine the adherence to training, we considered a session completed when ≥90% of the prescribed exercises were successfully performed (20).

Aerobic training. Each session started with aerobic training on a cycle-ergometer (Ergoselect 100P, Ergoline, Bitz, Germany). Aerobic exercise was preceded by an initial 10 min warm-up and a final 5 min cool-down period; the central component lasted only 20 min and was performed following a low-volume 'high intensity interval training' (HIT) protocol (21), where 1 min bouts at a target load were interspersed with recovery periods of the same duration. Although one possibility is to use 30-second, 'all-out' (*i.e.*, Wingate) repetitions, this might not be safe, tolerable or appealing for some individuals, such as patients (21). Thus, a more practical model of low-volume HIT that is still time-efficient (as it can be performed over 20 min) while also having wider application to different populations including patients, consists of 10 × 1 min work bouts at a constant-load, very high intensity (~90% of maximal heart rate), interspersed

with 1 min of recovery (21). Owing to the poor baseline physical condition of our participants and to the fact that HIT has not been previously applied to this patient population, we did not target high intensities until the second half of the program. Thus, the target power output (watts) was gradually and individually increased during the program as follows: weeks 1–2, 65% of peak power output (PPO) reached during the baseline cycle-ergometer test until exhaustion (see below); weeks 3–4, 75% of PPO; weeks 5–6, 85% of PPO; weeks 7–8, 90–100% of PPO. The load for recovery periods was kept below 65% of PPO.

Resistance training. This followed the aerobic training and included a three-time circuit of exercises involving large muscle groups and performed with specific weight training equipment (Gervasport, Pleven, Bulgaria) in the following order: leg and bench press, leg extension, lateral pulldown and abdominal crunches. Initial loads for each exercise were individually determined, using values of 6–7 in the Borg 1–10 scale of perceived exertion. The rate of load increases was also set individually as follows: when the patient reported a score <6 for a given exercise in two consecutive sessions, the corresponding load was increased (with the premise that the score for the new load=6–7). The number of repetitions for each exercise was decreased with corresponding increases in load (kg) as follows: 15 (weeks 1–4) and 7–8 repetitions (weeks 5–8).

Inspiratory muscle training. This included two daily sessions (morning and evening, all at the patient's home except for Monday-Wednesday-Friday where the evening session was performed inhospital after the aforementioned resistance training), 6 days/week (Monday–Saturday). Each of the two daily sessions consisted of 30 inspirations through a specific pressure-load device (Powerbreathe® Classic Medium Resistance, Powerbreathe International Ltd., Southam, UK) against 40% of PI_{max} (session duration ~5 min) (20). Paticipants' PI_{max} was reassessed by us at the beginning of each week to adjust the weekly load accordingly.

Endpoint assessment

All assessments were consistently performed over a 1-week period in the following days and order at each of pre-, post- and detraining, respectively, after familiarisation with the equipment and tests (Thursday and Friday): first assessment day (Monday of the next week), venous blood drawing, cycle-ergometer test followed by post-exercise blood drawing, and distribution of QoL questionnaires; second day (Wednesday), strength tests followed by functional tests of ADL, and PI_{max} test; and third day (Thursday or Friday), body composition analysis. In order to rule out a potential effect of changes in patients' levels of habitual physical activity (PA) on study outcomes, during the week prior to the aforementioned assessments at pre-, post- and detraining, we determined PA objectively, using a triaxial accelerometer (GT3X monitor device, Actigraph; Pensacola, FL) (22). For each study participant, a minimum of 5 days' monitoring (including 2 weekend days), and a minimum of 10 hours/day of complete accelerometry were considered necessary. Data were analyzed using ActiLife5 LITE software (Actigraph, Pensacola, FL). We determined average intensity (counts/min) by dividing the sum of the total counts per pre-defined epoch (15 seconds) for a valid day by the number of minutes of wear time in that day across all valid days. Thereafter counts were converted to average daily time engaged in moderate-vigorous PA (22).

Main endpoints

Aerobic power. Aerobic exercise testing was performed on a cycle-ergometer, following a ramp-like protocol (workload increases of 1 watt every 6 s (averaging 10 watts/min) starting from an initial load of 0 watts, with a pedal cadence of 60–70 rpm throughout the test). Gas-exchange variables were collected breath-by-breath with an automated metabolic cart (Quark CPET, COSMED, Rome, Italy). The VO_{2peak} and the peak pulmonary ventilation rate (VE_{peak}) were computed as the highest value of these two variables obtained for any 20-s period during the

tests. Two important physiological indicators of submaximal aerobic power, VT and respiratory compensation point (RCP), were detected using previously defined criteria (23).

Muscle strength/power. We assessed upper/lower-body muscle power with bench/leg press tests using the same equipment as for training, connected to a linear encoder (Power Encoder, Smartcoach Europe, Stockholm, Sweden). This methodology has proven valid to determine muscle force (N) and power (W) (24) and has been recently applied by us for assessing patients with different types of myopathy (20,25). Patients performed one set of three repetitions for each exercise at the maximum possible speed; each set was followed by a recovery period of 60–90 s. The load (or 'resistance') was increased by 5 or 10 kg (leg press) and by 2 kg (bench press) in each successive set. Because power is the product of force and velocity, it initially increases with resistance and then decreases when the resistance causes a substantial decrease in velocity. Thus, the test is stopped when the decrease in velocity is so pronounced that it also causes a decrease in average muscle concentric power. An advantage of this test is that it ends with a submaximal load, thereby avoiding major/haemodynamic problems. We recorded the highest value of average power (watts) and load (kg) in the concentric-propulsive phase, which typically coincides with the start of a decline in this variable together with the occurrence of the highest value of average force (newtons) (20, 25).

Maximal inspiratory capacity. We measured participants' PI_{max} at the residual volume using a mouth pressure metre (Micro Medical Inc., Chatham, Kent, UK) (20).

Secondary endpoints

Functional capacity during ADL. Walking capacity was evaluated with the six-minute walking distance test (6MWD) test in accordance with established standards (20). Mobility was assessed with the timed up and go test (TUG) and 15-step stair tests (26).

Body composition. Total and regional body composition was assessed by dual energy x-ray absorptiometry (DXA) (Hologic Serie Discovery QDR, Physician's Viewer, APEX System Software Version 3.1.2. Bedford, MA, USA) as described (27). An additional examination was conducted to estimate bone mass and femoral T-score (number of standard deviations above or below the young adult mean bone mineral density) at the proximal region of the femur. Femoral T-scores were calculated using the manufacturer's reference values based on the revised National Health and Nutrition Examination Survey III reference data (27). Patients were also compared with a sex and age-matched healthy control group (21 men and 12 women, age 45.7±2.0 years) previously assessed by us with the same equipment.

QoL. We used the Spanish version of the SF-36 questionnaire (version 2) to assess patients' health-related QoL (28).

Blood variables. Twenty myokines/cytokines were assessed in plasma samples before ('basal') and after aerobic power tests using the MILLIPLEX® MAP Human High Sensitivity T Cell Magnetic Bead Panel and the MILLIPLEX® MAP Human Myokine Magnetic Bead Panel with Luminex® Technology on a MAGPIXTM instrument (EMD Millipore Corporation, Billerica, USA). Basal plasma levels of creatine kinase (CK) [laboratory reference values <190 U/L (men) and <170 U/L (women)], creatinine [reference <1.2 mg/dL (men) and <0.9 mg/dL (women)] and lactate (reference 0.5–2.2 mmol/L) were also measured using a Cobas C spectrophotometer (Cobas® 8000 modular analyser series, Roche Diagnostics, S.L., Spain).

Statistical approach

We compared endpoint values over time with the non-parametric Friedman test. We also compared acute changes (pre- *vs.* post-exertion) in myokines at each of the three study time-points with the Wilcoxon test. Finally, DXA variables were compared between patients and controls with Mann–Whitney's *U* test. To minimise the risk of statistical error type I (given the large number of endpoints), analyses were corrected for multiple comparisons with the stringent

Bonferroni method in which the threshold *P*-value is obtained by dividing 0.05 by the number of comparisons; further, *post hoc* paired comparisons were performed only for those endpoints showing a significant time effect.

RESULTS

Valid accelerometry data in each of the three time points were obtained from 7 patients. Mean(±SEM) levels of MVPA did not differ (P=0.8426) between pre- (56±20 min/week), post- (56±21 min/week) or detraining (45±29 min/week). Vigorous PA was virtually absent in all individuals in the three points of assessment.

Adherence and safety

Mean adherence to the programme was 94±5% (range: 83–100). Main reasons for missing 1+ sessions were family (n=5 sessions) or work (n=4) obligations, medical visits (n=1) or minor health issues temporarily disabling exercise (n=7, *e.g.*, upper respiratory tract infection, migraine). No health issues other than the expected muscle discomfort were noted. In two subjects, the 15 repetitions that were originally planned for strength exercises during weeks 1–4 were divided in two sets of 7–8 repetitions to attenuate muscle pain.

Main endpoints

Peak values of heart rate (161±6, 164±6 and 161±7 beats/min at pre-, post- and detraining, respectively, P=0.242 for time effect) and respiratory exchange ratio (1.18±0.04, 1.18±0.02, and 1.23±0.03, P=0.368) reached by the participants in the aerobic power tests did not differ over time which, besides indicating a comparable level of effort, suggests that patients performed a maximal effort in the three assessments. A significant time effect was found for all endpoints indicative of aerobic power, muscle strengh and inspiratory muscle power (all $P \leq 0.004$) except for VO_2 at the RCP relative to muscle mass (P=0.006), indicating a consistent training improvement (**Table 2**). Statistical significance was also reached for numerous endpoints in

paired *post hoc* comparisons of pre- *vs*. post-training values. Importantly, some training improvements were not completely lost after detraining, with several values at detraining remaining significantly higher than at pre-training ($P \leq 0.004$). All main endpoints showed the same individual response (*i.e.*, training-induced improvement) for virtually all patients (see Figure, Supplemental Digital Content 1, Individual data for patients' main endpoints, http://links.lww.com/MSS/B189).

Secondary endpoints

A significant time effect ($P \leq 0.003$) was found for all outcomes indicative of functional capacity in ADL, total/trunk and leg muscles' muscle mass, total fat mass, femoral fracture risk (T-score) and general health perception in the SF-36 questionnaire. Similar to the main outcomes, this indicated a training-induced improvement (which for fat mass and T-score was reflected in a decrease in mean values) followed by a subsequent loss, at least partially, of such improvements after detraining (**Table 3**). In *post hoc* analyses, we found a significant improvement at post- compared with pre-training for all the functional tests as well as for total lean and leg muscle mass (all $P \leq 0.003$). Further, detraining values of 6MWD and 15-step stair tests, and total lean mass remained significantly higher than pretraining values ($P \leq 0.003$). For ADL tests and DXA variables, virtually all patients showed the same individual response *(i.e.,* training-induced improvement; see Figure, Supplemental Digital Content 2, Individual data for patients' secondary endpoints, http://links.lww.com/MSS/B190). Furthermore, lean mass was significantly lower in patients than in controls (see Table, Supplemental Digital Content 3, Results of dual energy x-ray absorptiometry in mitochondrial disease patients and age and gender-matched healthy controls, http://links.lww.com/MSS/B191).

No significant time effect was found for the basal levels of blood variables (all $P>0.002$) (**Table 4**). A significant acute exertional increase was found only for interleukin-8 (IL8) at post- and detraining (both $P=0.002$) and for fatty acid binding protein 3 (FABP3) at detraining ($P=0.002$) (**Table 4**).

DISCUSSION

The main finding of our study was that, despite its relatively short duration (8 weeks), a 'complete' and innovative exercise intervention including aerobic, resistance and specific inspiratory muscle exercises performed by patients with mitochondrial disorders produced significant benefits in numerous indicators of their physical capacity, including aerobic power, muscle strength and inspiratory muscle power. It also improved patients' ability to manage ADL and their general health perception. The training programme also induced a shift towards a healthier body composition phenotype, with increased and decreased muscle and fat mass, respectively. Importantly, despite the heterogeneity in clinical profiles, these improvements were corroborated in virtually all patients individually. Further, many of the training improvements were partly retained after detraining. Our intervention was also safe and well tolerated by the patients: no major health issues, no significant increases in muscle damage (CK levels) and no alterations in renal function (creatinine) were found. To the best of our knowledge, this is the first study to use such an 'integrative' training approach and to show benefits in essentially all of the functional endpoints we tested, including also improvements in important novel outcomes, muscle mass and bone fracture risk.

Peak aerobic power levels are usually poor in mitochondrial disorders (1,4-6). The VO_{2peak} levels varied widely among our patient cohort, which is likely attributable to the variability in the magnitude of OXPHOS impairment in this patient group (1). Nevertheless, before starting the training programme, the majority (75%) of patients had a VO_{2peak} level <25

$mL \cdot kg^{-1} \cdot min^{-1}$ (or 7 metabolic equivalents or MET, where 1 MET reflects resting metabolic rate or 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$ for most humans). The finding that mean VO_{2peak} approached 8 MET is clinically relevant because this is the minimum threshold for optimal health, above which the risk for all-cause mortality is significantly reduced (29).

Importantly, VO_{2peak} also increased significantly after training when normalised to leg muscle mass, which was most involved during the exercise mode of leg pedaling used here both for aerobic power assessment and aerobic training. This suggests an improved ability of the trained muscle tissue to extract and use O_2 during exercise, which is considered a major limiting factor of the VO_{2peak} that can be reached by patients (5,11,30). Our hypothesis of increased patient muscle aerobic power after training is consistent with previous studies reporting improved endothelial function, mitochondrial biogenesis and oxidative capacity (6–11,17), as well as increased peak oxygen extraction (as assessed by near-infrared spectroscopy) in the muscle tissue of trained patients (12). Unfortunately, we were not authorised by our ethics committee to collect muscle biopsies from the patients. Our observations of significant improvements in VO_{2peak} through training is in agreement with previous intervention studies of similar or longer duration that have focused on aerobic exercise (5–12). The success of our training programme despite its relatively short duration might be attributable to the low-volume HIT protocol we used, at least for the second half of the intervention, where participants performed ~10 repetitions of 1-minute intervals per session, at ~80% (weeks 5–6) and 90–100% of peak watts achieved during the baseline cycle-ergometer test (weeks 7–8). Indeed, growing evidence suggests this type of training stimulates physiological adapations (*e.g.*, VO_{2peak} increases) comparable with moderate-intensity continuous training despite a lower time commitment and exercise volume (21,31). Our findings are also important in practical terms given that 'lack of time' remains the most commonly cited barrier to regular exercise participation among westerners (21). By contrast, previous studies have used interventions of

continuous exercise during longer bouts but at lower intensities, such as 30 min at 70% of peak watts (13), 30–45 min at 65–80% of maximal heart rate (10-12), or 30 min at 70–80% of VO_{2peak} (5–7). Of note, here we chose to use the term 'VO_{2peak}' instead of *maximal* oxygen uptake ('VO_{2max}'). A second, constant work rate test performed at ~110% of the work rate achieved on the initial ramp test would have served as a validation tool to assess actual VO_{2max} levels (32). This validation method might be incorporated for the assessment of mitochondrial disease patients in future training studies.

Patients with mitochondrial disorders often show a deteriorated maximal inspiratory capacity, as manifested by a low PI_{max} (18). In this regard, the mean baseline values of PI_{max} in our patients (79±11 cmH_2O) were only slightly (+3.8%) higher than those we recently reported using the same method in patients of a very similar mean age (~46, *vs.* 47 years here) suffering a devastating disease, pulmonary arterial hypertension, which typically provokes marked exercise intolerance together with frequent dyspnea and also myopathy affecting ventilatory muscles (20). Yet, not only PImax (+29%, $P<0.001$), but also another indicator of muscle ventilatory capacity, VE_{peak}, showed remarkable increases with training (+25%, $P<0.004$). Two patients (#2 and #12, **Table 1**) required non-invasive nocturnal ventilatory support (with Bilevel Positive Airway Pressure, biPAP). Although after the training program they both reported feeling less ventilation-limited during daily living, the biPAP was still kept at night for safety purposes. Further, any improvement in respiratory muscle capacity as the one shown here is of potential medical relevance because in the long-term, respiratory failure is a main cause of death among adults with mitochondrial disorders (33).

Patients with mitochondrial disorders have high blood lactate levels at rest and especially during exercise, even of submaximal intensity, as a consequence of OXPHOS dysfunction (1). In this regard, we also found a significant training-induced improvement in two important indicators of submaximal exercise capacity, the VT (also referred to as the 'anaerobic threshold')

and the RCP (also known as 'second VT'). Previous studies have also found an increase in the VT with training in this patient group (5,13), but ours is the first study to assess training effects in the RCP. While the VT represents the first increase in pulmonary ventilation that is proportional to the increase in CO_2 output generated by the HCO_3^- buffering of lactic acidosis, the RCP represents a higher work intensity at which blood lactate accumulation increases considerably (*i.e.*, production exceeds clearance) and is accompanied by an additional hyperventilation in an attempt to buffer increasing blood acidosis (23). Therefore, the increments in VT and RCP provoked here were likely mediated by: (i) a higher ability to buffer lactic acidosis, and (ii) a higher ability of working muscles to rely on aerobic metabolism, as reflected by the finding that VO_{2peak} normalised to leg muscle mass increased with training.

We used a novel, practical approach to assess the effects of strength training on the ability of muscles to produce power by studying the force-velocity curve, allowing the patients to avoid performing maximal efforts and the Valsalva maneuver, and therefore limiting the risk of adverse events (as opposed to 'classical' 'one-repetition maximum' weight lifting exercises). Moreover, compared with conventional one-repetition maximum tests, muscle power improvements have more direct transference into patients' ability to perform ADL (*e.g.*, standing from a chair, stair climbing) (20). This was indeed reflected in the training-induced improvement we found for all outcomes indicative of functional capacity during ADL (6MWD, TUG and 15-step stair tests) in our patients, demonstrating that ADL was better tolerated after training.

The increments in strength/muscle power levels were accompanied by improvements in muscle mass measured by DXA. No study has assessed exercise training effects on the muscle mass in this patient group; yet, it is a potentially important issue as our patients showed significantly lower total lean mass (~18%) than their healthy peers. Thus, the training improvement in total lean mass is of special relevance, particularly the higher values at the lower extremities. This training effect remained after the detraining period. The phenotype of patients´

muscle tissue was likely changed with resistance training towards hypertrophy A previous study has reported that strength training increases the number of satellite cells (as a result of increased muscle damage) and of intermediate muscle fibres, while decreasing the number of cytochrome c oxidase-deficient fibres (14). Although the mechanisms involved in this process remain to be clearly elucidated, Murphy et al. proposed that the activation of quiescent mitotic cells by strength training results in the shifting of normal mitochondrial templates to mature muscle and restoration of a normal mitochondrial genotype and phenotype (14). However, a similar proportion of mitochondrial DNA (mtDNA) mutations (single large-scale deletions) has been reported in satellite cells and mature muscle of patients with mitochondrial disorders (34). Unfortunately, the fact that we did not perform muscle biopsies precluded assessing a potential training effect on mtDNA mutational load at the muscle tissue level.

Training-induced gains in muscle mass were also accompanied by a reduction in fat mass, and consequently a healthier body composition phenotype. While keeping in mind the limitation that we did not assess potential dietary effects on the body composition changes observed during the study, this is also an important finding given that many of these patients are at high risk of metabolic conditions such as obesity and type 2 diabetes (16,35–37), or impaired glucose tolerance and diminished insulin-stimulated glucose influx to skeletal muscle and adipose tissue (36). An additional novelty of our study was the finding that exercise training decreased the risk for femoral fractures (T-score), which is also clinically relevant in light of the fact that mitochondrial disorders are often associated with endocrine conditions that can adversely affect bone mass (diabetes mellitus, insulin resistance/glucose intolerance, hypoparathyroidism, renal tubular acidosis, chronic renal insufficiency and gastrointestinal disease) (38). Interestingly, one of our patients was reclassified from osteoporotic to osteopenic after the training period (T-score of -2.5 and -2.3, respectively). The finding that 7 patients were classified as having osteopenia or osteoporosis at the beginning of the study and the proposed link between mitochondrial

dysfunction and a bone phenotype (38) further support the need to implement exercise interventions such as those used here in this patient group.

Skeletal muscle can act as an endocrine organ, especially during contractions, by releasing molecules that are collectively known as 'myokines' (mainly proteins and peptides) into the bloodstream (15). Myokines account for some of the beneficial effects of exercise, such as anti-inflammatory effects, or healthy metabolic adaptations (*e.g.*, decreased insulin resistance). In this regard, patients with mitochondrial disorders can present high basal concentrations of several pro-inflammatory cytokines (such as TNFα) (36). Accordingly, we evaluated the effects of exercise training on basal myokine levels and found no statistically significant differences. By contrast, a significant acute increase in IL8 was found at post- and detraining, and FABP3 at detraining. Previous research on healthy adults has reported an increase in circulating levels of IL8 after an exercise bout (39). Similarly, IL8 receptor levels are elevated in the working skeletal muscles of healthy individuals (15) and in patients with mitochondrial disorders after an acute exercise bout (36), with IL8 receptor-mediated signaling potentially stimulating local angiogenesis (15). A stimulating effect of IL8 on angiogenesis after acute exercise might be mediated by CXC receptor 2 signalling, which in the longer-term may increase muscle capillarity (15). In a previous study, capillary density increased after 12-month aerobic training in four patients with different mtDNA mutations and high mtDNA mutational load (7), although the underlying mechanism remains unknown. However, the same authors found a training-induced decrease in capillary density after 12-week aerobic training (6). Clearly, more research is needed to examine the effects of exercise on muscle capillarisation in patients with mitochondrial disorders and to determine whether IL8-related pathways are involved. Our data on FABP3 are in agreement with a previous study in healthy individuals where a single bout of endurance cycle-ergometer exercise provoked an increase in FABP3 expression in *vastus lateralis* muscle (40). FABP3 facilitates the cytoplasmic transport of fatty acids through

intracellular membranes and provides the primary means for energy production in working skeletal muscle together with carbohydrates (40). Future studies might determine if longer exercise training interventions are needed to elicit significant changes in the myokine blood profile.

Besides unavailability of patients' muscle biopsies due to ethical constraints, a main limitation of our study was that we did not perform a randomised controlled trial on patients. This is partly compensated by the fact that we assessed patients at three time points (including detraining), thereby preventing subject bias as the same subjects were used as their own controls. In addition, the exercise intervention did not have a beneficial impact on patients' MVPA levels after it had ended (*i.e.*, detraining period). Nevertheless, our study has several strengths. It is the first to use an 'integrative' training approach in this patient group. Other strengths were: the myriad benefits obtained, the novelty of several endpoints (such as bone and muscle mass) and of our training methods, including low-volume HIT, 'specific' resistance training and inspiratory muscle exercises. Concerning the latter method, this was easy to perform, little time-consuming, and it induced a significant improvement in inspiratory muscle capacity. Finally, we minimised type I statistical error by adjustment for multiple comparisons.

In conclusion, an 8-week 'complete' exercise intervention, produced benefits in numerous indicators of physical capacity in patients with mitochondrial disorders, as well as a shift towards a healthier body composition phenotype, with increased and decreased muscle and fat mass, respectively.

Acknowledgments and Conflicts of interest

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The authors report no conflict of interest and they affirm that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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FIGURE LEGENDS

Table 1. Main clinical and diagnostic characteristics of the patients.

Table 2. Results of patients' main endpoints by training status.

Table 3. Results of patients' secondary endpoints by training status.

Table 4. Results of blood variables by patients' training status.

Fig. 1. Representation of the study design.

Supplemental Digital Content 1. Figure. Individual data for patients' main endpoints.

Supplemental Digital Content 2. Figure. Individual data for patients' secondary endpoints.

Supplemental Digital Content 3. Table. Results of dual energy x-ray absorptiometry in mitochondrial disease patients and age and gender-matched healthy controls.

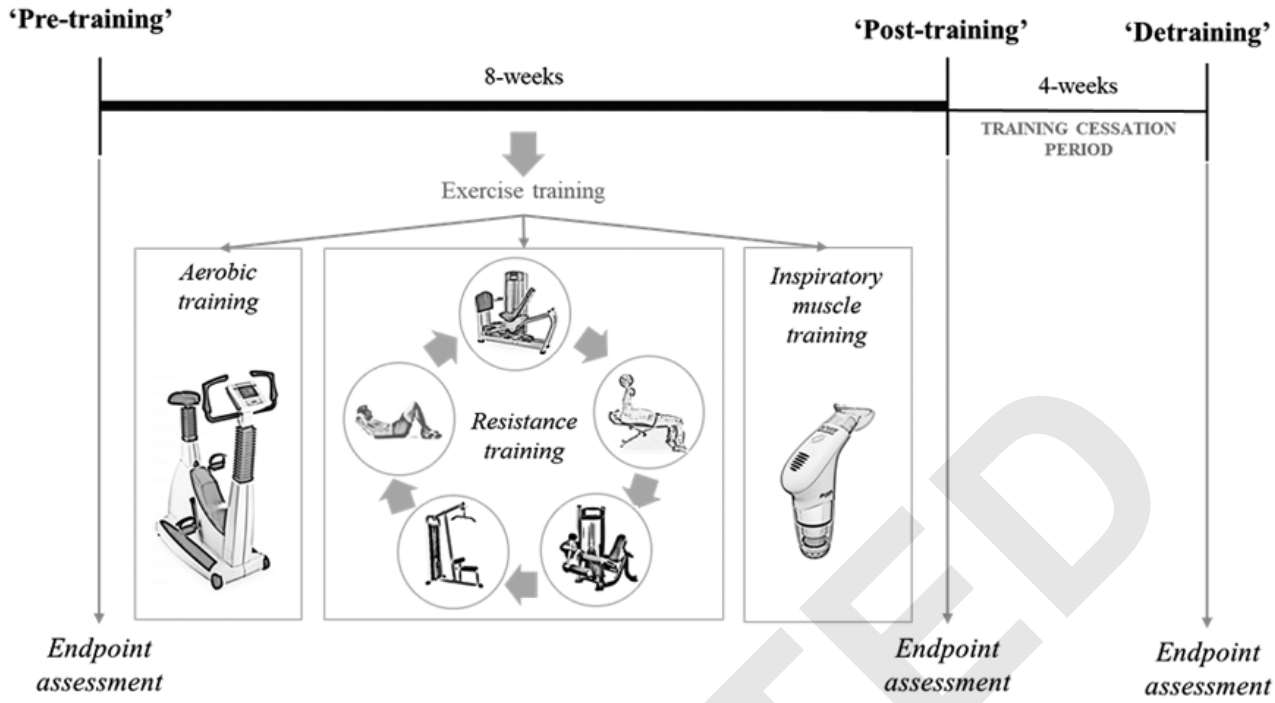


Figure 1

Table 1. Main clinical and diagnostic characteristics of the patients

ID	Sex	Age (years)	Main clinical symptoms	Serum Basal CK (U/L)	Muscle biopsy: Morphology	Muscle biopsy: Respiratory chain activity	Genetic alteration/s
1	M	59	CPEO, ptosis and bulbar weakness (dysphagia and dysphonia)	Normal (<200)	COX-, RRF	N/A	Multiple mtDNA deletions *TWNK*: c.1361T>G, p.Val454Gly, heterozygous*
2	F	53	Progressive myopathy with prominent ataxia, facial and respiratory muscle weakness*	400	COX-, RRF	Normal	Multiple mtDNA deletions
3	M	57	Fatigue and exercise intolerance	500–1 000	COX-, RRF	CI, CIII and CIV deficiency	Multiple mtDNA deletions, *POLG:* c.2573C>T, p.Thr858Ile*
4	F	40	MELAS, seizures, exercise intolerance, diabetes mellitus and hearing loss	300	COX-, RRF	N/A	*MTT1*: m.3243A>G, 89% heteroplasmy in skeletal muscle
5	M	41	Exercise intolerance	1 200	COX-, RRF	N/A	*MTTN*: m.5692T>C 73% heteroplasmy in skeletal muscle;
6	M	19	CPEO, exercise intolerance and migraines	400	COX-, RRF	Normal	Single large-scale mtDNA deletion Δ: 4.8 kb; 60% heteroplasmy in skeletal muscle;
7	M	50	CPEO and exercise intolerance	300	COX-, RRF	N/A	Single large-scale mtDNA deletion; Δ: 4.0 kb 78% heteroplasmy in skeletal muscle
8	M	52	Bilateral optic neuropathy, hearing loss, axonal sensory polyneuropathy and ataxia	Normal (<200)	Normal	CI deficiency	Multiple mtDNA deletions *OPA1:* c.1189G>A, p.Lys397Glu, heterozygous**
9	M	39	Retinitis pigmentosa and migraines	Normal (<200)	N/A	N/A	*MTATP6:* m.8993T>G, 81% heteroplasmy in blood
10	F	35	CPEO with asymmetrical ptosis and exercise intolerance	400	COX-	Normal	Single large-scale mtDNA deletion. Δ: 5.7 kb 34% heteroplasmy in skeletal muscle
11	F	57	CPEO and exercise intolerance	Normal (<200)	COX-, RRF	Normal. CS ↑	Single large-scale mtDNA deletion Δ: 4.8 Kb; 40% heteroplasmy in skeletal muscle
12	M	58	Progressive myopathy, bulbar weakness and respiratory failure*	400	COX-, RRF	Normal	Multiple mtDNA deletions *TK2*: c.604_606del, p.201-202del, homozygous

Abbreviations: CI, CIII, CIV, mitochondrial respiratory chain complex I, III and IV, respectively; CK, creatine kinase; COX-, fibres deficient in cytochrome c oxidase; CPEO, chronic progressive external ophthalmoplegia; CS, citrate synthase; F, female; ID, identification; M, male; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes syndrome; mtDNA, mitochondrial DNA; N/A, not available; *OPA1*a , optic atrophy 1 (autosomal dominant) gene (ref NM_130837); RRF, ragged-red muscle fibres; *TK2*, thymidine kinase 2 gene (ref NM_004614); *TWNK*, Twinkle helicase gene (ref NM 021830.4);.

Symbols: Δ: Estimated size of single mtDNA deletion on Southern blot;* required non-invasive nocturnal ventilatory support (with Bilevel Positive Airway Pressure, biPAP); **Novel unpublished mutation in a nuclear gene involved in maintenance of mtDNA defect: family segregation, population variant frequency in ExAC, EVS and 1000genomes databases, and pathogenicity scores of several predictors of missense variants (SIFT, Polyphen2, CADDPhred, M-CAP among others) suggested the variant was pathogenic.

Table 2. Results of patients' main endpoints by training status

Endpoint	Pre-training (t_0)	Post-training (t_1)	Detraining (t_2)	*P*-value for time effect	*P*-value for *post hoc* comparisons		
					t_0 *vs.* t_1	t_1 *vs.* t_2	t_0 *vs.* t_2
Aerobic power, peak values							
PPO (W)	98 ± 11	123 ± 13	112 ± 12	< **0.001**	**0.003**	**0.003**	**0.004**
PPO/legs muscle mass ($W\,kg^{-1}$)	15 ± 1	18 ± 1	17 ± 1	< **0.001**	0.005	0.008	0.007
VO_{2peak} [mL· kg (of whole body mass)$^{-1}$ min^{-1}]	22.1 ± 1.8	25.8 ± 1.8	24.2 ± 1.9	< **0.001**	**0.003**	0.008	**0.003**
VO_{2peak} [mL· kg (of legs' muscle mass)$^{-1}$ min^{-1}]	229 ± 13	259 ± 11	243 ± 15	**0.003**	0.005	0.028	0.037
VE_{peak} ($l\,min^{-1}$)	65 ± 7	87 ± 9	78 ± 9	**0.004**	**0.004**	0.037	0.016
Aerobic power, VT							
VO_2 [mL·kg (of whole body mass)$^{-1}$ min^{-1}]	14.0 ± 1.2	19.1 ± 1.5	16.9 ± 1.5	< **0.001**	**0.003**	**0.003**	**0.003**
VO_2 [mL· kg (of legs' muscle mass)$^{-1}$ min^{-1}]	152 ± 7	195 ± 9	170 ± 12	**0.002**	0.005	0.013	0.059
Aerobic power, RCP							
VO_2 [mL·kg (of whole body mass)$^{-1}$ min^{-1}]	17.8 ± 1.5	23.0 ± 2.2	21.1 ± 2.0	< **0.001**	**0.003**	0.033	**0.004**
VO_2 [mL· kg (of legs' muscle mass)$^{-1}$ min^{-1}]	186 ± 10	230 ± 16	215 ± 15	0.006	-	-	-
Strength tests, leg press							
Peak power (W)	281 ± 42	456 ± 72	473 ± 88	< **0.001**	**0.002**	0.155	**0.003**
Peak power [W·kg (of legs' muscle mass)$^{-1}$]	41 ± 5	65 ± 8	60 ± 8	< **0.001**	**0.003**	0.155	**0.003**
Strength tests, bench press							
Peak power (W)	127 ± 31	190 ± 41	150 ± 29	< **0.001**	**0.003**	0.021	0.182
Peak power [W·kg (of arms' muscle mass)$^{-1}$]	58 ± 12	79 ± 13	62 ± 10	**0.002**	0.005	0.028	0.386
Inspiratory muscle strength							
PI_{max} (cmH_2O)	79 ± 11	112 ± 15	107 ± 15	< **0.001**	**0.002**	0.084	**0.002**

Data are mean ± SEM. Significant *p*-values are in bold -threshold *p*-value was set at 0.004 (=0.05/14). Abbreviations: PI_{max}, maximal inspiratory pressure; PPO, peak power output; RCP; respiratory compensation threshold; VE_{peak}, peak pulmonary ventilation; VT, ventilatory threshold; VO_{2peak}, peak oxygen uptake.

Table 3. Results of patients' secondary endpoints by training status

Endpoint	Pre-training (t_0)	Post-training (t_1)	Detraining (t_2)	*P*-value for time effect	*P*-value for *post hoc* comparisons		
					t_0 *vs.* t_1	t_1 *vs.* t_2	t_0 *vs.* t_2
Functional tests							
6MWD (m)	464 ± 26	562 ± 28	530 ± 26	< **0.001**	**0.002**	0.010	**0.002**
TUG test (s)	5.7 ± 0.5	4.6 ± 0.4	5.1 ± 0.5	< **0.001**	**0.002**	0.013	0.006
15-step stair test (s)	38.7 ± 4.1	27.3 ± 2.8	29.7 ± 2.8	< **0.001**	**0.002**	0.015	**0.002**
DXA, lean mass							
Total lean mass (kg)	41.4 ± 2.8	42.5 ± 2.8	42.7 ± 2.8	< **0.001**	**0.003**	0.182	**0.003**
Trunk lean mass (kg)	20.9 ± 1.3	21.5 ± 1.3	21.6 ± 1.3	**0.003**	0.006	0.374	0.006
Leg muscle mass (mean) (kg)	6.6 ± 5.3	6.8 ± 5.2	6.8 ± 5.1	**0.001**	**0.003**	0.594	0.006
Arm muscle mass (mean) (kg)	2.0 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	0.009			
DXA, fat							
Total body fat mass (kg)	24.3 ± 2.1	23.4 ± 2.1	23.0 ± 2.1	**0.001**	0.010	0.050	0.021
DXA, bone							
Femoral neck bone mineral density (g/cm^2)	0.75 ± 0.14	0.76 ± 0.14	0.75 ± 0.14	0.125			
Femoral fracture risk (T-score)	-1.2 ± 0.3	-1.1 ± 0.3	-1.2 ± 0.3	**0.001**	0.026	0.136	0.918
Quality of life, SF-36							
Total physical component	34 ± 3	43 ± 3	40 ± 3	0.009			
Total mental component	46 ± 3	46 ± 3	43 ± 3	0.038			
Physical function	54 ± 9	68 ± 8	67 ± 7	0.008			
Physical role	51 ± 8	64 ± 9	57 ± 9	0.043			
Pain	41 ± 7	63 ± 9	50 ± 6	0.027			
General health	32 ± 6	47 ± 7	39 ± 7	**0.001**	0.008	0.007	0.044
Vitality	34 ± 7	47 ± 8	44 ± 7	0.159			
Social function	67 ± 8	79 ± 7	66 ± 9	0.032			
Emotional role	76 ± 7	78 ± 7	69 ± 6	0.123			
Mental health	63 ± 6	65 ± 5	64 ± 6	0.412			

Data are mean ± SEM. Significant *P*-values are in bold and threshold *P*-value was set at 0.003 (=0.05/20). Abbreviations: 6MWD, 6-minute walking distance; DXA, dual-energy x-ray absorptiometry; SF-36, Short Form-36 Item Health Survey; TUG, timed up and go test.

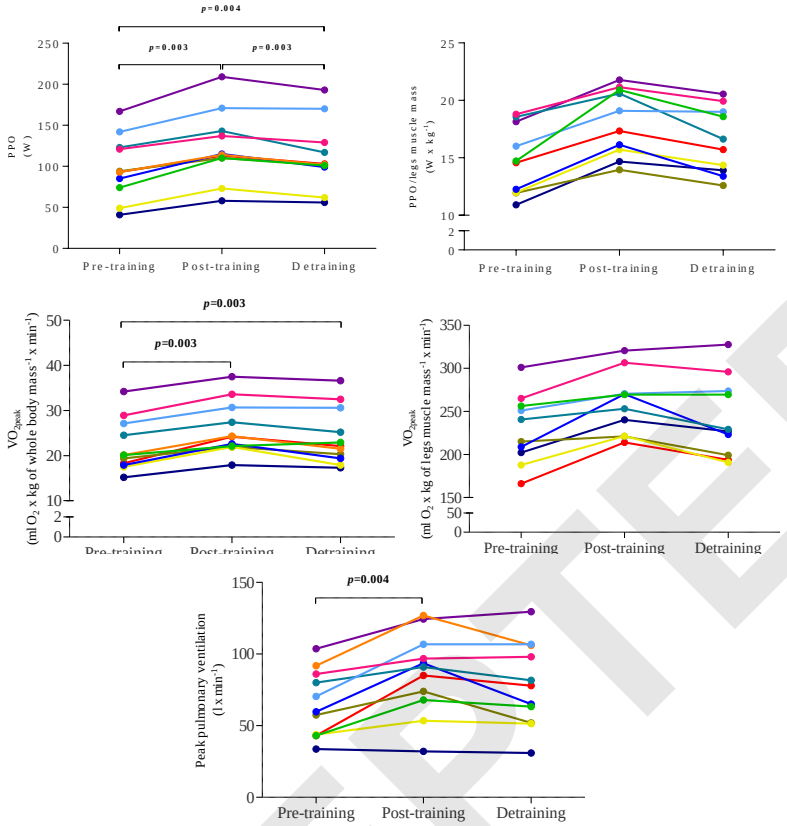
Table 4. Results of blood variables by patients' training status

	Resting levels				Acute change					
	Pre-training	**Post-training**	**Detraining**	***P*-value for time effect**	**Pre-training**	***P*-value**	**Post-training**	***P*-value**	**Detraining**	***P*-value**
Myokines/cytokines (pg/mL)										
Apelin	117 ± 43	153 ± 39	75 ± 22	0.030	-18 ± 11	0.419	-39 ± 27	0.102	22 ± 12	0.102
BDNF	1,269 ± 340	1,040 ± 159	689 ± 123	0.014	-367 ± 317	0.206	157 ± 239	0.206	390 ± 216	0.096
EPO	2,239 ± 352	2,381 ± 345	1,634 ± 212	0.030	-362 ± 199	0.414	-309 ± 144	0.102	317 ± 227	0.102
FABP3	1,588 ± 284	1,297 ± 190	1,511 ± 299	0.741	140 ± 86	0.058	211 ± 70	0.058	153 ± 50	**0.002**
FGF21	43 ± 7	48 ± 6	52 ± 10	0.565	-8 ± 3	0.059	4 ± 4	0.257	-8 ± 10	1.000
Fractalkine	297 ± 75	307 ± 70	221 ± 48	0.513	-20 ± 28	0.414	-33 ± 31	0.414	40 ± 36	0.414
FSTL1	5,685 ± 1 517	7,134 ± 1,317	3,443 ± 640	0.074	-1,075 ± 784	0.180	-2,297 ± 751	0.025	765 ± 927	0.317
IL1β	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.519	0.1 ± 0.1	0.480	0.1 ± 0	0.034	0.1 ± 0.1	0.480
IL2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.656	0.1 ± 0	0.058	0.1 ± 0	0.034	0.1 ± 0	0.034
IL4	7.7 ± 1.0	7.3 ± 1.7	5.3 ± 2.2	0.779	2.2 ± 0.8	0.046	4.3 ± 2.3	0.317	5.1 ± 2.6	0.317
IL6	8.0 ± 5.4	9.1 ± 5.9	7.8 ± 5.1	0.115	-0.3 ± 0.6	0.655	0.2 ± 0.4	0.655	0.7 ± 0.3	0.046
IL7	4.0 ± 1.3	4.3 ± 1.5	2.5 ± 1.3	0.325	0.8 ± 0.6	0.034	1.2 ± 1.1	0.480	2.1 ± 1.6	0.157
IL8	0.8 ± 0.3	0.7 ± 0.2	0.7 ± 0.3	0.122	0.1 ± 0.1	0.011	0.2 ± 0.1	**0.002**	0.2 ± 0.0	**0.002**
IL10	3.9 ± 1.1	3.8 ± 1.1	3.8 ± 1.2	0.867	0.5 ± 0.1	0.008	1.0 ± 0.4	0.014	0.5 ± 0.2	0.059
IL15	6.6 ± 5.6	8.3 ± 6.5	6.3 ± 5.8	0.449	-0.1 ± 0.3	0.655	-0.2 ± 0.5	0.655	0.4 ± 0.3	0.655
LIF	9.7 ± 3.5	8.7 ± 3.2	4.9 ± 1.5	0.057	-1.6 ± 0.7	0.144	0 ± 0.9	0.893	2.6 ± 0.9	0.043
Musclin	100 ± 13	108 ± 12	86 ± 9	0.066	-15 ± 9	0.059	-10 ± 8	0.705	12 ± 6	0.059
Oncostatin	3.3 ± 0.9	3.4 ± 1.0	2.7 ± 0.9	0.061	-0.1 ± 0.4	0.527	0.2 ± 0.2	1.000	0.8 ± 0.4	0.206
SPARC	56 ± 11	54 ± 10	42 ± 7	0.122	-16 ± 8	0.058	1 ± 6	0.527	9 ± 9	0.527
TNFα	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.926	0.4 ± 0.1	0.011	0.3 ± 0.1	0.011	0.3 ± 0.1	0.206
Lactate (mmol/L)	2.7 ± 0.3	2.9 ± 0.3	4.0 ± 0.9	0.739	4.0 ± 0.7	0.016	3.7 ± 0.1	0.003	3.2 ± 0.6	0.005
CK (U/L)	356.5 ± 86.2	344.2 ± 113.4	287.9 ± 62.9	0.441	28.8 ± 13.7	0.026	40.7 ± 12.6	0.003	20.2 ± 5.9	0.015
Creatinine (mg/dL)	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.739	0.04 ± 0.01	0.003	0.04 ± 0.01	0.003	0.02 ± 0.02	0.137

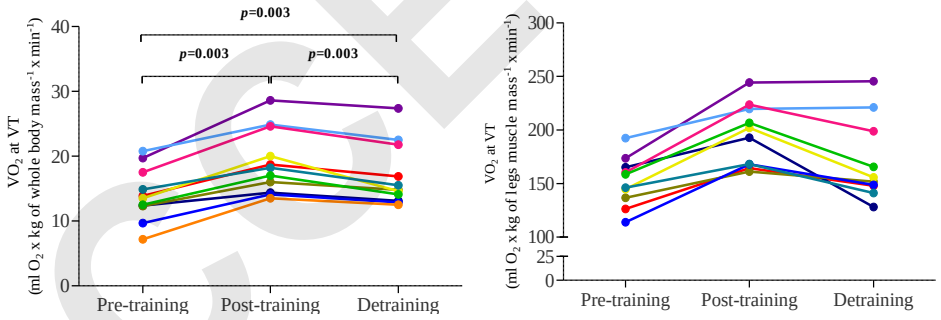
Data are mean ± SEM. Significant *P*-values are in bold -threshold *P*-value was set at 0.002 (=0.05/23). Acute change = value *after* maximal cycle-ergometer test minus value *before* maximal cycle-ergometer test. Abbreviations: BDNF, brain-derived neurotrophic factor; CK, creatine kinase; EPO, erythropoietin; FABP3, fatty acid binding protein 3; FGF21, fibroblast growth factor 21; FSTL1, follistatin-like 1 (also known as TSC-36); IL, interleukin; LIF, leukemia inhibitory factor; SPARC, secreted protein acidic and rich in cysteine (also known as basement membrane protein (BM)-40); TNFα, tumor necrosis factor α.

Supplemental Digital Content 1 (Figure). Individual data for patients' main endpoints.

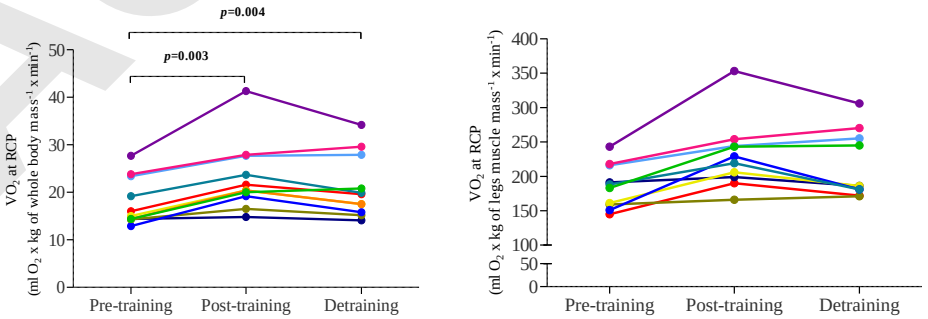
Aerobic power, peak values



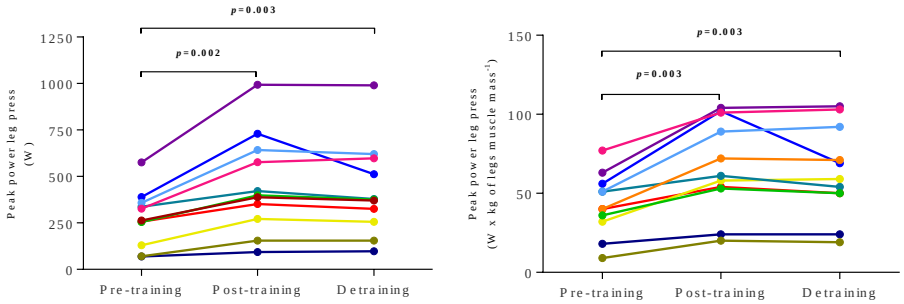
VT



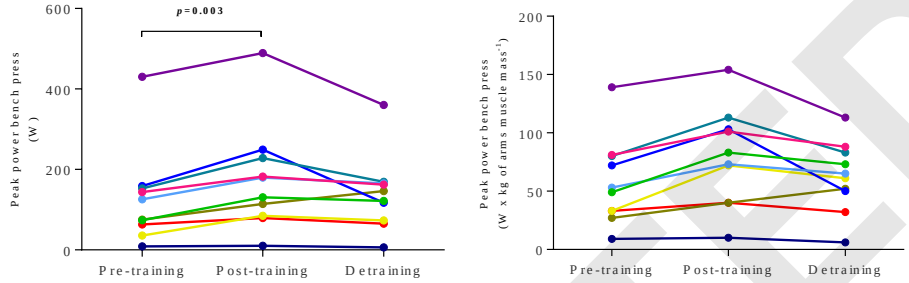
RCP



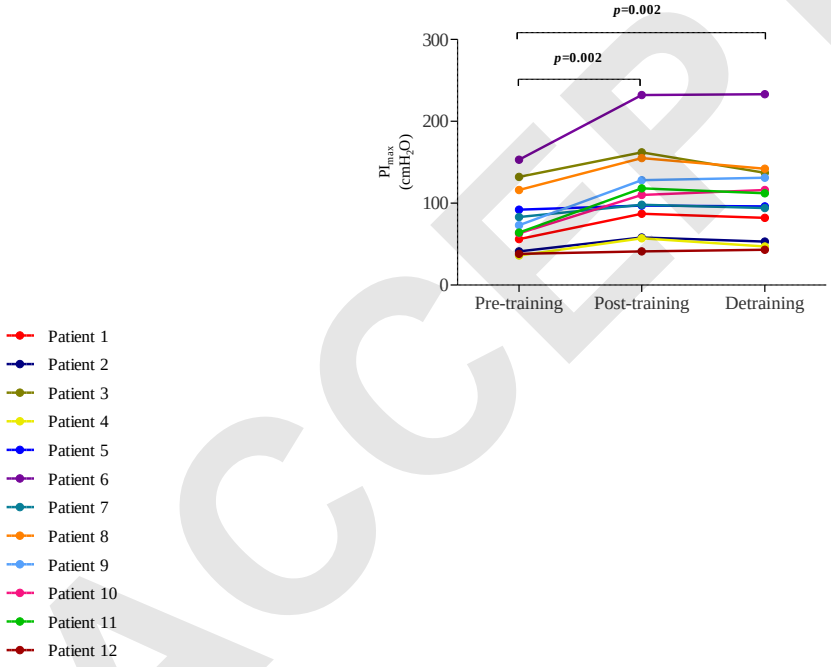
Strength test, leg press



Strength test, bench press



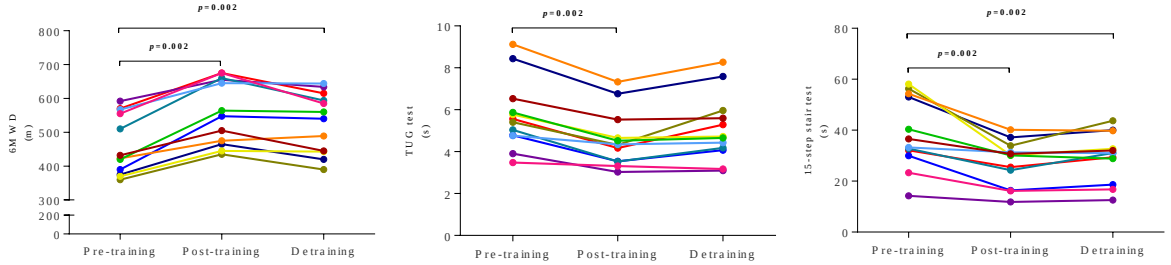
Maximal inspiratory muscle pressure



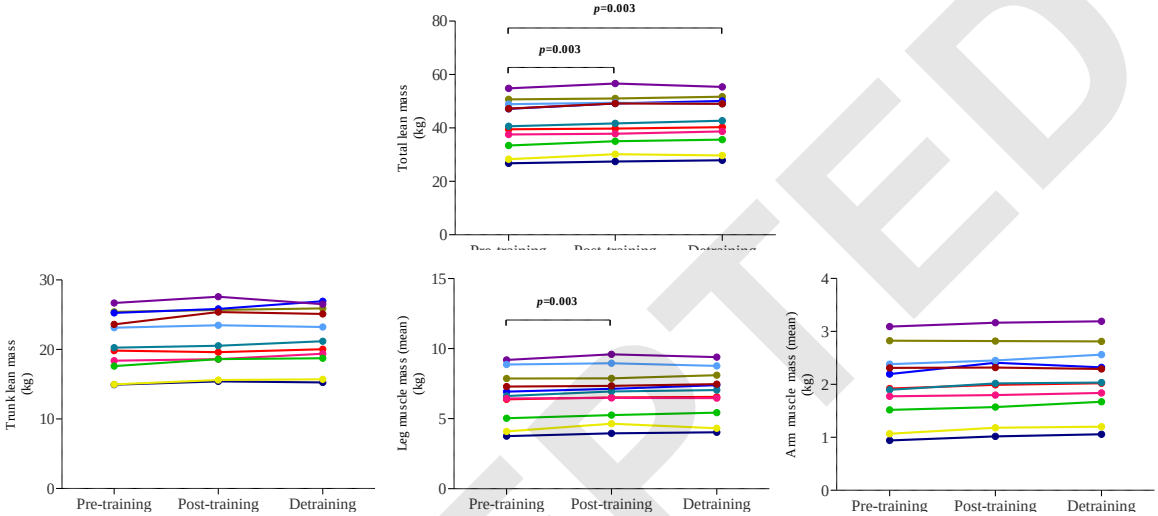
Patients´ identification follows the same order as that in Table 1. Abbreviations: PI_{max}, maximal inspiratory muscle pressure; PPO, peak power output; RCP, respiratory compensation point: VO_{2peak}, peak oxygen uptake; VT, ventilatory threshold.

Supplemental Digital Content 2, Figure. Individual data for patients' secondary endpoints.

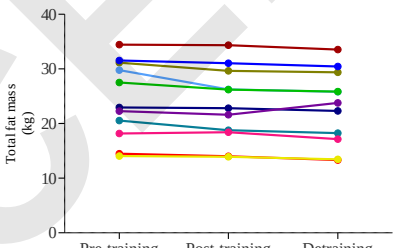
Functional tests indicative of ADLs



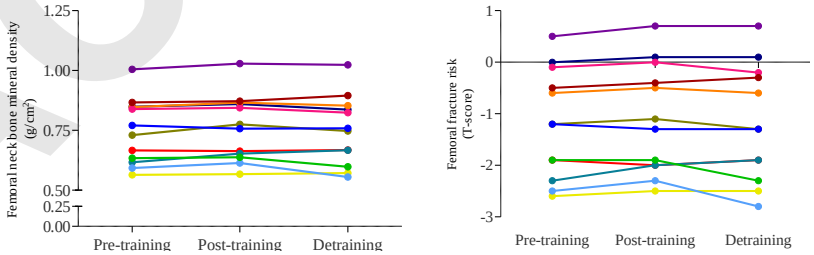
DXA, lean mass



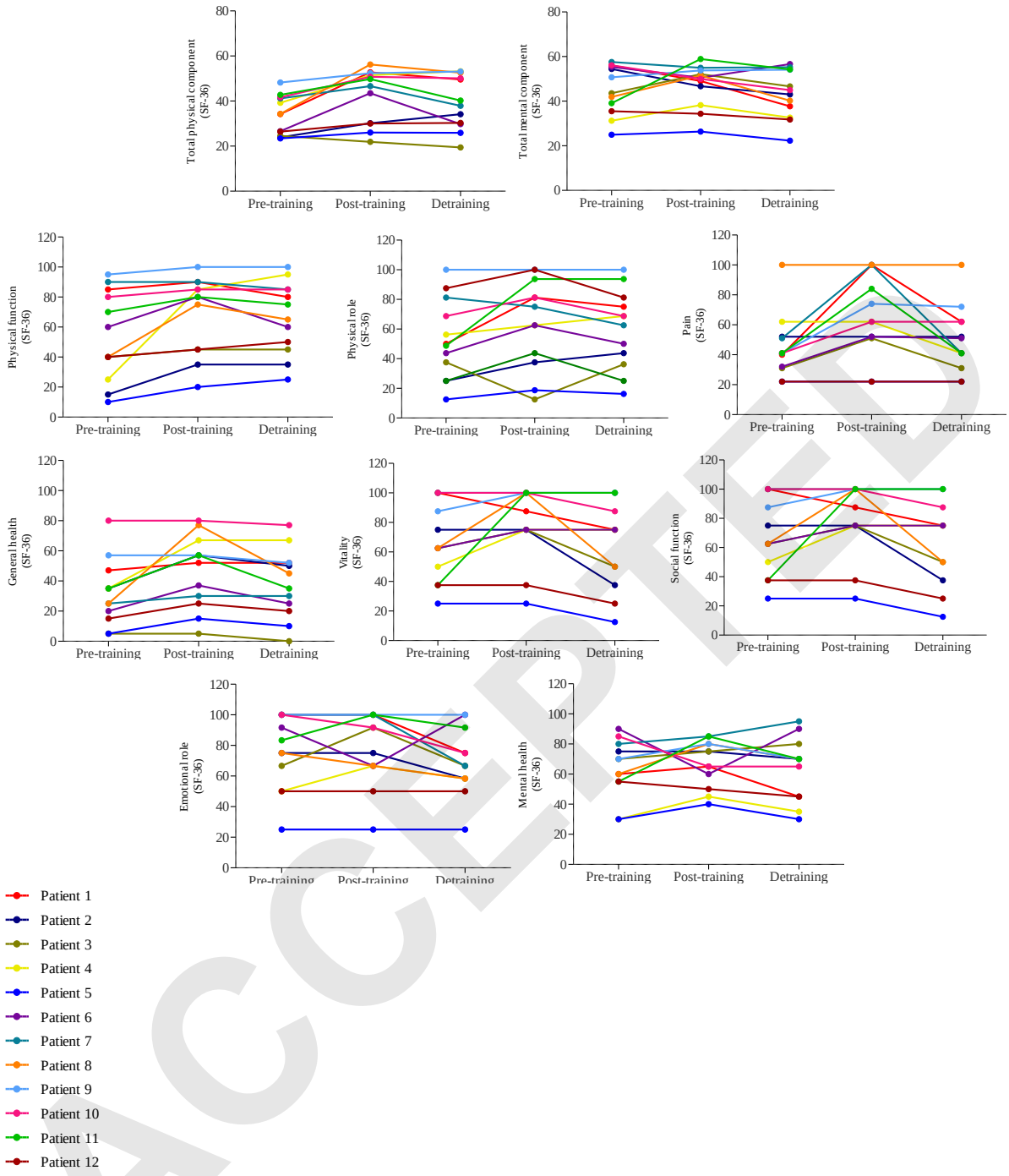
DXA, fat



DXA, bone



Quality of life, SF-36



Patients´ identification follows the same order as that in Table 1. Abbreviations: ADL, activities of daily living; DXA, dual energy x-ray absorptiometry; SF-36, Short Form-36; TUG, timed up and go test; 6MWD, six-minute walking distance test.

Supplemental Digital Content 3, Table. Results of dual energy x-ray absorptiometry in mitochondrial disease patients and age and gender-matched healthy controls

Outcomes	Patients (n=11)	Controls (n=33)	*P*-value
DXA, lean mass			
Total lean mass (kg)	41.4 ± 2.8	50.4 ± 2.0	**0.019**
Trunk lean mass (kg)	20.9 ± 1.3	25.5 ± 0.9	**0.025**
Leg muscle mass (mean) (kg)	6.6 ± 5.3	8.2 ± 0.4	**0.029**
Arm muscle mass (mean) (kg)	2.0 ± 0.2	2.6 ± 0.1	**0.038**
DXA, fat			
Total fat mass (kg)	24.3 ± 2.1	20.8 ± 1.4	0.198
DXA, bone			
Femoral neck bone mineral density (g/cm^2)	0.75 ± 0.14	0.77 ± 0.07	0.849
Femoral fracture risk (T-score)	-1.2 ± 0.3	-0.8 ± 0.5	0.649

Data are mean±SEM. Significant *P*-values are in bold and threshold *P*-value was set at 0.05. Abbreviation: DXA, dual energy x-ray absorptiometry.