

## **Physical exercise in Aging: Nine weeks of leg press or electrical stimulation training in 70 years old sedentary elderly people**

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### **Abstract**

Sarcopenia is the age-related loss of muscle mass and function, reducing force generation and mobility in the elderly. Contributing factors include a severe decrease in both myofiber size and number as well as a decrease in the number of motor neurons innervating muscle fibers (mainly of fast type) which is sometimes accompanied by reinnervation of surviving slow type motor neurons (motor unit remodeling). Reduced mobility and functional limitations characterizing aging can promote a more sedentary lifestyle for older individuals, leading to a vicious circle further worsening muscle performance and the patients' quality of life, predisposing them to an increased risk of disability, and mortality. Several longitudinal studies have shown that regular exercise may extend life expectancy and reduce morbidity in aging people. Based on these findings, the Interreg IVa project aimed to recruit sedentary seniors with a normal life style and to train them for 9 weeks with either leg press (LP) exercise or electrical stimulation (ES). Before and at the end of both training periods, all the subjects were submitted to mobility functional tests and muscle biopsies from the Vastus Lateralis muscles of both legs. No signs of muscle damage and/or of inflammation were observed in muscle biopsies after the training. Functional tests showed that both LP and ES induced improvements of force and mobility of the trained subjects. Morphometrical and immunofluorescent analyses performed on muscle biopsies showed that ES significantly increased the size of fast type muscle fibers ( $p < 0.001$ ), together with a significant increase in the number of Pax7 and NCAM positive satellite cells ( $p < 0.005$ ). A significant decrease of slow type fiber diameter was observed in both ES and LP trained subjects ( $p < 0.001$ ). Altogether these results demonstrate the effectiveness of physical exercise either voluntary (LP) or passive (ES) to improve the functional performances of aging muscles. Here ES is demonstrated to be a safe home-based method to counteract fast type fiber atrophy, typically associated with aging skeletal muscle.

**Key Words:** aging, physical exercise, electrical stimulation, leg press, skeletal muscle wasting  
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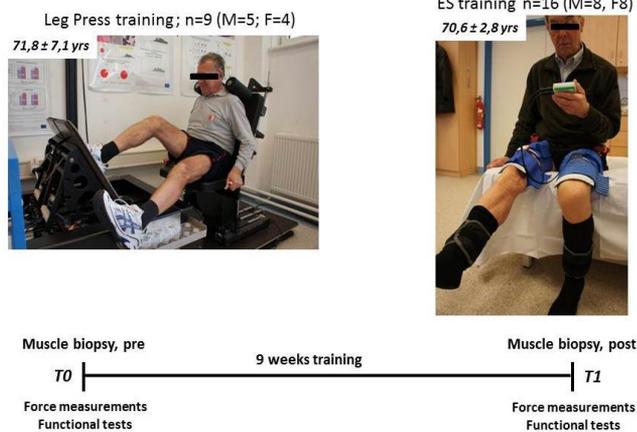
**I**n this study the efficacies of physical exercise and electrical stimulation to counteract skeletal muscle decline in seniors were compared. Skeletal muscle biopsies from 70 year old sedentary seniors were collected from *Vastus Lateralis* of both legs at the beginning and at the end of two different types of physical training: leg press (LP) as voluntary exercise or electrical stimulation (ES) as passive, home-based

exercise (Fig. 1).<sup>1-3</sup> In the LP group, nine subjects were recruited (muscle biopsies  $n=16$ ), while in the ES group sixteen elderly people were enrolled (muscle biopsies  $n=27$ ).

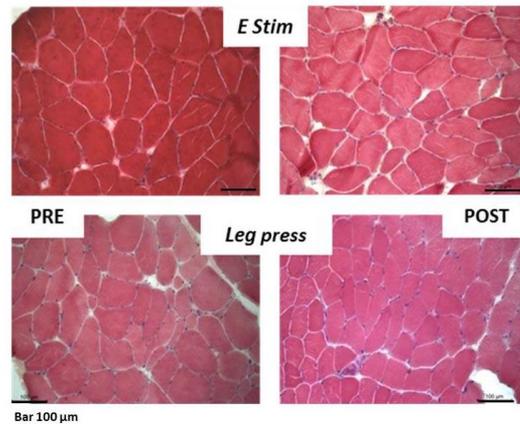
The LP program consisted of three training sessions a week, for a nine week period, using a computer controlled, linear motor-powered leg press machine.

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**Fig 1.** Interreg IVa “Mobility in Aging”. Study design. Leg press (left), Electrical stimulation (right)



**Fig 2.** Muscle structure and morphology before and after trainings: No myofiber damage and/or inflammatory cell response were observed

Functional and mechanical descriptions of the LP machine are described in details in the papers of Prof. Dusan Hamar and in that of Jan Cvečka, et al. included in this EJTM Special “Mobility in Elderly”.

The ES training was performed at home by the subjects themselves after receiving detailed instructions. The program consisted of three training sessions a week, for a period of nine weeks, using a stimulator device specifically designed for this project.<sup>2</sup> The current was delivered using two large rubber electrodes which

covered the entire quadriceps muscle (Fig. 1).

Together with muscle biopsies, force measurements and functional tests were performed in Vienna and Bratislava, at the beginning (T0) and at the end of the 9 week period of training (T1), as described in Zampieri et al. 2015.<sup>4</sup> From the functional point of view, positive improvements either in terms of muscle force, balance or mobility were observed at the end of the training in both groups, indicating that they were both effective (Table 1).

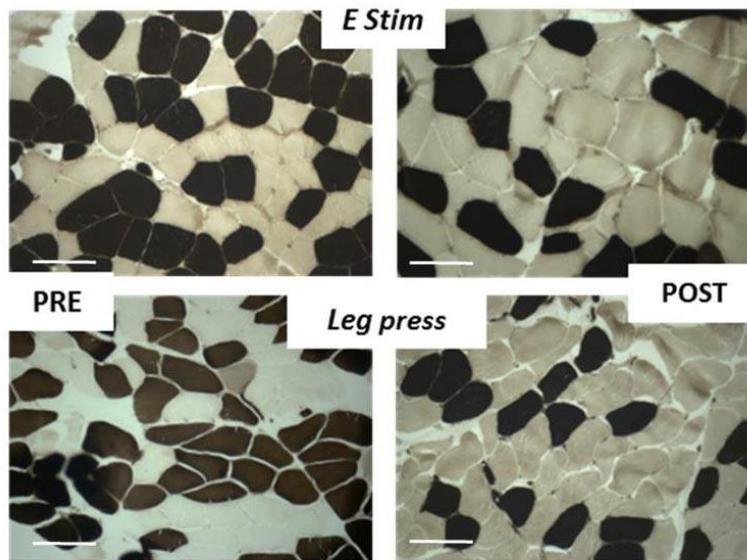
**Table 1.** E-Stim and LP trainings induced similar improvements in force and functional performances after 9 weeks

E-Stim	Before training	After training	t-test
Torque (Nm/kg)	1.42 ± 0.34	1.51 ± 0.38	p < 0.05
TUGT (s)	8.42 ± 1.95	7.04 ± 1.09	p < 0.0005
5x Chair Rise (s)	13.85 ± 3.33	10.53 ± 3.63	p < 0.005 <sup>§</sup>
SPPB Score	10.06 ± 1.39	11.19 ± 1.22	p < 0.005 <sup>§</sup>
10m Test habitual (m/s)	1.20 ± 0.19	1.26 ± 0.18	p < 0.05
10m Test fast (m/s)	1.58 ± 0.28	1.66 ± 0.24	p < 0.05
LP	Before training	After training	t-test
Torque (Nm/kg)	1.64 ± 0.44	1.73 ± 0.47	p = 0.231
TUGT (s)	6.16 ± 1.20	5.63 ± 0.58	p = 0.184
5x Chair Rise (s)	10.95 ± 1.75	9.54 ± 1.92	p < 0.05
SPPB Score	11.38 ± 0.74	11.88 ± 0.35	p = 0.157 <sup>§</sup>
10m Test habitual (m/s)	1.38 ± 0.19	1.43 ± 0.20	p = 0.123
10m Test fast (m/s)	1.90 ± 0.19	2.01 ± 0.23	p < 0.005

Values are given as mean ± SD; TUGT= timed up and go test; SPPB= short physical performance battery. § Wilcoxon-Test

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Slow type ATPase histochemistry (brown staining) , Bar 100  $\mu$ m

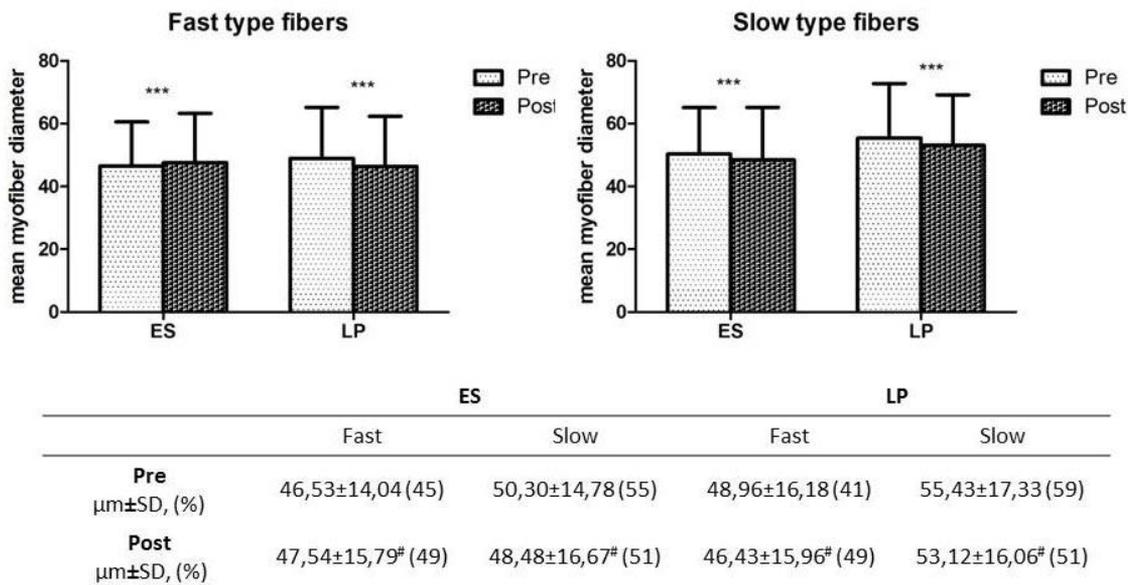
**Fig 3.** Distribution of fast and slow twitch fiber types by myofibrillar ATPase histochemistry. Slow muscle fibers are brown.

In the ES treated group, these improvements were all statistically significant, while in the LP trained group, even if positive changes were observed in all measurements, significant improvements were achieved only in the 5x chair rise and 10m walking test scores.

We analyzed muscle morphology before and after the training period by Hematoxylin and Eosin staining (Fig. 2) and myofibrillar ATPase histochemistry (Fig. 3) of sections obtained from elderly muscle biopsies.<sup>4,5</sup> As shown in the Figure 2, no myofiber damage, increased

fibrosis or inflammatory cell infiltration were detected in post training muscles either in the LP or ES treated groups, indicating that both trainings were safe and that the integrity of the senescent muscle was maintained.

We also calculated and compared the diameter of myofibers in pre- versus post-training biopsies. A mild decrease in the mean myofiber diameter was observed after LP training, while in the ES trained group the overall mean myofiber diameter remained unchanged after the training.<sup>4</sup> It is interesting to note that in the LP

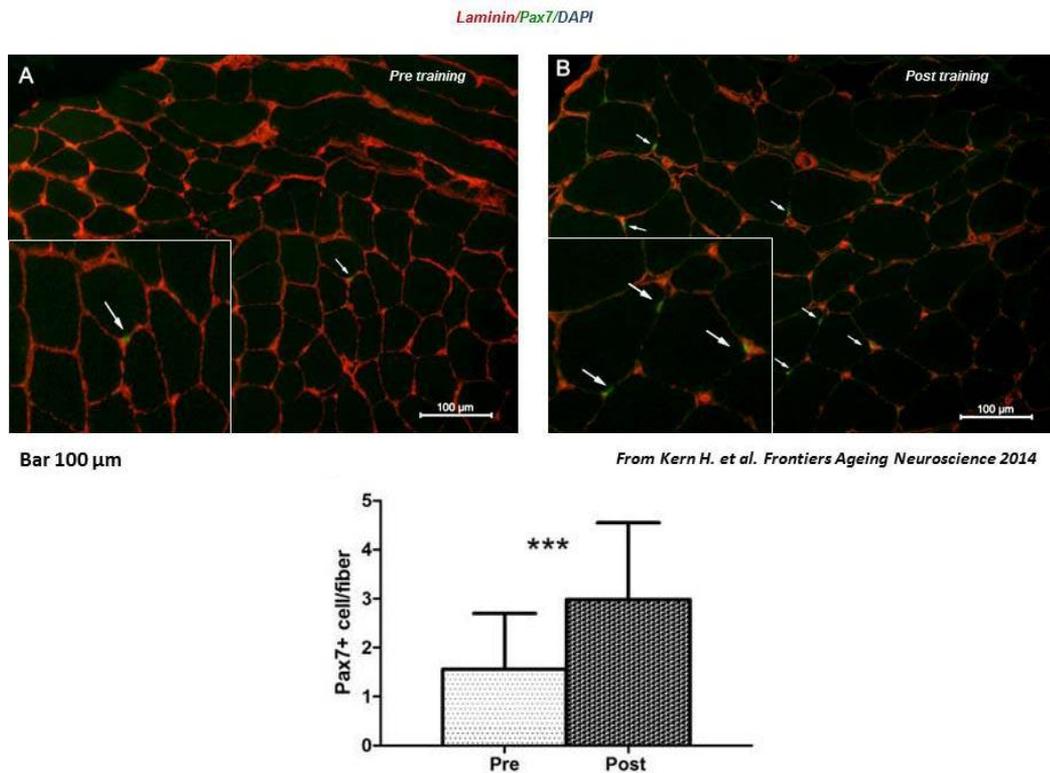


\*  $p < 0.001$  vs pre

**Fig 4.** Mean myofiber size in pre and post training biopsies

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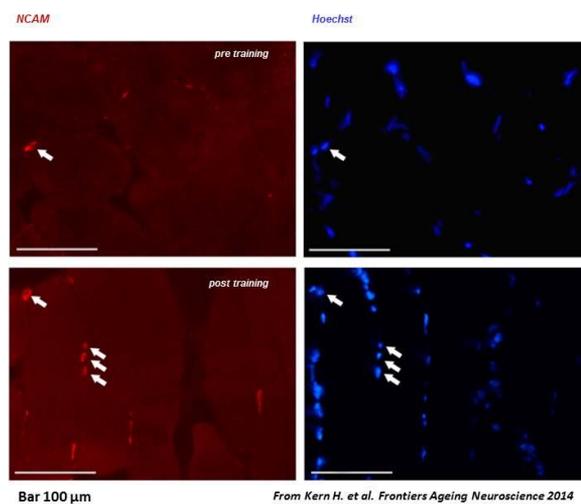
**Fig 5.** More Pax7 positive satellite cells were detected in muscle biopsies after ES training

trained group, despite the mild decrease in fiber diameter, the trained muscles improved in terms of both strength and mobility as shown by functional tests. This indicates that myofiber cross-sectional diameter does not necessarily correlate with muscle strength and performance. The decrease in cross sectional area that occurred in the LP trained group suggests that this activity regimen induced muscle adaptation differently

than the ES treatment in which myofiber size was maintained by the training. The decreased myofiber cross sectional area observed in LP trained group may be related to an increase in myofiber length, which is important for the development of longitudinal or lateral muscle force.

We also analyzed the effects of LP and ES trainings on muscle phenotype. By histochemical analyses testing

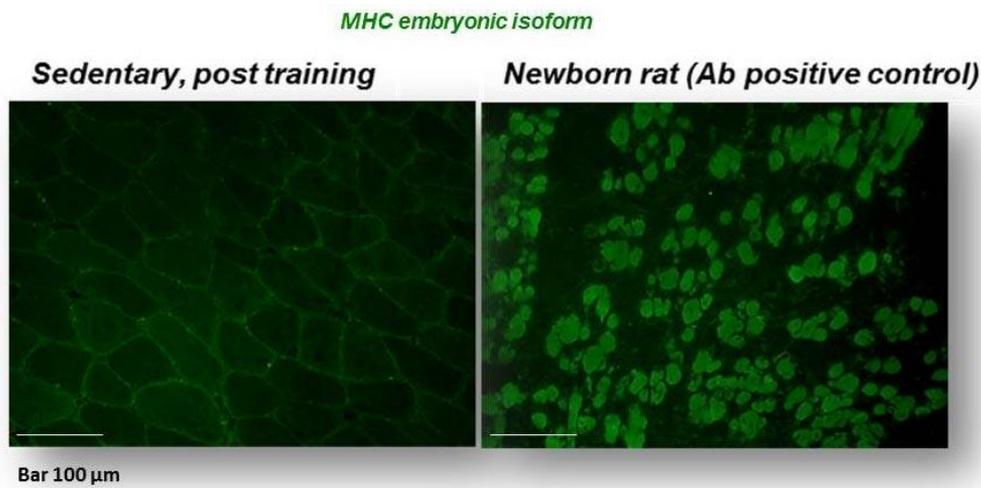
E-Stim induces an increase of N-CAM+ committed satellite cells



**Fig 6.** NCAM positive cells detected in muscle biopsies after ES training

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**Fig 7.** Absence of regenerating myofibers in post training muscle biopsies.

for the activity of myofibrillar ATPase (Fig. 3), we distinguished slow contracting and fatigue resistant fiber types from those which are fast contracting and most powerful.<sup>5</sup> In Figure 3, the slow type fibers are brown stained and the fast type fibers are white stained.

Comparing the post- to the pre- training muscle sections (Fig. 4), it can be observed that the diameter of the fast type fibers decreased after the training in the LP treated group, while it increased in the ES trained one. Conversely, slow type fibers decreased either in number or diameter in both groups. In the Figure 3, the bar histograms and the table report the mean myofiber diameters and the percentage of either slow or fast type myofibers for both groups of trained subjects, showing that the differences observed in post- vs pre- training conditions were all statistically significant. Muscle morphometry indicates that ES seems to be more effective in comparison to the LP training in recruiting fast type fibers, inducing their hypertrophy.

This is an important positive effect of this training, in particular taking into account that fast type fibers are those muscle fibers that predominantly decrease in size and number during aging, contributing to the characteristic loss of muscle mass and force observed in elderly people.<sup>6,7</sup>

We also wanted to test the possible contribution of satellite cells to the skeletal muscle adaptation induced by physical exercise in seniors. In order to do this, we tested the number of Pax7 positive cells, as a specific marker of activated satellite cells, in skeletal muscle biopsies from both groups, comparing the post-training biopsies to the pre-training ones (Fig. 5). In the post-training muscle biopsies from the ES treated subjects, an increase of Pax7 positive cells was observed, as shown by the higher number of green stained cells detected at the periphery of myofibers in comparison to those detected in pre-training muscle biopsies. The increased

number of Pax7 positive cells detected in post training biopsies was statistically significant.

These data indicate that satellite cells are activated in response to physical exercise, likely contributing to the fast fiber type hypertrophy observed in the post training biopsies of the ES treated subjects.

In the same group of muscle biopsies, we also observed an increase of small committed satellite cells expressing the neural cell adhesion molecules (NCAM), that are those stained in red in the Figure 6. In the same figure, myonuclei are counterstained in blue by Hoechst.

In post-training muscle biopsies (left panel of Fig. 7) fibers expressing the embryonic isoform of myosin heavy chain (a hallmark of myofiber regeneration) are absent, indicating no damage/regeneration events were occurring. The staining for embryonic MHC in newborn rat muscle is shown as an antibody positive control (right panel of Fig. 7).

The number of Pax7 cells and NCAM positive cells was unaffected by the LP training, and these findings are in line with morphometry data. Altogether these data demonstrate that both trainings induced similar force and functional improvements without damaging skeletal muscle fibers.

However, in comparison to LP, ES more efficiently attenuates the muscle mass decline associated with aging, maintaining the overall size of muscle fibers, increasing the number and the size of the fast type fibers, and activating satellite cells.<sup>8</sup>

In conclusion, our results provide evidence that ES performed three times a week is a safe and effective therapy to delay age related muscle decline, counteracting atrophy and improving functional outcomes balance tests included,<sup>9</sup> with a subsequent positive influence on the quality of life for seniors.

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### References

1. Kern H, Pelosi L, Coletto L, et al. Atrophy/hypertrophy cell signaling in muscles of young athletes trained with vibrational-

proprioceptive stimulation. *Neurol Res* 2011;33:998-1009.

2. Krenn M, Haller M, Bijak M, et al. Safe neuromuscular electrical stimulator designed for the elderly. *Artif Organs* 2011;35:253-6.

3. Kern H, Kovarik J, Franz C, et al. Effects in senior sportsmen of eight weeks of vibration at different frequencies (1 or 15 Hz) on force and of one year of training on muscle fibers. *Neurol Res* 2010;32:26-1.

4. Zampieri S, Pietrangelo L, Loeffler S, et al. Lifelong Physical Exercise Delays Age-Associated Skeletal Muscle Decline. *J Gerontol A Biol Sci Med Sci* 2015;70:163-3.

5. Mosole S, Carraro U, Kern H, et al. Long-term high-level exercise promotes muscle reinnervation with age. *J Neuropathol Exp Neurol* 2014;73:284-4.

6. Kim TN, Choi KM. Sarcopenia: definition, epidemiology, and pathophysiology. *J Bone Metab.* 2013; 20: 1-10.

7. Nilwik R, Snijders T, Leenders M, et al. The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size. *Exp Gerontol* 2013; 48: 492-8.

8. Kern H, Barberi L, Löffler S, et al. Electrical stimulation counteracts muscle decline in seniors. *Front Aging Neurosci.* 2014 Jul 24;6:189. doi: 10.3389/fnagi.2014.00189. eCollection 2014.

9. Sarabon N, Loeffler S, Cvečka J, Sedliak M, Kern H. Strength training in elderly people improves static balance: a randomized controlled trial. *Eur J Transl Myol - Basic Applied Myology* 2013; 23: 85-9.