

TRAINING IMPROVES OXIDATIVE CAPACITY, BUT NOT FUNCTION, IN SPINAL MUSCULAR ATROPHY TYPE III

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ABSTRACT: *Introduction:* In this study we investigated the effect of 12 weeks of cycle ergometer training in patients with spinal muscular atrophy type III (SMA III), a hereditary motor neuron disease with progressive muscle weakness and atrophy. *Methods:* Six SMA III patients and 9 healthy subjects completed a 12-week training program, performing 42 30-minute sessions exercising at 65–70% of maximal oxygen uptake (VO_{2max}). VO_{2max} , muscle strength, functional tests, and self-reported activities of daily living were assessed before and after the training. *Results:* Training induced a $27 \pm 3\%$ increase in VO_{2max} (17 ± 2 to 21 ± 2 ml/kg/min, $P < 0.001$) in patients. However, fatigue was a major complaint and caused 1 patient to drop out, increased the need for sleep in 3 patients, and led to training modifications in 2 patients. *Conclusions:* Cycle exercise improves VO_{2max} in SMA III without causing muscle damage, but it also induces significant fatigue. This warrants study into alternative training methods to improve exercise capacity in SMA III patients.

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Spinal muscular atrophy (SMA) is a motor neuron disease caused by autosomal recessively inherited mutations in the survival motor neuron gene (*SMN1*). Mutations in *SMN1* cause a loss of anterior horn cells in the spinal cord and lead to denervation of skeletal muscles and progressive muscle weakness and atrophy.

SMA type III (SMA III) is the mildest subtype of SMA. It is defined by onset of symptoms after age 18 months with patients retaining the ability to walk.¹

No effective treatment is available for SMA. We have previously shown that patients with muscular dystrophies improve oxidative capacity (VO_{2max}) and muscle strength by aerobic conditioning.^{2–4} Patients with SMA III share many clinical features with these conditions, although the mechanism of muscle weakness is different. In this study, we investigated how patients with SMA III respond to aerobic training.

Abbreviations: 5STST, 5 times sit-to-stand test; 6MWT, 6-minute walk test; 6SST, timed 6-step stair test; ADL, activities of daily living; ALS, amyotrophic lateral sclerosis; CK, creatine kinase; HR, heart rate; SMA III, spinal muscular atrophy type III; *SMN1*, survival motor neuron gene; TUG, timed up-and-go test VO_{2max} , maximal oxidative capacity; W_{max} , maximal workload capacity

Key words: exercise; fatigue; motor neuron disease; neuromuscular disorders; SMA III; spinal muscular atrophy type III; training

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METHODS

Subjects. Eight adult unrelated SMA III patients, with deletions of exon 7 in *SMN1*, were included (see demographics data in Table 1). Nine healthy, age- and gender-matched control subjects were included.

All control subjects lived sedentary lives. They exercised <60 minutes per week and commuted by bike <5 km/day for at least 6 months before inclusion.

Patients had the ability to walk ≥ 10 m with or without assistive devices. Two patients (patients 3 and 5) exercised regularly under the supervision of a physiotherapist for 30–60 minutes every week and every other week, respectively. None of the other patients exercised on a regular basis. Exclusion criteria were: (1) other serious medical conditions that could confound the interpretation of results; and (2) regular exercise of >1 hour weekly.

Ethics and Access. The Committee on Biomedical Research Ethics of the Capital Region of Denmark approved the study (Project ID H-KF297836). All subjects gave written consent to participate.

The study was conducted in accordance with the principles of the Helsinki Declaration.

The trial and study results have been registered in the trial database, which is available at www.clinicaltrials.gov (NCT02003937).

Incremental Exercise Test. After an overnight fast, the subjects performed an incremental exercise test to exhaustion on a cycle ergometer (Model 939E; Monark, Sweden) to determine maximal workload capacity (W_{max}) and maximal oxidative capacity (VO_{2max}). Pulmonary gas exchange was measured continuously with breath-by-breath indirect calorimetry (Cosmed Quark b²; Cosmed Srl, Italy).

Training Program. Subjects trained on a cycle ergometer for 12 weeks at home, performing 30-minute training sessions at their target heart rate (HR) throughout this period. The number of sessions was gradually increased from 2 to 4 times weekly, aiming to reach a total number of 42 sessions in 12 weeks.

The target HR corresponded to an oxygen uptake of 60–75% of VO_{2max} . All subjects monitored and recorded their HR during cycling using

Table 1. Secondary outcome measures before and after 12 weeks of cycle ergometer exercise in 6 patients with SMA III completing the study, 2 dropout patients, and 9 healthy controls.

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patients	Controls	D1	D2
		(W, 21 y)	(W, 19 y)	(W, 31 y)	(M, 19 y)	(M, 47 y)	(W, 58 y)	(mean ± SE)	(mean ± SE) (W: 4, M: 5; 21–52)	(W, 45 y)	(M, 43 y)
W_{max} (W)	Before	65	35	5	10	25	50	32 ± 10	178 ± 24	10	0
	After	90	35	5	20	25	70	41 ± 13	237 ± 21*	—	—
6MWT (m)	Before	527	387	180	90	375	424	330 ± 67	584 ± 37	376	306
	After	464	434	191	80	399	470	340 ± 67	630 ± 37	—	—
6MWT–RPE (Borg scale)	Before	9	10	15	19	10	6	12 ± 2	9 ± 1	15	13
	After	15	11	15	18	6	11	13 ± 2	11 ± 1	—	—
6MWT–dyspnea (Borg scale)	Before	3	2	4	7	1	8	4 ± 1	3 ± 1	3	1
	After	4	2	5	4	1	1	3 ± 1	3 ± 1	—	—
6SST (s)	Before	8	11	45	262	18	14	60 ± 41	7 ± 0	38	NA
	After	10	9	40	420	17	13	85 ± 67	6 ± 0	—	—
TUG (s)	Before	10	11	21	35	16	10	17 ± 4	8 ± 1	21	14
	After	11	9	20	34	13	9	16 ± 4	7 ± 0	—	—
5STST (s)	Before	29	20	64	67	40	23	41 ± 8	9 ± 1	51	23
	After	20	18	86	96	41	18	46 ± 15	8 ± 1	—	—
Body fat %	Before	46	55	44	35	51	46	46 ± 3	32 ± 4	53	56
	After	50	55	43	33	51	44	46 ± 3	29 ± 4	—	—
BMI (kg/m ²)	Before	24	25	16	17	24	24	22 ± 2	24 ± 1	25	33
	After	25	26	16	17	23	23	22 ± 2	24 ± 1	—	—
Gastrocnemius force (Newtons)	Before	276	244	140	103	204	259	204 ± 28	330 ± 30	207	279
	After	234	181	212	234	268	364	249 ± 26	393 ± 23*	—	—
Quadriceps force (Newtons)	Before	107	38	14	21	8	26	36 ± 15	341 ± 31	17	5
	After	52	47	10	19	27	19	29 ± 7	381 ± 26	—	—

W, woman; M, man; y, years of age; D1 and D2, dropout patients 1 and 2; W_{max} , maximum workload; 6MWT, 6-minute walk test; 6SST, 6-step stair test; TUG, timed up-and-go test; 5STST, 5 times sit-to-stand test; BMI, body mass index. 6MWT–RPE: rate of perceived exertion evaluated on a Borg scale (6 = lowest and 20 = highest) at the end of the 6MWT; 6MWT–dyspnea: subjective feeling of dyspnea evaluated on a Borg scale (0 = lowest and 10 = highest) at the end of the 6MWT; NA, not applicable, the subject was unable to perform the test.

*Difference vs. before training ($P < 0.05$).

a Polar Pulse watch. Patients were instructed to perform 3–5 minutes of warm-up, gradually increasing the workload until the target HR was reached ahead of each training session.

Compliance was monitored by: (1) calling or e-mailing subjects weekly; (2) subjects keeping a training diary; and (3) downloading exercise duration and HR from pulse watches from each training session (Polar Pro Trainer software, version 5).

Primary Outcome Measures. Primary outcome measures were VO_{2max} and activities of daily living (ADL). Subjects performed an incremental exercise test before and after the training period to determine VO_{2max} . Changes in ADL were assessed by a modified standardized questionnaire at the end of the training period in which patients rated indicators of physical function as being either worse, unchanged, or improved after training (Fig. 1).⁵

Secondary Outcome Measures. Secondary outcome measures were: changes in W_{max} , isometric leg muscle strength, and body composition; and performance on functional tests, after vs. before training.

Isometric Muscle Strength Testing. Isometric muscle strength was measured in 4 muscle groups (deltoid, biceps, gastrocnemius, and quadriceps) with a hand-held dynamometer (Citec, C.I.T. Technics). The better of 2 values was registered. The biceps and deltoid strengths were used as test controls.

Functional Tests. In a 6-minute walk test (6MWT), the subject walked as far as possible in 6 minutes with permission to rest during the test. The test was conducted on a flat, straight corridor with turnaround points at 30 m. All subjects wore shoes. One subject used a cane, and 1 used crutches. During the test, the examiner offered no verbal encouragement. The rates of perceived exertion (Borg scale, score range 6–20) and degree of dyspnea (Borg scale, score range 0–10) were recorded after the test.

A timed 6-step stair test (6SST) measured the time taken to climb up and down 6 stairs (height 18 cm, depth 30 cm). The subjects wore shoes, walked the stairs in their habitual manner, and could use the banisters at 1 side at a time.

The timed up-and-go test (TUG) measured the time taken to rise from a chair (45 cm high), walk 3 m in bare feet, turn around, return to the chair, and sit again. The pace was self-selected.

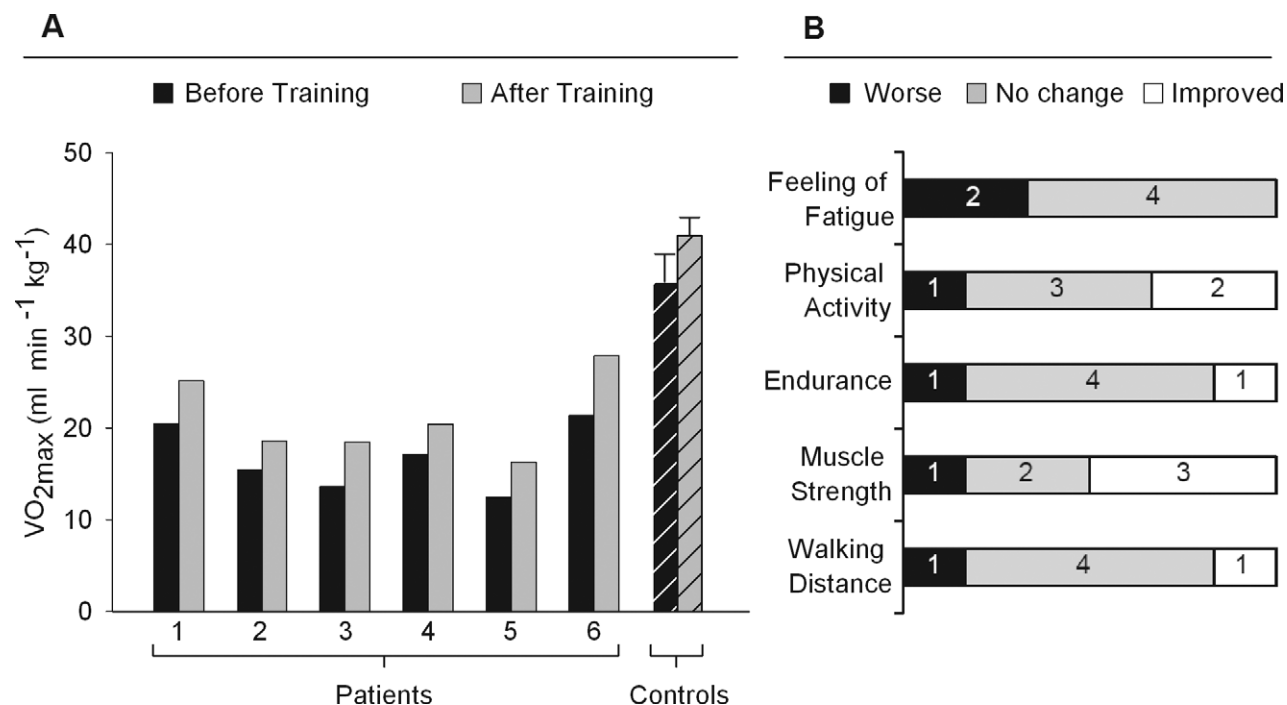


FIGURE 1. Primary outcome measures. **(A)** Maximal oxygen uptake (VO_{2max}) before and after 12 weeks of cycle ergometer training in 6 patients with SMA III and the average of 9 healthy controls (hatched bars, mean \pm SE). **(B)** Changes in activities of daily living (ADL) reported in an ADL questionnaire after 12 weeks of cycle ergometer training in 6 patients with SMA III.

In the *5 times sit-to-stand test* (5STST) the subject arose from a chair (45 cm high) as rapidly as possible 5 times in a row. Subjects were instructed to attempt to avoid using the arm-rests, but 5 had to use their arms to push themselves off the seat. Subjects performed the test in bare feet.

Training-induced alterations in body composition were measured by dual X-ray absorptiometry (Lunar; GE Medical Systems).

Safety Endpoints. Blood was drawn 3 times during the training period to assess plasma creatine kinase (CK) as a measure of muscle damage.

Statistical Analysis. Values are expressed as mean \pm standard error (SE). Changes within groups were analyzed using a Student *t*-test for paired data and between groups by a 2-sample Student *t*-test, assuming equal variance. $P < 0.05$ (2-tailed test) was considered significant.

RESULTS

Adherence to Training. Healthy controls completed 39 ± 1 sessions in 91 ± 4 days. Patients 1–4 completed 33 ± 4 sessions in 90 ± 5 days. The training program had to be modified in patients 5 and 6. Patient 5 needed 1-min breaks every 10 min during exercise due to fatigue. The training period was prolonged to obtain an effect comparable to other subjects. Patient 6 had 2 falls and dealt with a family death, which interrupted the training program. After these incidents, patient 6

completed 6 weeks of training 3 or 4 times weekly before performing the final tests. Patient 5 completed 62 sessions in 168 days, and patient 6 completed 50 sessions in 198 days.

Two patients, D1 and D2, dropped out. D1 dropped out after 4 weeks of training, because she felt excessively fatigued. D2 needed assistance to mount the bike, which he could not get frequently enough for training sessions. The reported results are based on the remaining 6 patients.

Safety Endpoints. Adverse Effects of Training. Patient 5 fell twice during training, which was considered unrelated to training (because he fell as frequently before the experiment). Patient 6 fell twice during the training period, and patient 4 had pain in the right hip after 6 weeks of training, which improved after 2 weeks. Both events were considered related to training.

All subjects found training 4 times weekly to be very strenuous. Patients 3, 4, and 6 reported the need for more sleep during the training period.

Plasma CK Levels. There were no training-induced changes in plasma CK levels in any of the patients. Mean values were 210 ± 45 U/L before, 215 ± 56 U/L after 4 weeks, and 214 ± 36 U/L at 12 weeks.

Primary Outcome Measures. VO_{2max} and ADL. Training improved VO_{2max} in the patients by $27 \pm 3\%$ ($P < 0.001$) and by $15 \pm 4\%$ in healthy controls ($P = 0.005$) (Fig. 1). Less compliant subjects had

the same magnitude of improvement as fully compliant subjects.

In the ADL questionnaire, 3 patients reported improved muscle strength, and 2 reported improved level of physical activity. However, all subjects reported either no change or an increased feeling of fatigue. On the remaining parameters there were no tendencies in ADL (Fig. 1). There was no correlation between muscle strength or exercise performance and reported changes in ADL.

Secondary Outcome Measures. W_{max} . W_{max} was unchanged in half of the patients, increased in the other half, and increased in the healthy subjects by $41 \pm 10\%$ ($P < 0.001$).

Isometric Muscle Strength. Among the patients, the average pretraining force generated in the quadriceps was 11% of the force generated by the controls ($P < 0.001$). There were no training-induced changes in muscle strength in the patients. The healthy controls improved strength in the gastrocnemius muscle by $23 \pm 7\%$ ($P = 0.007$).

Functional Tests. Training caused no significant change in total walking distance or self-reported dyspnea and fatigue in any subjects in the 6MWT. There were no training-induced changes in performance of the 6SST, TUG, and 5TST in any subject.

Body Composition. Training induced no changes in total fat or fat-free body mass in any subject. The body fat percentage was higher ($P = 0.03$) in the patients compared with healthy participants, whereas the body mass indices were comparable in the 2 groups.

Dropout Patients. One dropout patient, D2, had very little thigh muscle strength. He generated 5 W force with his quadriceps; he initially exercised without resistance on the ergometer but reported exercising with 15-W resistance at the time of dropout. The other dropout, D1, had leg muscle strength comparable that of several of the patients who completed the training program but did not increase resistance on the ergometer (Table 1). D2 was unable to climb stairs and perform the 6SST. Otherwise, the dropout patients did not perform differently from the other patients on the functional tests (Table 1) or the incremental exercise tests (VO_{2max} : D1, 10.4 ml/kg/min; D2, 18.2 ml/kg/min).

DISCUSSION

The principal new finding of this study is that 12 weeks of training can significantly improve VO_{2max} in patients with SMA III, but also induces fatigue and has no obvious beneficial effects on

physical functioning. These results are very much in contrast to findings that training equally weak muscular dystrophy patients did not produce the same exercise-induced fatigue.²⁻⁴ The data suggest that the mechanism of training-induced fatigue in SMA III may relate to the different underlying disease mechanisms in dystrophies and motor neuron diseases, while also suggesting that other modes of exercise should be investigated in SMA III to circumvent fatigue.

A possible mechanism of exercise-induced fatigue in SMA III could relate to the enlarged motor units resulting from degeneration of the motor neurons with subsequent collateral reinnervation.⁶ In line with this, enlarged motor units in patients with amyotrophic lateral sclerosis (ALS) have been shown to be mechanically less efficient and show increased fatigability.⁷ The only other study addressing aerobic conditioning in those affected by a loss of spinal cord motor neurons was an investigation of Kennedy disease. That study also demonstrated excessive fatigue in patients to the extent that there were no beneficial effects of training, even regarding oxidative capacity.⁸ Studies in SMN-deficient mice suggested that damage to mitochondria and depleted adenosine triphosphate production play a major role in the loss of motor neuron cells in SMA III, which may also affect the physiological response to exercise and post-exercise fatigue in these patients.⁹

Should exercise then not be recommended in motor neuron diseases? Our study has clearly shown a marked physiological improvement with training and, based on CK levels, there was no muscle damage. Physical activity prevents the development of risk factors for chronic diseases and reduces mortality and morbidity in SMA III patients as in others.^{10,11} Furthermore, SMA mouse models have shown a protective effect of exercise training on spinal cord motor neurons.^{12,13}

To circumvent the development of pronounced fatigue in SMA III patients, other potential training methods could involve strength-oriented exercise or less frequent or shorter bouts of high-intensity training with breaks, allowing time for physiological adaptation. Also, a gradual increase in intensity or exercise time over the course of the study could perhaps reduce muscle fatigue. Supervised training sessions would likely increase adherence to training, and training on a recumbent ergometer may offer better support for weaker SMA III patients who may fear falling from an upright ergometer.

Collectively there is evidence to suggest a role for exercise in SMA III, but alternative training regimes should be investigated among the approaches to avoid fatigue.

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