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Long-term Exercise Intervention in Patients with McArdle Disease: Clinical and Aerobic Fitness Benefits

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ABSTRACT

Introduction: The long-term effects of exercise in patients with McArdle disease—the paradigm of 'exercise intolerance'—are unknown. This is an important question as the severity of the disease frequently increases with time. **Purpose:** To study the effects of a long-term exercise intervention on clinical and fitness-related outcomes in McArdle patients. **Methods:** Seventeen patients (exercise group: N=10, 6 male, 38±18yrs; control: N=7, 4 male, 38±18yrs) participated in a two-year unsupervised intervention including moderate-intensity aerobic (cycle-ergometer exercise for 1h) and resistance (high load-low repetition circuit) training on 5 and 2-3 days/week, respectively. Patients were assessed at baseline and postintervention. Besides safety, outcomes included clinical severity (e.g., exercise intolerance features) on a 0-3 scale (primary outcome), and aerobic fitness, gross muscle efficiency, and body composition (total/regional fat, muscle, and bone mass) (secondary outcomes). **Results:** The exercise program was safe and resulted in a reduction of one point (-1.0, 95% confidence interval -1.6—-0.5, p=0.025) in clinical severity vs. the control group, with 60% of participants in the exercise group becoming virtually asymptomatic and with no functional limitation in daily life activities. Compared with controls, the intervention induced significant and large benefits (all p<0.05) in the workload eliciting the ventilatory threshold (both in absolute (watts, +37%) and relative units (watts·kg^{-1} of total body mass or of lower-limb muscle mass, +44%)), peak oxygen uptake (ml·kg^{-1}·min^{-1}, +28%) and peak workload (absolute (+27%) and relative units (+33%)). However, no significant changes were found for muscle efficiency nor for any measure of body composition. **Conclusions:** A two-year unsupervised intervention including aerobic and resistance exercise is safe and induces major benefits in the clinical course and aerobic fitness of patients with McArdle disease.

Key Words: MYOPHOSPHORYLASE; EXERCISE IS MEDICINE; GLYCOGENOSIS TYPE 5; GLYCOGEN STORAGE DISEASE

INTRODUCTION

McArdle disease (glycogen storage disease type V; OMIM® 232,600; estimated prevalence of ~1/140,000 people) (1) is an autosomal recessive disorder caused by deficiency of the skeletal-muscle isoform of glycogen phosphorylase (myophosphorylase), the enzyme that catalyzes the breakdown of glycogen into glucose-1-phosphate and is encoded by the *PYGM* gene (2). Patients are thus unable to obtain energy from muscle glycogen stores, with most symptoms essentially due to energetic deficit. This disorder is indeed considered the paradigm of 'exercise intolerance', characterized by early fatigue, muscle weakness, myalgia and contractures associated with physical tasks (such as brisk walking or carrying/lifting weights) (2, 3). These episodes are often accompanied by 'crises' of rhabdomyolysis (1, 2, 4, 5), as reflected by an increased release of intra-muscle proteins into the bloodstream, such as creatine kinase (CK) and myoglobin (1, 2, 4, 5). Thus, very high levels of serum CK ('hyperCKemia', e.g., >5,000 U/L) caused by exercise is a common finding in these patients, which can be accompanied by myoglobinuria — typically referred to as 'dark urine' (1) On the other hand, an almost unique feature of McArdle disease that limits adherence to an active lifestyle is that the first minutes of exercise commonly trigger myalgia and tachycardia that are not attenuated until 7-10 minutes have elapsed — the so-called 'second wind phenomenon' (6).

Due to risk of severe rhabdomyolysis and eventually of acute renal failure, patients with McArdle disease have traditionally been advised to refrain from any type of physical exercise. Thus, not surprisingly, they typically show low levels of physical activity (1) together with poor aerobic fitness (7). Yet, peak maximum oxygen uptake (VO_{2peak}) is significantly higher in physically active McArdle patients than in their inactive peers (1, 6, 8), and preliminary non-

controlled studies in small patient cohorts (N=7-9) have shown the benefits of low-moderate intensity (60-70% of maximum heart rate) aerobic training (cycle-ergometer exercise or brisk walking on three to seven days/week) of short (four-week) to medium (eight-month) duration for improving VO_{2peak} (9-11). We recently reported, on the other hand, that adult patients had significantly lower muscle mass values in whole-body and regional sites (as determined by dual-energy x-ray absorptiometry (DXA)) than their age- and sex-matched healthy controls (12). This was also accompanied by a worsened bone mass. In this regard, we previously analyzed the effects of a four-month, individually-supervised resistance (weight lifting) training program in seven adult patients with McArdle disease of both sexes and found significant increases in total and lower-limb lean mass (13).

There is, however, to the authors' knowledge no evidence on the potential long-term (i.e., one year or more) effects of exercise intervention in patients with McArdle disease. This is an important consideration in light of the evidence that the severity of the disease frequently increases with time, especially in inactive patients (8). The purpose of this study was to determine the safety and effects of a long-term (two-year) unsupervised exercise intervention (combining aerobic and muscle resistance or 'strength' exercises) on: clinical severity (*primary outcome*), and aerobic fitness, muscle efficiency (i.e., the ratio of work accomplished to energy expended), and body composition (total and regional fat, muscle and bone mass) (*secondary outcomes*) in patients with McArdle disease.

METHODS

Participants

This concurrent prospective cohort study adhered the ethics guidelines of the Declaration of Helsinki, and was approved by the Ethics Committee of *Hospital 12 de Octubre*, Madrid, Spain (approval number 16/081). All participants were informed about the study procedures and signed a written informed consent. Eligibility criteria were: man/women aged 18 years or older genetically diagnosed with McArdle disease, that is, with identification of a pathogenic mutation in each allele of the gene (*PYGM*) encoding myophosphorylase (as explained below); having no condition contraindicating maximal exercise testing (notably, severe cardiopulmonary disease) or DXA assessment (notably, pregnancy); attending our talks (AS, AL) on lifestyle recommendations (mostly regarding pre-exercise nutrition and physical activity) specifically addressed to patients with McArdle disease in the meeting organized by the 'group of glycogenosis type V' within the frame of the annual meeting of the Spanish Association of Patients with Glycogenoses held in the city of Toledo (Spain) in year 2017. All the patients attending our talks and meeting the aforementioned inclusion criteria were fully informed of the details of the present study and invited to participate.

Potentially eligible patients with McArdle disease who agreed to participate were offered the possibility to enroll in a combined (aerobic + strength) unsupervised training program based on previous studies (10, 13) and according to Word Health Organization (WHO) recommendations for an active lifestyle (14): ≥150 min·week^{-1} in moderate-intensity aerobic activities or ≥75 min·week^{-1} in vigorous-intensity aerobic activities, as well as strength training (involving large muscle groups) at least twice a week. Subjects were placed in an intervention (exercise) group or

control group non-randomly, that is, if they had agreed or not, respectively, to participate in the intervention, and were assessed at baseline (year 2017) and after two years. Based on previous research from our group (10) and attending to the effect size observed for major outcomes also assessed in the present study (e.g., Cohen's d=1.17-1.18 for VO_{2peak} or peak workload), using GPower (version 3.1.9.2, Universität Düsseldorf, Germany) we considered that a sample size of N ≥10 in the exercise group and a total sample size (combining exercise and control groups) of N ≥13 would be enough to detect statistically significant within-group (Student's paired samples t-test) and between-group differences (ANCOVA with two groups, two measurement points and one covariate), setting α and β thresholds at <0.05 and >0.95, respectively.

Diagnosis

The presence of McArdle disease in all the participants was ascertained by genetic diagnosis (Neuromuscular disease laboratory, Hospital 12 de Octubre; Madrid, Spain), that is, by identification of a documented pathogenic mutation resulting in McArdle disease (among the 179 pathogenic variants that have been described) in each of the two *PYGM* alleles (i.e., with the presence of the same mutation in homozygosis or of two different mutations in heterozygosis), as per international recommendations (15). Thus, mutant *PYGM* alleles were identified in muscle or blood samples using SNaPShot minisequencing (Thermo Fisher Scientific; Waltham, MA) (16), followed by Sanger sequencing of the entire coding region and intron/exon boundaries of the *PYGM* gene (17). Alternatively, we used a next-generation sequencing-customized gene panel on a PGM-IonTorrent platform (ThermoFisher), consisting of 35 genes (including *PYGM*) associated with metabolic myopathies.

Outcome assessment

All participants arrived at the exercise laboratory (Faculty of Sport Sciences; Toledo, Spain) in the morning after an overnight fast and were assessed (within a single day, with assessors blinded to group allocation) at baseline and after the two-year intervention period, respectively, as shown below.

Safety. We recorded episodes of rhabdomyolysis (as reflected by self-reported episodes of dark urines and/or 'hyper-CK-emia' (serum CK >5,000 U/L) accompanied or not with fever or hospitalization), contractures, or severe myalgia (i.e., rate of perceived pain [RPP] (18) >5) associated with the exercise sessions during the study period.

Clinical severity. Clinical severity was assessed with the most commonly used phenotype severity scale (ranging from 0 [lowest] to 3 [highest]) for this disease (19), where: 0 = asymptomatic or virtually asymptomatic (mild exercise intolerance, but no functional limitation in any daily life activity); 1 = exercise intolerance, contractures, myalgia, and limitation of acute strenuous exercise, and occasionally in daily life activities; no record of myoglobinuria, no muscle wasting or weakness; 2 = same as 1, plus recurrent exertional myoglobinuria, moderate restriction in exercise, and limitation in daily life activities; 3 = same as 2, plus fixed muscle weakness, with or without wasting and severe limitations on exercise and most daily life activities.

Body composition. Total and regional body composition were assessed using a DXA instrument (Hologic QDR Discovery Wi; Bedford, MA) (12, 20) that was calibrated with a lumbar spine phantom following the Hologic guidelines. All scans were analyzed using Physician's Viewer,

APEX System Software version 3.1.2. (Bedford, MA) that was daily calibrated as part of the quality assurance program. This calibration was performed to ensure that the equipment was operating at a level consistent with Food and Drug Administration (FDA) and manufacturer's standards for diagnostic quality and patient safety. This test was run each day before any patient examination was conducted, using a lumbar spine phantom (ASY-01564) with known values and following the Hologic guidelines. The subject was placed in a supine position in the center of the table, aligned with the long axis of the scanner and with the head near the end of the table. The head was facing upwards, without turning left or right; the arms were pronated to the side maintaining the space between the arms and trunk; the hands were placed with palms flat against the scan table; the legs were internally rotated at 45° and fixed together with a Velcro strap around the ankles to minimize involuntary movement and feet were to be kept relaxed with the toes pointing upwards. Scans were made with participants wearing light clothing with no metal and no shoes or jewelry.

Lean mass (kg), bone mineral content (BMC, g) and density (BMD, $g \cdot cm^{-2}$) were calculated from total and regional analysis of the whole-body scan. Whole-body scans were submitted to a regional analysis to determine the composition of the arm, leg, and trunk regions. The arm region included the hand, forearm, and arm and was separated from the trunk by an inclined line crossing the scapulohumeral joint, such that the humeral head was located in the arm region. The leg region included the foot, the lower leg, and the upper leg. It was separated from the trunk by an inclined line passing just below the pelvis, which crossed the neck of the femur. The trunk region included the rest of the body excluding the arms, legs, and head regions. The head region comprised all skeletal parts of the skull and cervical vertebra above a horizontal line

passing just below the jawbone. All scans were analyzed using Physician's Viewer, APEX System Software version 3.1.2. (Bedford, MA) by the same researcher and all separation lines were examined and adjusted to guarantee maximum accuracy.

Aerobic fitness. After DXA assessments, participants performed an exercise test for VO_{2peak} determination. All the tests were performed using the same cycle-ergometer (Ergometrics 800, Ergoline GmbH; Bitz, Germany) and metabolic cart (Oxycon Pro, Jaeger, Hoechberg, Germany) following previous methodology from our group (6, 10). Concisely, the test was preceded by a five-minute free-wheel pedaling warm-up, after which the workload was increased following a ramp protocol (10 $W \cdot min^{-1}$) until volitional exhaustion. The peak workload was the wattage reached at exhaustion. VO_{2peak} was determined as the highest VO_2 value (20-second average) recorded during the tests, whereas the workload eliciting the ventilatory threshold (VT) was visually identified by two independent investigators (AS, AL) or by a third one in case of disagreement (CFL) as the workload eliciting an increase in the 20-second average value of the ventilatory equivalent for oxygen, with no concomitant increase in the ventilatory equivalent for carbon dioxide (10).

Muscle efficiency. In both baseline and postintervention tests, gross muscle efficiency (%) was calculated at the workload eliciting the VT in the baseline test as the ratio (x 100) of work accomplished per minute (i.e., watts converted to kilocalories per minute) to energy (kilocalories) expended per minute (10).

Exercise training intervention

Participants in the intervention group performed a two-year intervention. They, like participants in the control group, were periodically (every two weeks) contacted by telephone by one of the researchers (AS) to gather details on the number of aerobic and resistance training sessions they performed every week and were free to call him at any time to solve any potential question. Subjects were instructed (in the aforementioned preliminary talks on lifestyle recommendations) and reminded (in the biweekly telephone conversations) to perform all exercise sessions ~one hour after a meal containing approximately 100 g of complex carbohydrates (e.g., one large plate of white pasta or rice, three to four slices or white bread, or four servings of boiled potatoes without skin). In addition, before each strength training session (see below), patients performed two consecutive warm-up bouts of 12-minute duration each, the first on an arm-crank ergometer and the second on a cycle-ergometer, in order to trigger the occurrence of the second wind in both upper and lower body muscles, respectively, and the end of the second warm-up was followed by ingestion of a commercialized sports drink (250 mL, ~20 g of sucrose).

Aerobic training. This included five sessions/week. After two familiarization sessions in our laboratory, participants pedaled on stationary bicycle (at home or in a local gym/fitness center) at moderate intensity while avoiding pain (i.e., at a workload eliciting a Borg's rating of perceived exertion (RPE) (21) and pain (RPP) (18) on a 0 (minimum) to 10 (maximum) scale of 5-7 and 0-1, respectively) (15). Session duration was gradually increased (starting with ~15-20 minutes), such as to complete one hour per session at the aforementioned intensity (i.e., RPE level consistently of 5-7) by the fourth week of the program.

Strength training. Following previously methodology published by our group (13), a basic high load – low repetition circuit training was prescribed, using large muscle group machines. This strength training methodology has proven effective and safe (i.e., with no major increases in CK levels) (13). After two familiarization sessions in our laboratory, subjects were encouraged to perform two to three strength training sessions per week (with a minimum of 48 hours of recovery between two consecutive sessions) in their local gym of fitness center. Only four exercises were performed using a circuit (three rounds per session) involving large muscle groups in the following order: bench press, leg press, pull down and abdominal crunches. The exercises were performed for three sets of a low number of repetitions (i.e., six) using a load (kg) eliciting an OMNI-RPE (22) and RPP (18) value of 6-7 and 0-1, respectively, at the end of each set. When the ONMI-RPE value for a given exercise was <6 in two consecutive training sessions, subjects were advised to increase the load (kilograms) such as to reach again an ONMI-RPE-value of 6-7. The low number of repetitions allows the use of muscle phosphocreatine (PC) as the main energy substrate to fuel contraction, with no major reliance on muscle glycogen deposits, and the circuit structure, with two-to-three–minute rest periods between each set of repetitions and exercises, was designed to allow PC to be resynthesized in a given muscle before this muscle was utilized again (13, 15). Passive stretching exercises were performed after each set of an exercise to attenuate muscle stiffness (10 to 30 seconds for each muscle group).

Of note, we used RPP as a safety measure (in both aerobic and strength training sessions) due to the usefulness of this indicator for accurate pain perception. Thus, besides avoiding reaching muscular failure in each of the strength exercises, participants were clearly instructed to stop the relevant aerobic or strength exercise if they noticed a rapid increase in RPP and to perform light

stretching exercises until pain disappearance. Thereafter, they could continue the aerobic session or move to the next strength exercise. After each session, patients were encouraged to drink plenty of water to protect kidney function, as per international clinical guidelines (15).

Statistical analyses

Data are shown as mean ± standard deviation (SD) unless otherwise stated. Baseline differences between groups on descriptive variables were assessed through Students' unpaired t-tests or chi-squared tests for continuous and dichotomous variables, respectively. The effects of the intervention on study outcomes (postintervention *minus* baseline data) were assessed through a one-way ANCOVA, including baseline results as a covariate. The Mann Whitney's U test was used for the analysis of non-parametric outcomes. Effect sizes (partial eta squared, η_p^2) were also computed and interpreted as trivial ($\eta_p^2 < 0.01$), small ($\eta_p^2 > 0.01$), medium ($\eta_p^2 < 0.06$), or large ($\eta_p^2 > 0.14$). Statistical analyses were performed with SPSS 23.0 (IBM statistics; Armonk, NY) setting the level of significance at 0.05.

RESULTS

A consort diagram is shown in Figure 1. From 31 potentially eligible patients 20 agreed to participate. Seventeen patients diagnosed with McArdle disease were assessed at both baseline and postintervention (N = 10 and N = 7 in the exercise and control group, respectively). The different *PYGM* genotypes of the 17 participants resulting in McArdle disease are shown in Supplemental file 1 (see Supplemental Digital Content 1, http://links.lww.com/MSS/C564). Each of these genotypes results in total absence whatsoever of myophosphorylase activity (and thus in total inability to use muscle glycogen as a substrate) (23-25) and all the pathogenic *PYGM* mutations

shown by the patients of the present study have been previously reported in the last update of the Spanish registry of patients with McArdle disease (1).

There were no between-group differences at baseline for demographic variables between the exercise and the control group (Table 1).

Participants in the exercise group performed on average 5 ± 1 ($96 \pm 8\%$ of maximum number of planned sessions) and 2 ± 0 ($74 \pm 13\%$) sessions per week of endurance and resistance exercise, respectively, during the study, whereas participants in the control group performed 2 ± 2 days per week of moderate physical activity (mostly walking for 30 minutes to one hour) and no resistance exercise at all ($p<0.001$ for both between-group comparisons). No exercise-related adverse events were reported during the exercise intervention sessions (notably, no episode of dark urine or 'hyper-CK-emia' and no medical visit associated to the exercise sessions).

Primary outcome. The exercise program resulted in a significant reduction (Mann Whitney's $p=0.025$) of one point (-1.0, 95% confidence interval -1.6 to -0.5) in clinical severity scale compared with the control group (Figure 2). Importantly, six of the 10 participants in the exercise group (60%) had changed to the lowest clinical severity grade (= 0) by the end of the intervention (i.e., becoming virtually asymptomatic and with no functional limitation in any daily life activity). In fact, one of these six subjects moved from severity class 2 at baseline to class 0 after the two-year intervention. By contrast, the most common trend in the control arm (71% of cases) was to remain within the same severity class throughout the intervention period.

Secondary outcomes. The change observed in the control and exercise groups as well as between-group differences in all secondary outcomes as well are shown in Table 2 (see also Supplementary file 2, Supplemental Digital Content 2, for all mean values, http://links.lww.com/MSS/C565). No significant changes were found for gross muscle efficiency or DXA-determined total or regional indicators of body composition. On the other hand, compared with the control group the intervention induced significant and large benefits (all $p<0.05$ and $\eta_p^2 > 0.35$) in virtually all aerobic fitness-related parameters: workload eliciting the VT (expressed both in absolute (watts) and relative units (i.e., with respect to kilograms of total body mass or of lower-limb muscle mass)) (Figure 3), VO_{2peak} in relative units ($ml \cdot kg^{-1} \cdot min^{-1}$) (Figure 4) and peak workload (expressed both in absolute and relative units) (Figure 5).

DISCUSSION

The main finding of our study was that a long-term (two year) unsupervised intervention including aerobic and resistance exercise training is safe in patients with McArdle disease and induces major benefits in the clinical course of the disease as well as in aerobic fitness indicators. These results are medically relevant, as discussed below.

The exercise program markedly reduced clinical severity, with more than half of the participants in the intervention arm becoming essentially asymptomatic and not limited anymore during daily life activities by the end of the intervention. By contrast, the most common trend in their controls was to remain within the same severity class throughout the two-year period. Our results are of clinical relevance when considering the epidemiological evidence that the vast majority (92%) of patients with McArdle disease report functional limitations during daily life

activities such as household tasks (e.g., bed-making, sweeping, washing dishes), personal care (e.g., hair combing), lifting/carrying weights during shopping, or carrying children and one third show a worsening in the clinical course of the disease after only four years (1, 8). Yet, regular physical exercise during half this period can restore almost full functional capacity in these patients, at least during activities of daily living, as suggested by the present findings. This is an important consideration when keeping in mind that no molecular therapy is predicted to have such beneficial effect in the foreseeable future — that is, no drug is likely to ensure long-lasting restoration of myophosphorylase (even residual) activity, as recently reviewed by us in depth regarding published and ongoing preclinical therapeutic trials (26). Our results are consistent with previous noncontrolled research from our group, where a resistance training-only intervention reduced clinical severity in one adolescent (27) or in seven adults with McArdle disease (13). In addition, there is epidemiological evidence that meeting minimum WHO-determined guidelines for aerobic physical activity is associated with a better clinical outcome (1, 8).

Our results indicate a significant improvement in aerobic fitness indicators after the intervention, with a mean VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$) increase of ~23% (or ~1.6 metabolic equivalent of task (MET)) in the intervention arm. Importantly, none of the participants showed a VO_{2peak} value ≥ 8 MET at baseline, whereas three participants in the exercise arm (vs. none in the control group) reached or surpassed the 8-MET cutoff after the intervention period — with one in fact reaching 10 MET. In this regard, 8 MET is the minimum threshold for optimal health, above which the risk for cardiovascular mortality in adults is significantly reduced compared with lower values (28). This is an important consideration in light of recent data from the European registry of patients with McArdle disease, indicating an overall unhealthy cardiometabolic profile for these

individuals, with two-thirds showing high BMI values and ~12% having cardiovascular disease (8% with coronary artery disease) despite the relatively young age of the cohort (median age 46 years) (5). On the other hand, the result that three patients reached VO_{2peak} values ≥8 MET values after training despite having total absence of myophosphorylase deficiency is a remarkable finding when considering that these values are above those (~7 MET) previously reported in two patients with a 'mild' form of McArdle disease owing to residual myophosphorylase activity (29) — which, as mentioned above, would be a quite successful outcome for future molecular therapies if eventually available.

Our data thereby reinforce the rationale for increasing the levels of exercise in patients with McArdle disease. One of the hallmarks of this condition, as confirmed here, is the very poor aerobic fitness of affected patients — with a mean VO_{2peak} at baseline for all participants in our study of barely ~19 ml·kg^{-1}·min^{-1} or ~5 MET, which is very similar to previous data showing that the VO_{2peak} of these patients is barely 50% of age and gender-predicted values (1, 5, 7, 10). In fact, the findings in patients have been replicated in the genetically manipulated mouse model of McArdle disease that we generated (harbouring the commonest *Pygm* genotype causing the disorder among Caucasians, p.R50*/p.R50*), with the endurance capacity of p.R50*/p.R50* mice ~50% lower compared to healthy wild-type (WT) mice (30, 31). Although the maximal aerobic capacity of p.R50*/p.R50* mice increases significantly, like in patients, with regular endurance training (for eight weeks, which would be equivalent to ~one-year duration when translated to 'human lifespan'), it still remains lower than that of untrained WT mice (30, 32), which is in line with present and previous findings (10) in patients. These preclinical and clinical results reflect the importance of myophosphorylase activity and thus of the glycogenolytic pathway to reach normal

levels of muscle oxidative capacity in muscle tissue. In fact, although in mice heterozygous for the p.R50* mutation myophosphorylase activity as well as the ability to utilize glycogen is not totally blocked (i.e., ~50 % of normal), their maximal aerobic capacity remains considerably reduced (by ~18 %) when compared with their WT peers (31).

The low VO_{2peak} of patients has been traditionally associated to the reduced flux of substrates along the glycolytic pathway, which limits the supply of substrates to the tricarboxylic acid cycle, thereby impairing oxidative metabolism — as reflected for instance by the slow VO_2 kinetics during the transition from rest to exercise (33) and by the slow PC recovery kinetics following exertion (34). Thus, the production of pyruvate, a molecule that plays an anaplerotic role in the Krebs cycle, would be severely reduced (4). Nonetheless, data from our group in the mouse model of the disease do not support that myophosphorylase deficiency produces a complete impairment in muscle oxidative phosphorylation capacity and as such the patients' muscle would be able to adapt favorable to aerobic exercise (32). In fact, other researchers have reported training-induced improvements in patients' muscle oxidative capacity (as reflected by higher activities in citrate synthase and hydroxyacyl coenzyme A dehydrogenase) with aerobic training (9).

Another important finding is the training-induced improvement in the workload (wattage) eliciting the VT as well as in peak workload, including when both variables were expressed relative to the amount of lower-limb muscle mass. This would suggest, at least partly, an improved ability of trained patients to produce power at both submaximal and maximal intensities for the same amount of muscle mass perhaps due to an improved muscle contractility. In this effect, previous research from our laboratory (using surface electromyography (EMG)) has indicated that, for the

same degree of muscle recruitment, untrained patients with McArdle disease are able to produce only about one-third of the power produced by age- and sex-matched healthy controls during the same ramp cycle-ergometer protocol that we used here, and also showed higher muscle recruitment levels for the same relative submaximal workload, thereby reflecting impaired muscle contractility (35). One explanation for a potential low muscle contractility in patients with McArdle disease might be down-regulation of calcium and sodium-potassium pumps, since both are highly dependent on glycogenolysis-derived ATP (35). We have in fact corroborated alteration of sarcoplasmic calcium handling (through glycolytic-ATP-dependent—sarco(endo)plasmic reticulum ATPase) in the mouse model of McArdle disease (32). This is an important consideration as 80% of the ATP consumed during muscle contraction is required for adequate removal of calcium from the sarcoplasmic reticulum (36). In addition, the documented deficient glycogen-dependent ATP supply to the sodium pumps in the skeletal muscle fibers of McArdle patients might result in down-regulation of these pumps and thus in reduced membrane excitability (37). These could all increase required motor unit recruitment for a given muscle task.

Our exercise program failed, however, to induce significant improvements in the patients' total or regional fat, muscle or bone mass. This is in contrast with the findings of a previous resistance exercise-only intervention (with no control group) performed by us following a similar protocol (13) but with all sessions individually supervised, where significant improvements were reported at postintervention for total and lower-limb muscle mass — but not for upper-limb/trunk muscle mass or for total fat mass. It therefore seems that individual supervision of resistance training is needed to induce a significant gain in the muscle mass of these patients. Individual supervision would seem particularly important when considering that owing to the risk of

rhabdomyolysis associated to exercises imposing high mechanical loads on muscle fibers (particularly lifting or carrying weights) and the inherent metabolic limitation in these patients (which can rely on muscle PC but not on muscle glycogen), resistance exercise sessions (especially if unsupervised) should not be focused on gaining muscle mass — for which imposing high mechanical stress and subsequent muscle damage would be needed (38). The intervention also failed to improve muscle efficiency, which is in accord with previous research from our group using an eight-month aerobic training intervention (10). The low levels of gross muscle efficiency (average of ~12%) are in line with the results previously reported by us (12-13%, vs. 19-20% in healthy controls) (10) as well as with the higher-than-normal VO_2:power output relationship reported for these patients during ramp cycle-ergometer tests (35, 39). Although some mechanisms like the aforementioned excessive muscle recruitment for a given workload and alteration of calcium handling can be involved, the main reason for the fact that the muscles of these patients are only able to convert a much lower portion of the total oxygen they consume into mechanical work than healthy age-matched controls is likely the major dependence on fat metabolism owing to the inherited block in muscle glycogen metabolism (2). This metabolic block cannot be overcome by exercise training in the event of total absence of myophosphorylase activity (since even trained patients would have to rely inevitably on fat oxidation as a main metabolic pathway) — only so by eventual restoration of enzyme activity with molecular therapies. Carbohydrates are indeed much more efficient fuels than fat in terms of rate of ATP generation per unit of oxygen consumed. Indeed, since the oxidative phosphorylation yield is higher when NADH is the electron donor — three coupling sites — compared with FADH2 — two coupling sides — and carbohydrate metabolism produces a greater ratio of the reducing equivalents NADH/FADH2 than

β-oxidation, carbohydrates are able to produce a greater ATP yield per unit of oxygen consumption than fat despite the greater ATP production per unit of substrate from the latter (40).

Our findings are limited by the fact that we did not perform a randomized controlled trial or molecular assessments at the muscle tissue level (notably, molecular markers of oxidative phosphorylation, sarcoplasmic reticulum calcium handling or sodium potassium pump function) due to ethical constraints. In addition, we did not assess muscle strength as a study outcome or use surface EMG and did not study intervention effects in participants of the highest severity class (=3), which affects one out of five patients in general (1). The sample size was overall limited, although not in the context of a rare condition such as McArdle disease (estimated prevalence of ~1/140,000 people) (1). In turn, major strengths were the fact that this is the first exercise intervention with these patients using a control group as well as the first to combine aerobic and resistance exercises, the long duration of the program (two years), and that we provided medical evidence for important exercise benefits (i.e., *exercise is medicine*) in the context of a disorder which is arguably considered the 'paradigm of exercise intolerance' (4), with patients traditionally advised to refrain from exercise and certainly resistance exercise.

CONCLUSIONS

In conclusion, a two-year unsupervised exercise training intervention including aerobic and resistance exercises is safe in patients with McArdle disease and induces major benefits in the clinical course of the disease as well as in aerobic fitness despite the major metabolic limitation associated with this condition.

Acknowledgements

Conflicts of interest

The authors declare no conflicts of interest. The results of the present study do not constitute endorsement by ACSM, and are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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FIGURE LEGENDS

Figure 1. Participants' flow diagram.

Figure 2. Effects of exercise training on clinical severity. Bars and lines represent mean ± SD and individual results, respectively. Between-group analyses were performed by comparing the change (post-intervention minus baseline) through the non-parametric Mann-Whitney U test, with the p-value shown in the Figure ($\eta_p^2 = 0.514$). Clinical severity was assessed on a 0-3 scale as explained elsewhere (Martinuzzi et al) (18) (see text for more details).

Figure 3. Effects of exercise training on the ventilatory threshold (VT). Between-group comparisons (adjusted for baseline data) were performed with a one-way ANCOVA, with the p-value shown in each panel ($\eta_p^2 = 0.292$, 0.318 and 0.360 for VT expressed in watts (**A**), watts relative to total body mass (**B**) or watts relative to lower-limb muscle mass (**C**)).

Figure 4. Effects of exercise training on peak oxygen uptake (VO_{2peak}). Between-group comparisons (adjusted for baseline data) were performed with a one-way ANCOVA, with the p-value shown in each panel ($\eta_p^2 = 0.172$ and 0.379 for VO_{2peak} expressed in ml·min^{-1} or ml·kg^{-1}·min^{-1}, respectively).

Figure 5. Effects of exercise training on peak work capacity (PWC). Between-group comparisons (adjusted for baseline data) were performed with a one-way ANCOVA, with the p-value shown in each panel ($\eta_p^2 = 0.172$, 0.379 and 0.459 for peak work capacity expressed in watts (**A**), and watts relative to total body mass (**B**) or lower-limb muscle mass (**C**)).

SUPPLEMENTAL DIGITAL CONTENT

SDC 1: Suppl File 1.docx – Table, Pathogenic *PYGM* genotype indicative of McArdle disease identified in all the participants

SDC 2: Suppl File 2.docx – Table, non-adjusted results
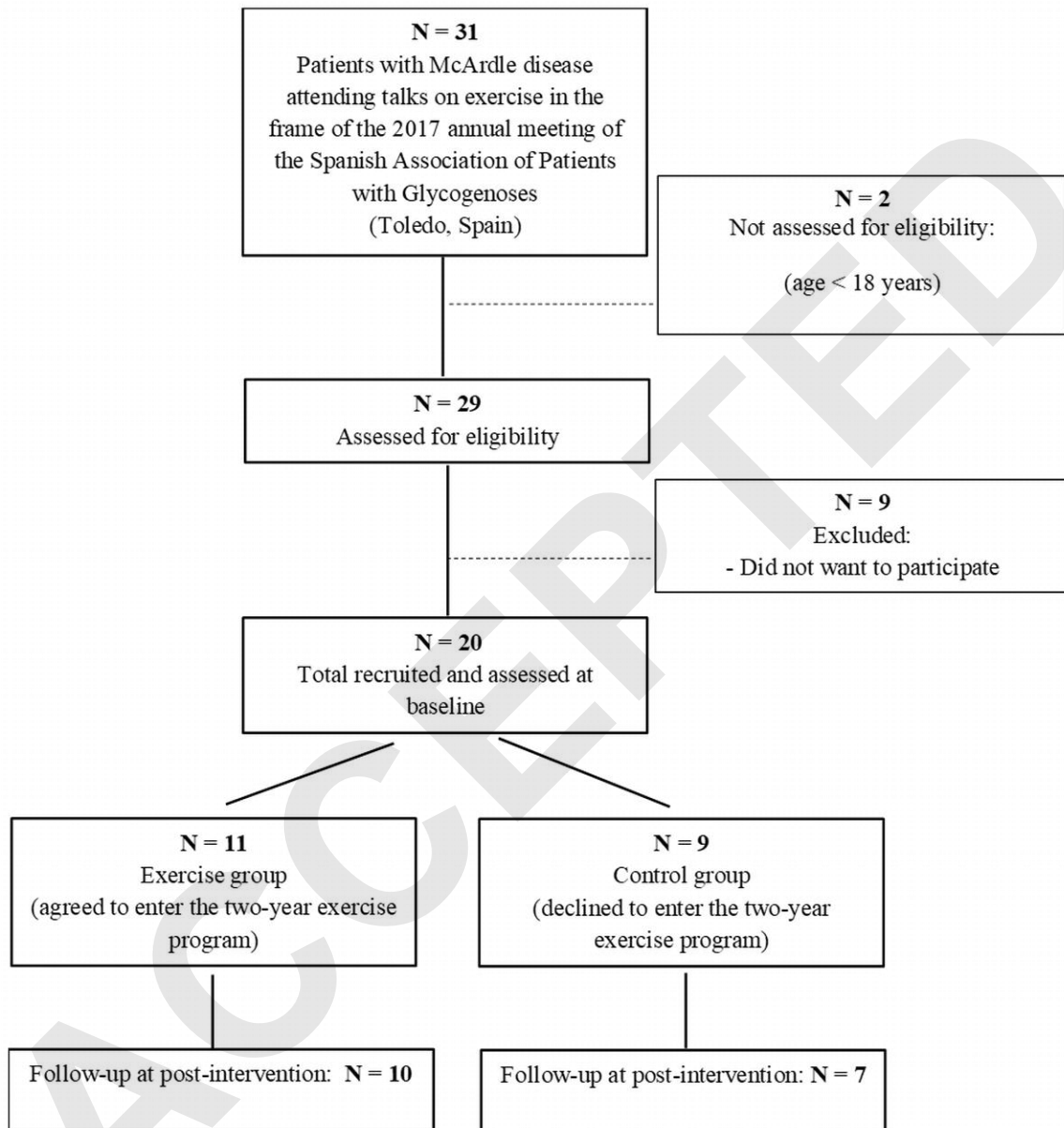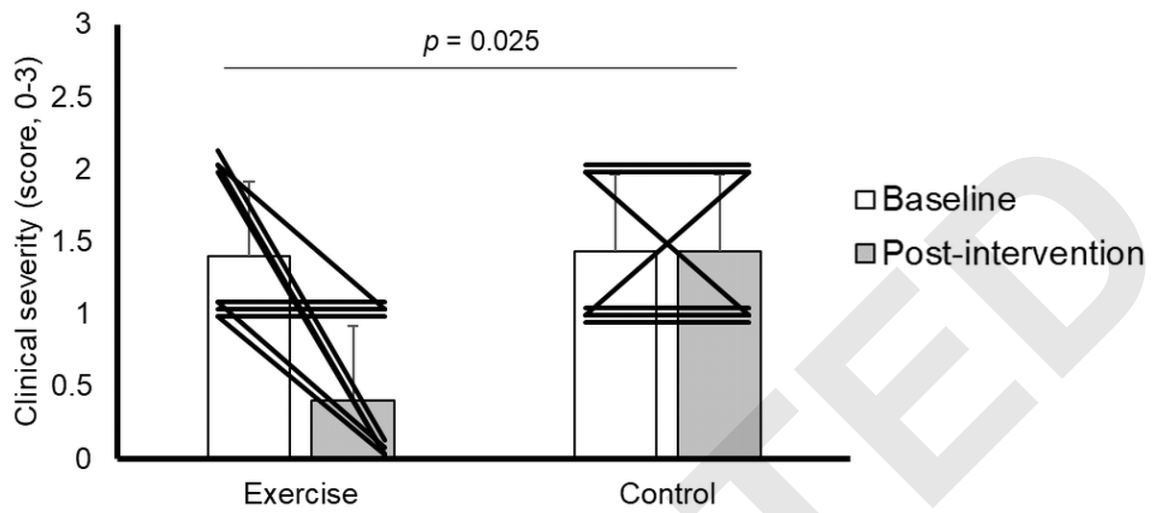
Figure 1

Figure 2

Figure 3
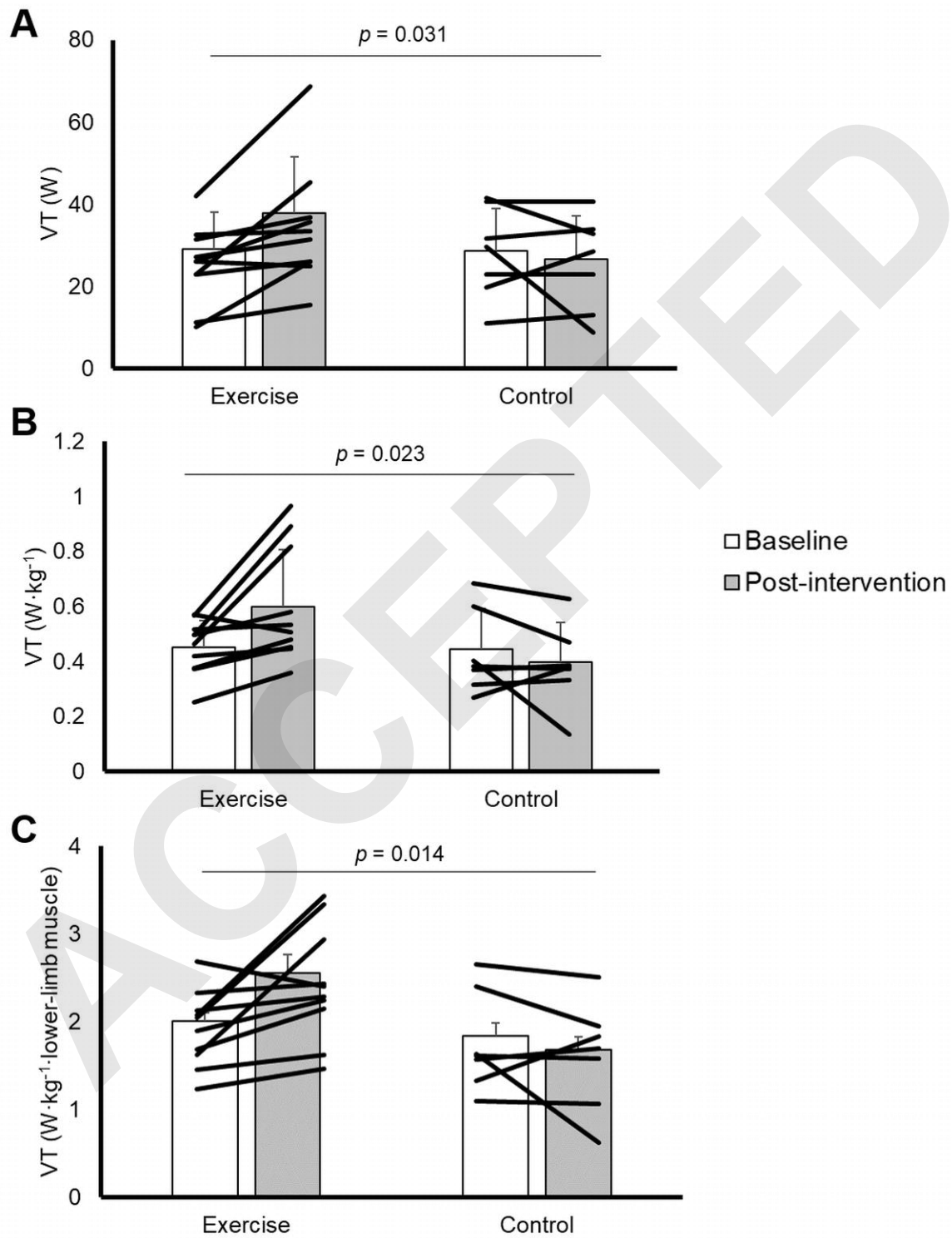
Figure 4
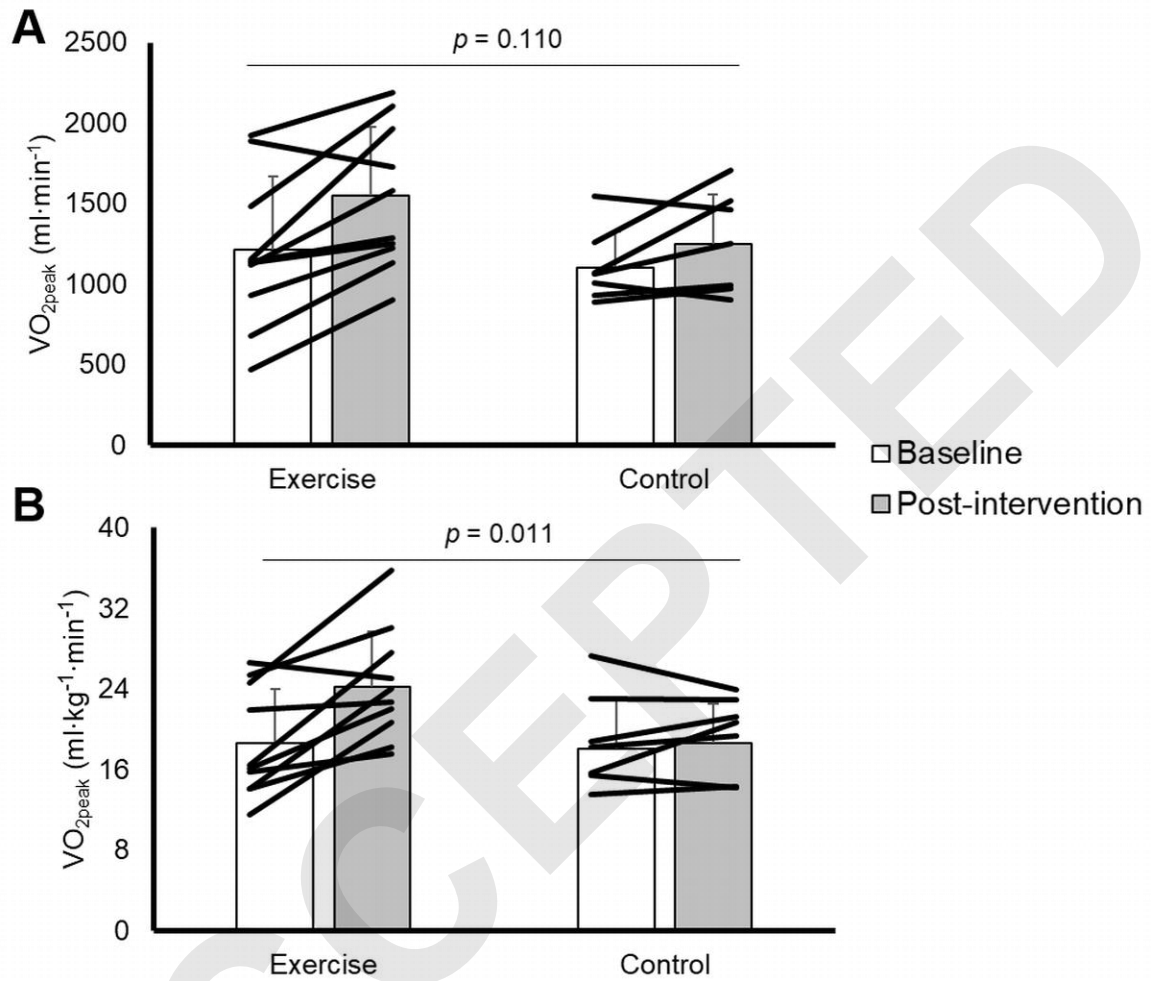
Figure 5
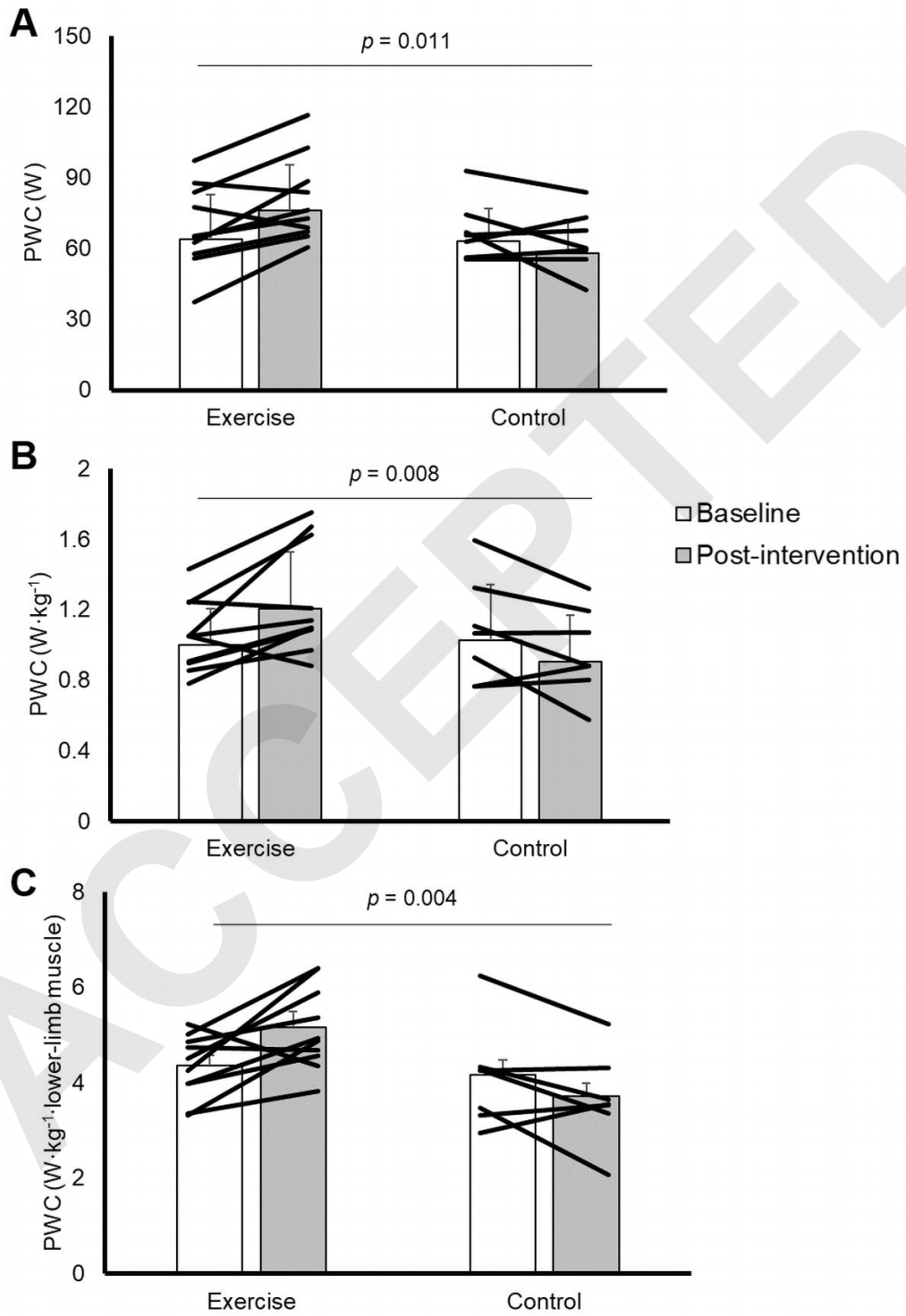
Table 1. Descriptive characteristics of the participants by group.

Variable	Exercise (N = 10)	Control (N = 7)	p-value
Sex (N, % male)	6 (60%)	4 (57%)	1.000
Age (years)	38 ± 18	37 ± 15	0.863
Weight (kg)	65.4 ± 15.1	65.0 ± 14.3	0.959
Height (cm)	163 ± 11	169 ± 8	0.241
Body mass index (kg/m^2)	24.2 ± 4.1	22.5 ± 4.0	0.429

Data are mean ± SD.

Table 2. Effects of exercise intervention on fitness- and body composition-related outcomes.

Outcome	Exercise change (95% CI)	Control change (95% CI)	Adjusted difference (95% CI)	p-value	η_p^2
VT (W)	8.6 (2.4, 14.8)	-2.2 (-9.5, 5.2)	10.8 (1.1, 20.4)	**0.031**	0.292
VT (W·kg^{-1})	0.15 (0.04, 0.25)	-0.05 (-0.17, 0.08)	0.20 (0.03, 0.36)	**0.023**	0.318
VT (W·kg^{-1} lower-limb muscle mass)	0.15 (0.05, 0.26)	-0.05 (-0.18, 0.07)	0.21 (0.05, 0.37)	**0.014**	0.360
VO$_{2peak}$ (ml·min^{-1})	345 (175, 515)	133 (-70, 336)	212 (-54, 478)	0.110	0.172
VO$_{2peak}$ (ml·kg^{-1})	5.70 (3.26, 8.15)	0.51 (-2.41, 3.43)	5.20 (1.39, 9.00)	**0.011**	0.379
PWC (W)	12.3 (4.2, 20.4)	-5.1 (-14.8, 4.6)	17.4 (4.7,30.0)	**0.011**	0.383
PWC (W·kg^{-1})	0.21 (0.06, 0.35)	-0.12 (-0.30, 0.05)	0.33 (0.10, 0.56)	**0.008**	0.402
PWC (W·kg^{-1} lower-limb muscle mass)	0.84 (0.31, 1.37)	-0.49 (-1.13, 0.14)	1.33 (0.50, 2.16)	**0.004**	0.459
Gross muscle efficiency (%)	0.74 (-0.43, 1.90)	0.27 (-1.13, 1.66)	0.47 (-1.36, 2.29)	0.593	0.021
Whole-body lean mass (kg)	0.67 (-0.28, 1.62)	2.02 (0.88, 3.15)	-1.35 (-2.83, 0.14)	0.072	0.213
Head lean mass (g)	33 (-83, 147)	106 (-31, 244)	-74 (-253, 105)	0.391	0.053
Upper-limb lean mass (g)	78 (-95, 251)	90 (-117, 296)	12 (-257, 281)	0.927	0.001
Lower-limb lean mass (g)	73 (-221, 367)	405 (53, 757)	332 (-128, 793)	0.144	0.146
Trunk lean mass (g)	643 (202, 1083)	1126 (599, 1653)	-483 (-1172, 204)	0.154	0.140
Whole-body fat mass (kg)	-0.39 (-1.98, 1.20)	0.48 (-1.42, 2.39)	-0.88 (-3.36, 1.61)	0.463	0.039
Head fat mass (g)	35 (-2, 69)	25 (-18, 67)	9 (-47, 4)	0.734	0.009
Upper-limb fat mass (g)	-41 (-210, 128)	-39 (-241, 164)	-2.33 (-269, 264)	0.985	0.000
Lower-limb fat mass (g)	-138 (-805, 530)	-11 (-809, 787)	-127 (-1168, 914)	0.798	0.005
Trunk lean mass (g)	-181 (-966, 605)	278 (-662, 1218)	-459 (-1690, 772)	0.437	0.044
Whole-body BMD (g·cm^{-2})	0.012 (-0.005, 0.029)	0.008 (-0.012, 0.028)	0.004 (-0.023, 0.030)	0.757	0.007
Hip BMD (g·cm^{-2})	0.107 (-0.250, 0.036)	-0.007 (-0.178, 0.163)	0.100 (-0.123, 0.322)	0.353	0.062
Bone mineral content (g)	12.8 (-16.1, 41.6)	26.2 (-8.3, 60.6)	-13.4 (-58.4, 31.6)	0.533	0.028

Data are shown as mean (95% confidence interval [CI]). Abbreviations: η_p^2, partial eta squared; BMD, bone mineral density, BMD; VT, ventilatory threshold; PWC, peak work capacity; VO$_{2peak}$, peak oxygen uptake. Between-group analysis were performed with a one-way ANCOVA adjusting for baseline results. Non-adjusted results at baseline and post-intervention are available in **Supplementary File 2**.

Supplementary File 1. Pathogenic *PYGM* genotype indicative of McArdle disease identified in all the participants (N = 17).

Type of mutation	N
p.R50* (c.148C > T) / p.R50* (c.148C > T)	6
p.R50* (c.148C > T) / p.W798R (c.2392 T > C)	2
p.G205S (c.613G > A) / p.G205S (c.613G > A)	2
p.R50* (c.148C > T) / p.G205S (c.613G > A)	1
p.R50* (c.148C > T) / p.K754fs*49 (c.2262delA)	1
p.R50* (c.148C > T) / p.R602W (c.1804C > T)	1
p.R50* (c.148C > T) / p.A660D (c.1979C > A)	1
p.R50* (c.148C > T) / p.E383K (c.1147G > A)	1
p.G205S (c.613G > A) / c.1768 + 1G > A	1
p.R50* (c.148C > T) / p.A55Gfs*21 (c.163_167delGCTCT)	1

Supplementary File 2. Non-adjusted results.

Outcome	Exercise		Control	
	Baseline	**Postintervention**	**Baseline**	**Postintervention**
VT (W)	29.2 ± 8.9	37.8 ± 13.6	28.7 ± 10.2	26.6 ± 10.5
VT (W·kg^{-1})	0.45 ± 0.10	0.60 ± 0.21	0.44 ± 0.15	0.40 ± 0.15
VT (W·kg^{-1} lower-limb muscle mass)	2.01 ± 0.46	2.56 ± 0.69	1.84 ± 0.60	1.69 ± 0.65
VO$_{2peak}$ (ml·min^{-1})	1211 ± 454	1546 ± 432	1100 ± 225	1246 ± 311
VO$_{2peak}$ (ml·kg^{-1}·min^{-1})	18.6 ± 5.4	24.2 ± 5.5	18.1 ± 4.8	18.7 ± 3.9
VO$_{2peak}$ (ml·kg^{-1} lower-limb muscle mass)	81.5 ± 18.6	103.8 ± 16.1	72.5 ± 17.4	79.1 ± 17.9
PWC (W)	63.9 ± 18.9	76.1 ± 19.2	63.1 ± 13.7	58.1 ± 14.2
PWC (W·kg^{-1})	1.00 ± 0.21	1.21 ± 0.32	1.03 ± 0.32	0.90 ± 0.26
PWC (W·kg^{-1} lower-limb muscle mass)	4.36 ± 0.67	5.17 ± 0.87	4.17 ± 1.08	3.72 ± 0.96
Gross muscle efficiency (%)	12.2 ± 1.3	13.0 ± 1.6	12.7 ± 2.6	12.9 ± 2.7
Weight (kg)	65.4 ± 15.1	64.7 ± 13.9	65.0 ± 14.3	67.2 ± 13.0
Whole-body lean mass (kg)	42.08 ± 9.61	42.85 ± 8.13	43.53 ± 7.35	45.40 ± 6.07
Head lean mass (kg)	3.19 ± 0.38	3.22 ± 0.34	3.22 ± 0.24	3.32 ± 0.22
Upper-body lean mass (kg)	3.96 ± 1.20	4.04 ± 1.18	4.05 ± 1.03	4.14 ± 0.89
Lower-body lean mass (kg)	14.75 ± 3.96	14.89 ± 3.23	15.47 ± 2.58	15.78 ± 1.86
Trunk lean mass (kg)	20.18 ± 4.39	20.86 ± 4.02	20.80 ± 3.87	22.16 ± 3.59
Whole-body fat mass (kg)	20.40 ± 7.34	19.98 ± 7.55	18.67 ± 7.61	19.22 ± 7.32
Head fat mass (g)	830 ± 98	864 ± 129	838 ± 68	864 ± 59
Upper-body fat mass (kg)	2.27 ± 0.94	2.23 ± 1.00	1.91 ± 0.84	1.87 ± 0.82
Lower-body fat mass (kg)	7.18 ± 2.48	7.04 ± 2.46	7.02 ± 2.74	7.01 ± 2.81
Trunk fat mass (kg)	10.12 ± 4.18	9.93 ± 4.38	8.92 ± 4.59	9.21 ± 4.45
Whole-body BMD (g·cm^{-2})	1.04 ± 0.12	1.05 ± 0.11	1.02 ± 0.13	1.03 ± 0.13
Hip BMD (g·cm^{-2})	0.79 ± 0.17	0.76 ± 0.15	0.78 ± 0.09	0.77 ± 0.09
Bone mineral content (g)	2023 ± 433	2038 ± 399	2049 ± 458	2074 ± 420

Data are shown as Mean $\pm$ SD. Abbreviations: PWC, peak work capacity; VT, ventilatory threshold; VO$_{2peak}$, peak oxygen uptake.