Research Article

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Effects of Exercise Intensity in Experimental Autoimmune Encephalomyelitis

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Abstract

Background: Research on muscle contractile properties and disease progression following experimental autoimmune encephalomyelitis (EAE) and physical exercise remains conflicting.

Objective: To investigate the effect of different exercise intensities on muscle contractile properties and hindquarter paralysis during EAE in Lewis rats.

Methods: A control and EAE group were divided in sedentary, light, moderate and high intensity running subgroups. During EAE course, hind limb paralysis, body weight and food intake were registered. Following EAE recovery isokinetic foot extensor strength was measured during 115 maximal contractions and fiber characteristics of m. tibialis anterior (TA) and m. extensor digitorum longus (EDL) were analysed.

Results: EAE reduced CSA of type IIb+x fibers of TA and EDL, while type I and IIa fibers CSA were not affected by EAE. Exercise did not change CSA of type I, IIa and IIb+x fibers of EDL nor TA, except for TA type IIa fibers CSA, which increased in EAE moderate and EAE high intensity groups. Muscle work peak was absent in all EAE animals during isokinetic muscle contractions. Intense exercise delayed onset of hindquarter paralysis in EAE, while disease peak and remission were not affected by exercise.

Conclusion: This study suggests that EAE reduces CSA of type IIb+x fibers of TA and EDL. This possibly explains the absence of peak muscle work during the first of a series of isokinetic muscle contractions. Furthermore, exercise was not able to reduce muscle fiber atrophy, whereas high intensity exercise delayed onset of hindquarter paralysis.

Keywords EAE; Rehabilitation; Muscle strength; Muscle contractile characteristics; Treadmill running

Abbreviations

CON: Healthy Control Group; CSA: Cross Sectional Area; EAE: Experimental Autoimmune Encephalomyelitis; EDL: Extensor Digitorum Longus; MS: Multiple Sclerosis; SED: Sedentary Group; TA: Tibialis Anterior; TR[H]: Treadmill High Intensity Group; TR[L]: Treadmill Light Intensity Group; TR[M]: Treadmill Moderate Intensity Group; TR[SED]: Treadmill Sedentary Group

Introduction

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease model of the central nervous system (CNS) often used to investigate demyelination in the CNS in general, and Multiple Sclerosis (MS) in particular [1]. So far, research investigating the impact of EAE and/or physical exercise during EAE on muscle contractile properties and EAE progression is scarce, conflicting but also promising [2-5]. De Haan and co-workers explored the impact of EAE on muscle contractile properties. Compared to healthy controls, EAE rats were not only less physically active, they also showed significant loss of body weight and gastrocnemius muscle mass (-21 and 33% respectively), reduced muscle fiber cross sectional area (CSA) of all fiber types (-40 to 50%), as well as lower maximal muscle force and power (-58 and 73% respectively) [2]. Furthermore, Le Page and co-workers demonstrated that treadmill running after immunization, delayed the onset and duration of hindquarter paralysis, associated with chronic EAE. However, maximal clinical scores were not affected and these investigators did not examine changes in muscle CSA [4]. More recently, in mice with chronic EAE less severe neurological deficits and a less pronounced spinal loss in the striated neurons after voluntary, not structured, wheel running have been reported [5].

In MS, muscle contractile property research is conflicting [6-10] and only the impact of moderate progressive strength training on it has been investigated [11]. Interestingly, several authors [12-15] suggested that MS patients could further benefit from higher intensity exercise, but it is unclear whether this could be tolerated. To further investigate the above described issues the use of an animal MS model seems appropriate [16].

In accordance with the above line of reasoning, the current study aimed to investigate the impact of low to high intensity exercise on muscle contractile properties and disease progression of healthy and

acute EAE rats. It is hypothesized that exercise improves muscle contractile properties and delays the onset of hindquarter paralysis.

Materials and Methods

Animals

In total 64 female Lewis rats (age 6-7 weeks, body weight 120-170 g, Harlan CPB, Zeist, The Netherlands) were maintained on a constant light:dark cycle (12:12), a temperature of 22°C and a relative humidity of 22-24%, in the animal facilities of Hasselt University. Rats were fed ad libitum with normal rat pellets (Carfil RN-01-K12, Harlan). The animal Ethics Committee of Hasselt University approved the study protocol in accordance with the national and European legislation. Furthermore, the National Research Council's guide for the care and use of laboratory animals was followed.

Study design

Following acclimatization and adaptation rats were enrolled in a treadmill running training program (TR, n=64, low to high intensity training) and subdivided in a sedentary (TR^{SED}), light (TR^L), moderate (TR^M) and high intensity (TR^H) running group (Figure 1). During habituation animals were familiarized to treadmill running (day -14 to -1) at progressively increased training durations and intensities. After habituation TR^L rats were able to walk 1 hour at 5m/min (0° inclination). TR^M and TR^H rats ran 1 hour at respectively 11m/min (15° inclination) and 18m/min (25° inclination). Although not measured, running intensity for TR^H rats was probably just below the anaerobic threshold [17,18]. Animals were encouraged to walk/run by means of low intensity electrical shocks. Shocks, if required, lasted <1s and usually occurred, maximally, a few times per training session. TR^{SED} animals did not receive any electrical shocks; however they were subjected to similar daily handling. From the habituation period onwards, daily food intake and body weight were registered. Following habituation (day 0), all sedentary and training groups were divided in a healthy control (CON) and EAE group (EAE induction). Hereafter TR^L, TR^M and TR^H rats were subjected to daily (1 hour/day) physical exercise, until progressive hindquarter paralysis (~day 11) prevented this. However, if an animal developed hindquarter paralysis before day 11, preventing daily exercise, the exercise program was immediately terminated, in accordance with the designated endpoint of exercise, whereafter the animal was excluded and humanely euthanized. After (partial) recovery (day 17) animals were anaesthetized using an intraperitoneal injection of pentobarbital sodium (5 mg.100 g^{-1} BW). Following determination of repetitive isokinetic foot extensor performance of the left hind limb, m. extensor digitorumlongus (EDL) and m. tibialis anterior (TA) of the right hind limb were dissected and freed from connective tissue and visible blood. Hereafter, the mid-part of each muscle was mounted in embedding tissue (Tissue-Tek® OCTTM Compound, Miles Laboratories, Inc., Elkhart, Indiana, USA), frozen in isopentane (Sigma-Aldrich, St. Louis, MO, USA), cooled in liquid N_2, and stored at -80°C until further analysis was performed. Finally, rats were sacrificed by an intracardial injection of pentobarbital sodium.
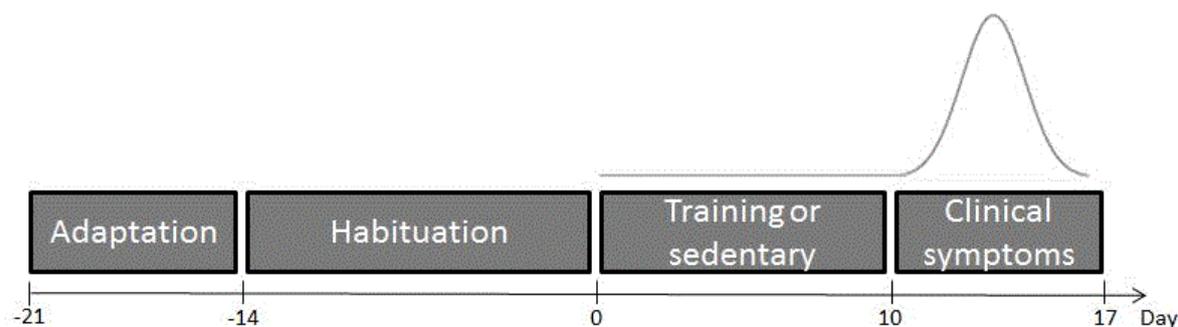

Figure 1: Study design. Following adaptation rats were familiarized to treadmill running during habituation. After EAE induction (day 0) TR^L, TR^M and TR^H rats were subjected to daily physical exercise, until progressive hindquarter paralysis prevented this (day 10). After (partial) recovery (day 17) isokinetic foot extensor performance was measured, where after muscle samples of EDL and TA were collected. Finally, rats were sacrificed.

EAE induction

EAE was induced in EAE subgroups by a single percutaneous injection in both footpads (100µl/foot) under isoflurane anesthesia and consisted, per animal, of 24µl purified myelin basic protein (MBP, 25mg/ml) in combination with 25µl 7RA heat-killed mycobacterium tuberculosis (20mg/ml, Difco), 120µl complete Freunds adjuvant (CFA, Difco) and 31µl phosphate-buffered saline (PBS) [19].

Primary outcome measures

Fiber CSA and distribution: To quantify type I and IIa muscle fiber CSA and distribution, serial transverse sections (8µm) from the obtained muscle samples were cut at -20°C and stained by means of triple-staining. Air-dried (30min) cryosections were washed (5min) with 0.5% Triton-100, added to phosphate-buffered saline (PBS) and then washed (5min) with PBS. Next, sections were incubated 60 minutes at room temperature with a mix of 2 mouse monoclonal antibodies against myosin heavy chain I (1:25; A4.840 supernatant, Developmental Studies Hybridoma Bank, Iowa, USA) and IIA (1:25; N2.261 supernatant, Developmental Studies Hybridoma Bank, Iowa,

USA) and 1 rabbit polyclonal laminin antibody (1:100; L-9393, Sigma, Zwijndrecht, The Netherlands). Then, slides were washed 3 times (5 min) with PBS, followed by an incubation period of 45 minutes at room temperature with a mixture of secondary antibodies (1:500, Goat anti-Mouse IgM AlexaFluor 555; 1:200 Goat anti-Mouse IgG1 AlexaFluor 488 and 1:130 Goat anti-Rabbit IgG AlexaFluor 350; Molecular probes, Invitrogen, Breda, The Netherlands) diluted in PBS. Hereafter, sections were washed 3 times (5 min) with PBS and mounted in Fluorescent Mounting Medium (Dako, North America, California, USA). Muscle fibers were examined and recorded using a Nikon Eclipse 90i fluorescence microscope (Nikon, Boerhavedorp, Germany). The fluorescence signals were recorded using a TRITC and FITC filter for type I and IIa muscle fibers, respectively, and DAPI filter for cell membrane. The regions that were not fluorescent, including type IIb and IIx fibers, were grouped together and called type IIb+x fibers. Digital images (x20 magnification, exposure time for TRITC and FITC 400ms, DAPI 800ms) were analysed using NIS Elements® BR 3.0 software (LIM, Prague, Czech Republic).

Hindquarter paralysis: After EAE induction all EAE rats were examined daily at 8.30 a.m. for the development of clinical symptoms. Typically, hindquarter paralysis developed 12 to 14 days after induction, where after rats partly recovered (day 17). Symptoms were blinded scored on a scale ranging from 0 to 5: 0, no signs; 0.5: partial loss of tail tonus (defined as the disease onset); 1.0: complete loss of tail tonus; 2.0: hind limb paresis; 3.0: hind limb paralysis; 4.0: moribund; 5.0: death due to EAE [19]. The clinical endpoints were based on the clinical scores. If an animal was not able to eat or drink independently, the animal was excluded and euthanized. Overall hindquarter paralysis was expressed as the average of the summated daily scores per group.

Secondary outcome measures

Isokinetic foot extensor strength: After general anesthesia at day 17, left hind limb foot extensor muscle strength was assessed during fatiguing isokinetic muscle contractions, as described elsewhere [20,21]. Briefly, percutaneous needle electrodes were placed on the common peroneal nerve fusing 115 consecutive concentric isokinetic foot extensions (50°/s, 1 mA, 250ms, 3s rest intervals) after standardized fixation of knee and ankle on a custom build Ashton-Miller like rat dynamometer [22]. Work fatigue was expressed as a percentage, compared to the highest work, which was performed during the first 30 consecutive contractions and set at 100%.

Body weight & food intake: Daily body weight and food intake was registered using an automatic/ digital balance (Sartorius®, Goettingen, Germany) at 8 a.m.

Statistical analyses

All data were analysed using SAS software (SAS Institute Inc, Cary, USA). First normality was checked using the Shapiro-Wilk test for all variables. Body weight and food intake were analysed by a (Group [CON; EAE] x Activity [TR^{SED}; TR^L; TR^M; TR^H] x study phase [habituation phase; induction phase; exercise phase; paralysis phase]) mixed model ANOVA. Muscle fatigue of isokinetic foot extensor data was analysed using a (Group [CON; EAE] x Activity [TR^{SED}; TR^L; TR^M; TR^H] x Contraction [number of dynamic muscle contractions]) mixed model ANOVA. Muscle fiber type area and distribution were analysed using a 2x4 (Group [CON; EAE] x Activity [TR^{SED}; TR^L; TR^M; TR^H]) mixed model ANOVA. Hindquarter paralysis was analysed using a (EAE Group [EAE-TR^{SED}; EAE-TR^L; EAE-TR^M; EAE-TR^H] x Time [Days 0-17]) mixed model ANOVA, with body weight as confounding factor. To analyse disease onset, which was defined as a hindquarter paralysis score equal to 0.5, a time to event analysis was used for all EAE groups. When appropriate, post hoc pre-planned contrast tests were applied. All data are presented as mean ± SE, the threshold for statistical significance was set at $p < 0.05$.

Results

Primary outcome measures

Muscle fiber cross sectional area and distribution: Figure 2 shows a representative image of muscle fiber types of CON and EAE. Type I and IIa muscle fiber CSA of both muscles in all groups were not affected by EAE.
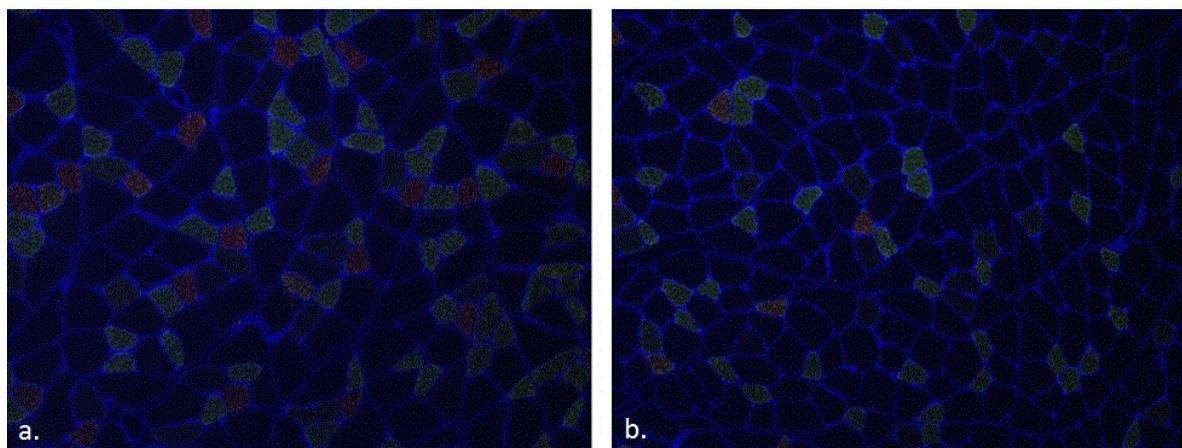

Figure 2: Representative image of fiber type analysis of CON (a) and EAE (b) animals. Fiber types are distinguished by colour (red: type I, green: type IIa, non-coloured regions: type b+x). Type I and IIa muscle fiber CSA were not affected by EAE, whereas type IIb+x fiber CSA was significantly reduced due to EAE. Fiber type distribution did not differ between CON and EAE.

Furthermore, type IIb+x fiber CSA of EDL and TA were significantly reduced, respectively ~22% and ~40%, due to EAE ($p<0.05$). Fiber type distribution in EDL and TA did not differ between CON and EAE (Table 1).

In CON and EAE exercise did not affect CSA of type IIb+x fibers of EDL nor TA, compared to corresponding TR^{SED}. Comparable results were detected, in all groups, for type I and IIa fibers, except for TA type IIa fiber CSA of EAE-TR^{M} and EAE-TR^{H} which increased, respectively, 16% and 23% ($p<0.05$), compared to EAE-TR^{SED}. Furthermore, exercise did not affect fiber distribution in EDL nor TA (Table 1).

		Type I					Type IIa					Type IIb+x				
		EDL														
	n	Area (µm²)		p	Distr (%)		p	Area (µm²)		p	Distr (%)		p	Area (µm²)		p	Distr (%)		p
CON-TR^{SED}	8	511 ± 46.6	0.64	5.7 ± 0.9	0.18	699 ± 55.7	0.65	26.1 ± 2.4	0.70	996 ± 59.2	0.04	68.1 ± 2.4	0.94
CON-TR^{L}	8	516 ± 42.7		5.0 ± 0.8		693 ± 44.3		26.9 ± 2.4		1137 ± 63.4		68.2 ± 2.8	
CON-TR^{M}	7	514 ± 46.9		6.4 ± 0.8		654 ± 39.7		20.0 ± 3.1		988 ± 57.5		73.7 ± 3.3	
CON-TR^{H}	8	559 ± 63.0		6.0 ± 0.8		658 ± 60.5		26.3 ± 2.9		1003 ± 62.7		67.8 ± 3.0	
EAE-TR^{SED}	8	552 ± 24.8		5.9 ± 0.9		680 ± 55.6		24.9 ± 2.0		772 ± 51.2*		69.2 ± 1.8	
EAE-TR^{L}	5	562 ± 95.0		7.2 ± 1.2		685 ± 24.4		23.6 ± 3.6		800 ± 109.8*		69.2 ± 3.9	
EAE-TR^{M}	7	624 ± 43.2		4.6 ± 0.5		735 ± 60.9		23.4 ± 3.6		832 ± 74.2		72.0 ± 3.6	
EAE-TR^{H}	6	539 ± 35.0		6.3 ± 1.4		619 ± 35.8		25.5 ± 3.2		767 ± 72.1*		69.2 ± 2.7	
		TA														
	n	Area (µm²)		p	Distr (%)		p	Area (µm²)		p	Distr (%)		p	Area (µm²)		p	Distr (%)		p
CON-TR^{SED}	7	682 ± 62.6	0.45	1.3 ± 0.3	0.15	797 ± 40.1	0.08	16.8 ± 2.9	0.88	1384 ± 84.2	0.03	81.9 ± 2.9	0.87
CON-TR^{L}	7	724 ± 29.8		1.3 ± 0.2		723 ± 36.1		15.5 ± 1.4		1198 ± 37.2		83.2 ± 1.4	
CON-TR^{M}	8	875 ± 40.8		3.2 ± 1.2		774 ± 39.3		15.3 ± 1.7		1267 ± 35.4		81.5 ± 1.5	
CON-TR^{H}	8	746 ± 43.5		2.0 ± 0.3		774 ± 22.1		19.5 ± 2.4		1244 ± 64.8		78.5 ± 2.5	
EAE-TR^{SED}	8	807 ± 56.8		2.6 ± 1.0		751 ± 41.2		18.2 ± 2.9		762 ± 47.4*		79.2 ± 3.1	
EAE-TR^{L}	6	740 ± 89.4		1.7 ± 0.5		778 ± 37.3		18.7 ± 2.8		786 ± 73.2*		79.6 ± 2.6	
EAE-TR^{M}	7	804 ± 95.2		1.4 ± 0.3		872 ± 42.3†		18.9 ± 2.4		786 ± 78.9*		79.8 ± 2.4	
EAE-TR^{H}	6	769 ± 54.5		1.6 ± 0.2		922 ± 46.3*†		19.5 ± 2.7		818 ± 72.4*		78.9 ± 2.7	

Values are means ± SE and express muscle fiber cross sectional area (µm²) and fiber type composition (Distr %) in Extensor Digitorumlongus (EDL) and Tibialis Anterior (TA) of sedentary (TR^{SED}), low- (TR^{L}), moderate- (TR^{M}) and High- (TR^{H}) training intensity healthy (CON) and experimental autoimmune encephalomyelitis (EAE) rats. p-values represent group (CON, EAE) x activity (TR^{SED}, TR^{L}, TR^{M}, TR^{H}) effects.

* $p<0.05$ compared to corresponding CON value. † $p<0.05$ compared to EAE-TR^{SED}

Table 1: Effect of EAE and treadmill exercises on CSA and muscle fiber composition of EDL and TA.

Hindquarter paralysis: Compared to the corresponding SED groups, disease onset, peak and remission did not differ between intensity groups, except for EAE-TR^{H} disease onset, which was significantly delayed (EAE-TR^{H}: day 11.6±0.3, EAE-TR^{SED}: day 11.0±0.1, $p<0.05$, Figure 3).

Secondary outcome measures

Isokinetic muscle strength: Group x contraction analysis, indicated that the muscle work curves of EAE and CON rats significantly differed (Figure 4). More particular, for the treadmill groups, muscle work of CON peaked and then declined (-36±1%) during the first 30 contractions while in EAE muscle work remained stable (-5±0.2%).

Body weight and food intake: EAE decreased body weight and food intake (data not shown) by, respectively, ~10% and ~57%, immediately after EAE induction ($p<0.05$). Hereafter, body weight and food intake gradually recovered until the onset of hindquarter paralysis at day 11. Then, body weight and food intake of EAE groups decreased ($p<0.05$), on average, by ~19% and ~69% respectively. Furthermore, exercise was able to reduce body weight loss and food intake drop during the paralysis phase. Here, an exercise effect ($p<0.05$) between EAE-TR^{H} and EAE-TR^{SED} on body weight and food intake was detected (Figure 3).
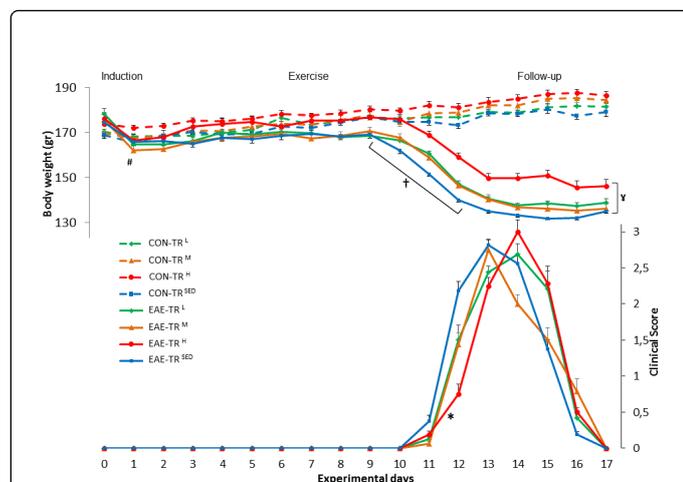
Figure 3: Effect of experimental autoimmune encephalomyelitis and different exercise intensities on body weight and hindquarter paralysis. Body weight immediately decreased after EAE induction (#), where after it gradually recovered until the onset of hindquarter paralysis at day 11. Then, body weight of EAE groups decreased (†). Exercise was able to reduce body weight loss during the paralysis phase. Here, an exercise effect between EAE-TR^H and EAE-TR^{SED} was detected (γ). The level of hindquarter paralysis is presented on a scale from 0 to 5 in sedentary and exercised EAE animals. Disease onset (score 0.5) of EAE-TR^H was significantly delayed, compared to EAE-TR^{SED} (*). Values are means ± SE.
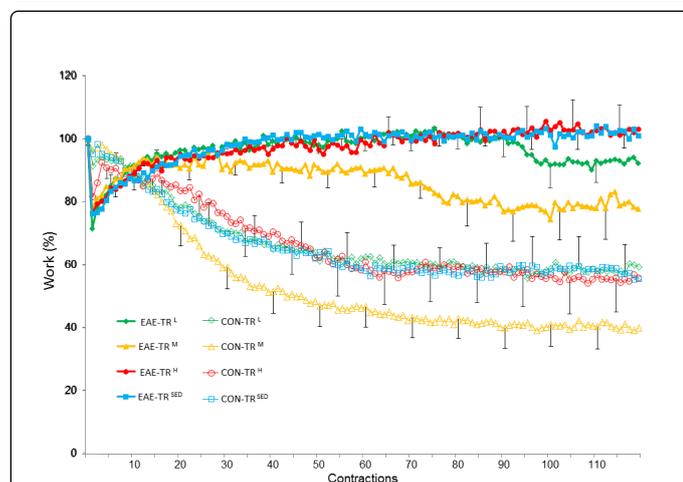

Figure 4: Effect of different treadmill exercise intensities on isokinetic muscle work in healthy and EAE rats. Values are means ± SE and express muscle work (%) during 115 consecutive maximal muscle contractions (1mA, 150Hz, 250ms) in healthy (CON) rats and EAE rats, subdivided in different exercise intensity groups (TR^{SED}: sedentary; TR^L: low; TR^M: moderate; TR^H: high intense running exercise).

Drop out

In total, five EAE animals died (EAE-TR^L n=2; EAE-TR^M n=1; EAE-TR^H n=2) during the study course with no significant differences in survival rate between groups.

Discussion

The current study was the first to investigate the impact of low to high intensity endurance training on muscle contractile characteristics and hindquarter paralysis during/following acute EAE. EAE reduced CSA of type IIb+x muscle fibers in TA and EDL, which probably explains the absence of peak force during a series of 115 isokinetic muscle contractions in EAE [23,24]. Under the conditions of the present study, treadmill exercise was not able to reduce type IIb+x muscle fiber atrophy. Finally, and with the exception of high intensity running exercise that was able to delay paralysis onset, peak and remission of hindquarter paralysis did not change after low and moderate intensity exercise.

Under the conditions of the present study the CSA of EDL and TA type IIb+x fibers in EAE animals was decreased. Muscle fiber distribution was not affected by EAE. De Haan and co-workers previously investigated the impact of EAE on the medial gastrocnemius, a muscle comprising ±80% type I fibers, and also demonstrated no muscle fiber shifts but reduced CSA of all fiber types [2]. In rats it is documented that running exercise can reverse muscle fiber atrophy [25-27]. In the present study high intensity running exercise tended to reduce muscle fiber atrophy only in EAE-TR^H rats, suggesting an exercise effect. However, other exercise intensities were not able to reduce EAE-induced fiber atrophy in any fiber type of the other groups. Interestingly, in the healthy control rats exercise did not alter muscle fiber characteristics. This suggests that one week of inactivity during the paralysis period of EAE rats, which was also applied in the control rats, may have tempered training effects. Therefore, muscle sampling immediately after the training period and before the onset of hindquarter paralysis might give better insight into the effect of exercise on muscle contractile properties during EAE. Furthermore, it is also interesting to investigate the effect of resistance training on muscle fiber characteristics, since EAE is able to reduce the CSA of type IIb muscle fibers, that are more susceptible to strength training [28-30].

As depicted in Figure 4, and as previously reported by Wakatsuki et al. [31] in healthy rats, muscle work in CON subgroups peaked during the first 20-30 contractions and then progressively declined. This was also reported by De Haan et al. in control and EAE rodents during a series of repeated maximal isometric contractions of m. gastrocnemius [2]. In the EAE subgroups of the present study muscle work did not peak. This might be explained by the fact that different muscle groups were analysed. More specific, TA and EDL with predominately glycolytic muscle fibers [32] were used in the current study, versus medial gastrocnemius with a proximal region, containing all muscle fibers, and a distal region, containing only type IIx and IIbfibers were investigated in the study of De Haan and co-workers [2]. Because fast glycolytic type IIb muscle fibers largely contribute to peak force production [23;24] the decreased CSA of type IIb+x fibers probably explains the absence of peak muscle work in EAE during the first 30 repetitive isokinetic contractions in the present study.

Interestingly, high intensity treadmill running was able to delay the onset of EAE induced hindquarter paralysis in EAE-TR^H. This was paralleled by reduced body weight loss during the paralysis period.

Low and moderate intensity endurance training, however, did not change EAE disease course. Other authors also reported delayed onset of clinical EAE signs following voluntary wheel running in chronic EAE mice [5] and treadmill running in rats [3,4]. It is difficult to compare different studies due to application of different exercise intensities (lower versus higher intensities) or due to the use of voluntary exercise versus quantified training load and intensity. The training intensities used in the present study were probably higher compared to the work of Le Page and Rossi. In fact, in the present study EAE-TR^H and CON-TR^H exercise intensity is probably near the anaerobic threshold [17,18]. Therefore, the present findings and the above-described work of others suggest that exercise intensity determines the impact of training on the course of EAE and that the optimal endurance training intensity in EAE is probably near the anaerobic threshold. As such and given the fact that the effects of exercise therapy on a variety of functional parameters in MS is at present under investigation, it is worthwhile to investigate this hypothesis in MS patients.

The present study had some limitations, resulting in a few recommendations for future research. The absence of an explicit exercise effect could, possibly, be explained by the sedentary week after a relatively short training period. Therefore, it is suggested to collect muscle samples immediately after the training period and before the onset of hindquarter paralysis, to further investigate the influence of exercise on muscle contractile properties in EAE. Furthermore, this research and the work of others suggested that the optimal exercise intensity is near the anaerobic threshold. However, during the present study lactate concentrations and/or VO$_2$ kinetics were not measured. Therefore, it is recommended to quantify exercise intensity in future studies. Finally, since forced treadmill running can induce stress, which could influence the EAE symptoms, it is recommended to measure stress hormone levels in future research.

In conclusion, the present study showed that intense treadmill running delayed the onset of hindquarter paralysis in EAE. Low and moderate running exercise had no effect. Furthermore, EAE reduced rat CSA type IIb+x fibers of TA and EDL. This probably explains the absence of peak muscle work during the first 30 contractions of a series of isokinetic muscle performance in EAE.

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