

# Muscle histology changes after short term vibration training in healthy controls

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**In search for additional counter measures of muscle atrophy vibration exercise training may have substantial effort for patients with neuromuscular disorders. To cover safety aspects and obtain muscle morphology data, a pilot study was performed in eleven healthy men. Countermovement jump, squat jump, drop jump and one repetition maximum test (1RM) were performed on a force platform before and after a 6 week training period. No severe side effects were found. Repeated needle muscle biopsies of the vastus lateralis muscle revealed a selective pre- to post-training type-2 myofiber hypertrophy of up to 50 %. The hypertrophy factors were 160 and 310, for type-2 myofibers. The mechanography system showed a significant increase in the 1RM maximum weight lifted (pre: 111,8 kg ± 11,5; post: 140,9 kg ± 13,00; p < 0,001). Vibration exercise is a safe and effective technique which desires further approval as counter measure in different types of neuromuscular atrophy.**

**Key words:** muscle atrophy, muscle biopsy, muscle morphometry, vibration exercise

## Introduction

Vibration stimulation exercise is a valuable training technique for athletes and counter measurement for any type of induced muscle atrophy. Vibration training is widely used as one of the prevention strategies of muscle aging. Only limited data are available about vibration induced effects on morphology of normal human skeletal muscle. Vibration applied to muscle or tendon induces a non-voluntary muscular contraction termed the “tonic vibration reflex” (1). Combined with substantial voluntary effort it was shown to elicit movements in neuromuscular patients who were unable to contract their paretic muscles (2). Vibratory stimulation of the muscle tendon evokes an excitation of muscle spindles mediated by Ia afferents and alpha-motor neurons. Additionally, it

is suggested that central motor neuron control organization is activated (3, 4). Vibration stimulation of muscle may therefore enhance contraction and post-stimulation facilitation. Subsequently, vibration stimulated training may have positive effects in counter measures of muscle atrophy in spaceflight and disease related muscle atrophy. The aim of this pilot study was to establish morphological and morphometric data on vibration related effects in human muscle in healthy controls.

## Probands and methods

The study was approved by the local Institutional Review Board of the University of Munich (vote 103/04). All participants gave written informed consent before they were included in the study.

### *Probands*

11 healthy, non-elite sportsmen, mostly sport students (mean ± SD age, 26 ± 8.0 years, mean ± SD height, 1.83 m ± 0.06 m, mean ± SD weight; 80.3 ± 3.2 kg) were investigated. All probands had repeated needle muscle biopsies of the lateral vastus muscle before and after a 6-week course of high-frequency vibration training on a vibration platform (Galileo, Novotec medical, Germany). Testing was performed before and after the training intervention jumping power and force were measured by performing a countermovement jump (CMJ), a squat jump (SJ) and a drop jump (DJ), according to Schmidtbleicher (5), on a ground reaction force platform (Leonardo, Novotec Medical, Pforzheim, Germany), and a one repetition maximum test (1RM) was performed. The knee ankle during the 1RM squat set at 90° to be accepted. After each successful attempt the load was increased until failure in lifting the load. The probands followed a

minimum of two minutes rest between the attempts in the 1RM. The pre test was performed at least two days after the biopsy and the post test at least two days after the last training.

### *Training*

The 6-week training period consisted of a one week familiarisation period and 5 weeks of training with additional weight, starting with 40% of the body weight. Workouts with 2 sets of squats and a five minute warm up before the workout were performed twice a week. The participants trained in every set until exhaustion, with a two minute rest between the sets. The athletes had to be exhausted after a maximum of three minutes; therefore the weight was adapted to the progress. The weight was carried around the hip, with a special center - of mass dumb-bell. The participants performed the squats on the vibration device with 25 Hz. The position from the foot varied between the athletes (15-21 cm with the bunion from the center of the platform), according to the body proportions. Due to the construction (seesaw) of the vibration device, the amplitudes varied according to the foot position between a minimum of 2.9-3.9 mm (5.8-7.8 mm peak to peak).

### *Muscle biopsy specimens*

Twenty-two needle samples were taken from the left vastus lateralis muscle. The first and second biopsy was done within the same area of about a 5-10 cm distance of the vastus lateralis muscle in all probands. All muscle specimens were processed using standard histological procedures. After biopsy, all parts were frozen in liquid nitrogen. Cryosections (8  $\mu$ m) were routinely stained, including haematoxylin & eosin, reduced nicotinamide adenine dinucleotide-tetrazolium reductase (NADH), adenosine triphosphatase reactions (ATPase) at pH 4.6 and pH 10.4, modified Gomori trichrome, van Gieson, cytochrome C oxidase, succinic dehydrogenase, Sudan black B, acid phosphatase, and periodic acid-Schiff. All sections were evaluated semiquantitatively by light microscopy according to the techniques and methods given by Dubowitz (6).

### *Morphometry*

Quantitative morphometry was done on 20 stained samples. In six consecutive microphotographs of stains for type 1 or 2 using ATPase at pH 4.6 and 10.4, the diameters of atrophic and hypertrophic myofibers (normal control range 40-80  $\mu$ m for males) were measured. The number of muscle fibers evaluated in each sample ranged from 60 to 212. The hypertrophy factors were calculated according to Brooke and Engel using an imaging software

(UTHSCSA ImageTool, alpha3, version 2c; San Antonio, Texas) (7, 8). Upper limits for the hypertrophy factor for normal quadriceps muscle are 150 for type 1 myofibers and 400 for type 2 myofibers for males (6-8).

### *Statistical analysis*

All values for the performance tests and for the training in the text and tables are given in mean  $\pm$  SD. Wilcoxon test with Monte Carlo significance was used for the comparison between pre and post test results. Significance was accepted at  $P < 0.05$ .

## **Results**

### *Side effects*

The repeated needle muscle biopsies of the lateral vastus muscle were without any notable side effects for the probands. Vibration stimulation exercise was very well tolerated by all participants. No adverse events directly related to the vibration were recordable.

### *Training results*

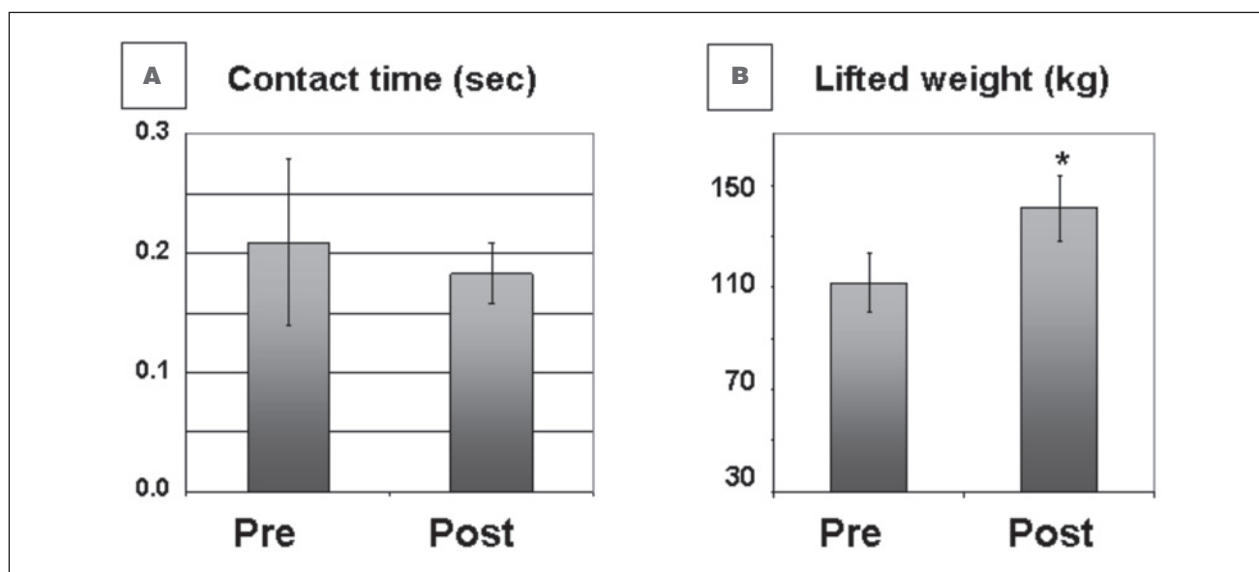
The average training time on the vibration platform was 150 seconds per set, with an average of 63 squats until exhaustion. The participants started with an additional weight of  $30.5 \pm 2.8$  kg, which conformed to 40 % of the body weight and increased the weight to  $65.5 \pm 8.2$  kg in their last workout. Due to personal reasons two probands performed no training in the last week.

### *Test results*

All participants performed pre and post testing on the force platform and the 1RM test. There were no significant changes between pre and post testing on the force plate (Fig. 1A). Only the weight lifted in the 1RM test did increase significantly (pre:  $111.8 \pm 11.5$ , post:  $140.9 \pm 13.0$ ;  $p < 0.001$ ) (Fig.1B).

### *Muscle biopsy*

Nineteen out of 22 performed muscle specimen were included for the final analysis. Three needle biopsies showed mainly connective tissue and only a few muscle fibers, thus they were therefore excluded. Routine histology and histochemistry revealed complete normal muscle biopsy finding in all analysed specimen. Histochemistry did not show significant post-training alterations (e.g. subsarcolemmal mitochondrial increase). Before training, the hypertrophy factor of type 1 myofibers was 120, and 160 for type 2 myofibers respectively. After a 6-week high-frequency vibration stimulation exercises (frequen-



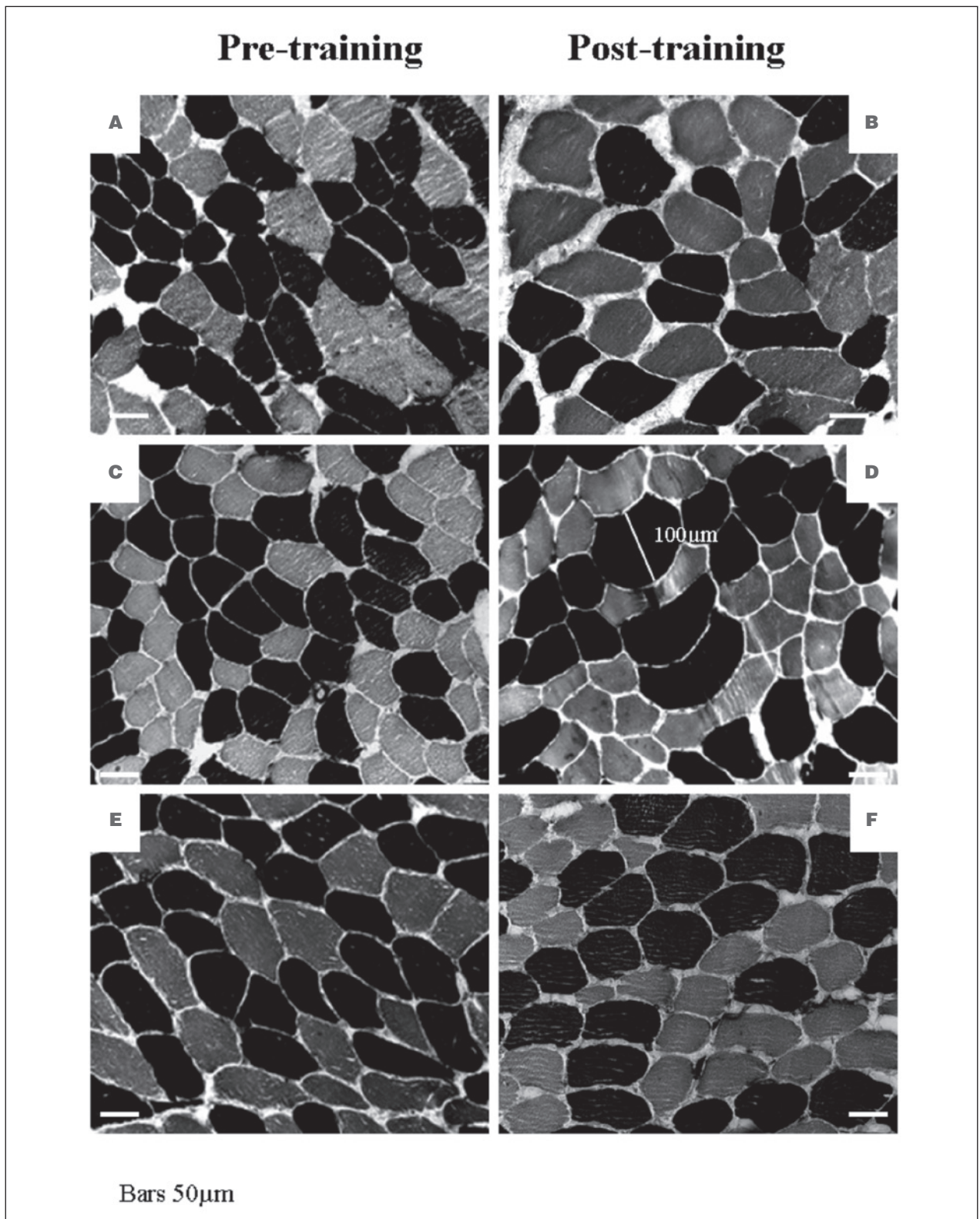
**Figure 1.** Training effects in vibration stimulated muscles. **A:** Non-significant reduction in measured contact time in the drop jump test (Pre  $0.209 \pm 0.07$  sec.; Post  $0.183 \pm 0.025$ ;  $P = 0.06$ ). **B:** Significant increase in the lifted weight (kg) in the 1RM test (Pre  $111.8 \pm 11.5$ ; Post  $140.9 \pm 13.0$ ;  $P < 0.001$ ).

cy 25 Hz, minimum amplitudes 2.9-3.9 mm), the type 1 fiber hypertrophy factor was 150, and the type 2 fiber hypertrophy factor was 310 (Figs. 2, 3).

## Discussion

This study evaluates the influence of high-frequency vibration stimulation exercise training in healthy probands on muscle fiber type hypertrophy controlled by morphometrically analyzed repeated muscle biopsies. Compared to given values of normal controls, an statistically non-significant myofibers type related muscle hypertrophy was found (6-8). However, this study revealed, that high-frequency vibration training as short as 2 sets with a maximum of 3 minutes 2-times a week, induced a type-2 myofiber hypertrophy with an increase of up to 50% of the total number of hypertrophied type-2 myofibers beyond 80 $\mu$ m in diameter. Significant changes in three standard jumping tests were not found, although there are tendencies towards a reduction of contact time in the DJ. This increase could be related to an improvement in neuromuscular performance, documented in acute and chronic enhancements in strength and power (4, 9-11). The significant change in the 1RM test may be mainly related to the squats performed on the platform, but also strengthened by the vibration applied to the body. Campos et. al found that three different exercise protocols accomplished for 8 weeks (low 3-5 repetitions, intermediate 9-11 repetitions; high repetitions 20-28 repetitions) resulted all in an increase of the dynamic strength in the

squat test. Their results showed, that the lower repetition group with more load improved the most. Although all three groups improved in the strength tests, significant changes in the fiber types were only found in the low and intermediate repetition groups with more resistance (12). Contrary to this, our study used a training protocol which, concerning the squat exercise, might only increase the muscular endurance according to other reports (13, 14). If any changes in muscular composition would occur, the changes could be more likely related to the vibration applied to the body. The participants in this study also trained with a shorter duration (6 weeks) and with less workouts (only twice a week). The 26 % increase in the 1 RM Test is comparable to the reported increase of 30% (12). Although vibration is known to improve jumping performance (11), the present study showed only tendencies towards a change. This might be related to a relatively short training time, which did not result in a significant improvement of neuromuscular performance. Finally, the optimal frequency of the vibration platform is still under debate. There is evidence that high-frequencies (> 50 Hz) might evoke a proportional increase in muscle tension, but this is almost absorbed by soft tissue (4). Additionally, increasing vibration frequency induced an increasing co-activation of antagonistic muscles (15). In contrast to this low-frequencies (<20 Hz) propagate through the kinetic chain to proximal muscles and may be absorbed by the human body tissue (4). Finally, we hypothesize, that different frequencies may stimulate distinct muscle fiber types in different muscles, which has



**Figure 2.** Pre and Post vibration training muscle morphology in 3 healthy sportsmen. Vastus lateralis muscle biopsy samples (ATPas histochemistry at pH 10.4) of three probands pre (**A, C, E**) and post (**B, D, F**) 6-week vibration platform training. **B, D, F** reveals an significant increase in type-2 myofiber diameters up to 100µm (**D**). Bars in A,B,C,E and F = 50µm.

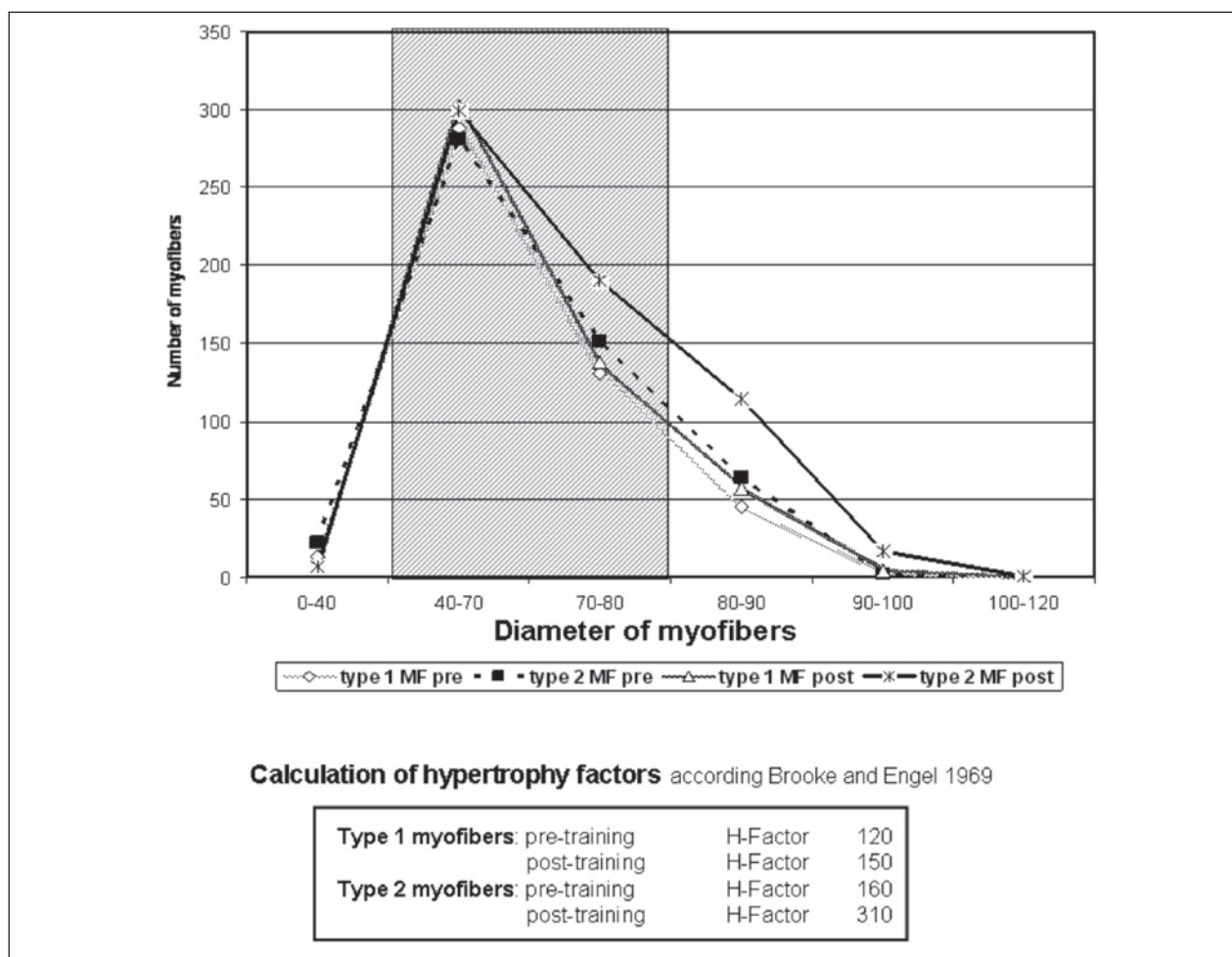


Figure 3. Morphometric histogram and hypertrophy factor calculation (details see text).

to be further analysed. In this study we showed, that a 25 Hz vibration stimulus induced a type-2 myofiber hypertrophy in human vastus lateralis muscle. In order to improve neuromuscular performances we believe that different frequencies (slow versus fast) within a training period have to be tested. Moreover, this protocol has to be adapted to distinct types of muscle atrophy with a more proximal or distal pronounced weakness.

In summary, vibration stimulation exercise is a safe and effective technique which may help to improve neuromuscular performance in health, mitigate spaceflight muscle atrophy, and muscle atrophy associated with neuromuscular disorders and muscle ageing.

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