THE EFFECTS OF LOCAL MECHANO-ACOUSTIC VIBRATIONS ON UPPER LIMB SPASTICITY

*R. Casale MD**, C. Foti MD FP*, T. Sciarra MD*,M. Castellana Vitaterna PT***, C. Damiani MD****

****San Raffaele Portuense Rehabilitation Hospital - Rome*
**Tor Vergata University of Rome Physical and Rehabilitation Medicine*
***Foundation Salvatore Maugeri IRCCS Montescano Pavia*

SPASTICITY CONTROL IS A MAJOR TASK IN NEURO-MOTOR REHABILITATION

BACKGROUND

REHABILITATION PROGRAMS TO CONTROL SPASTICITY ARE OFTEN ASSOCIATED WITH THE USE OF DRUGS EITHER SYSTEMICALLY GIVEN OR LOCALLY INJECTED

PHARMACO-THERAPY IS GENERALLY EFFICACIOUS, ALTHOUGH SOME LIMITATIONS EXIST

WEAKNESS
DROWSINESS
SEDATION
DEVELOPMENT OF IMMUNO-MEDIATED UNRESPONSIVENESS

T. Sciarra et Al
Tor Vergata University of Rome

BACKGROUND (Ctd)

A FURTHER POSSIBLE WAY OF CONTROLLING SPASTICITY, BY MEANS OF PHYSICAL MODALITIES SUCH AS VIBRATION, HAS ALSO BEEN SUGGESTED.

HOWEVER, VERY FEW AND DATED CLINICAL REPORTS ON THE USE OF PHYSICAL MODALITIES FOR THE TREATMENT OF SPASTICITY EXIST.

T. Sciarra et Al
Tor Vergata University of Rome

BACKGROUND (Ctd 1)

RECENTLY, MECHANICAL VIBRATION HAS BEEN SUCCESSFULLY PROPOSED TO REDUCE LOWER LIMB SPASTICITY IN SPASTIC DIPLEGIA

VIBRATION APPLIED TO THE ANTAGONIST FOREARM EXTENSOR MUSCLE INCREASE THE CENTRAL SILENT PERIOD OF THE FOREARM EXTENSOR MUSCLES AS A SIGN OF ENHANCED INHIBITION / REDUCED EXCITABILITY OF THE CORTICO-SPINAL ACTIVITY 31 (BINDER, 2009).

STUDY OBJECTIVES

PRIMARY OBJECTIVE:

CLINICALLY TEST, IN A GROUP OF HEMIPLEGIC PATIENTS, THE HYPOTHESIS THAT THE APPLICATION OF A SELECTIVE VIBRATION ON THE UPPER LIMB FLEXOR ANTAGONIST (I.E. THE TRICEPS BRACHHI) CAN REDUCE THE SPASTICITY OF THE FLEXOR AGONIST (BICEPS BRACHII)

SECONDARY OBJECTIVE :

TEST THE DIFFERENCE BETWEEN EXPERIMENTAL AND THERAPEUTIC EXERCISES TREATMENTS.

METHODOLOGY

SAMPLE SIZE 30 PATIENTS

TREATMENT ALLOCATION RATIO 1:1

INCLUSION CRITERIA:
UPPER LIMB SPASTICITY (MAS 1-4)

AGE RANGE 18-70

EXCLUSION CRITERIA:
STROKE OCCURRENCE >12 MONTHS
MMSE < 22
ALL PATHOLOGIES THAT COULD AFFECT THE TRIAL

RANDOMIZATION

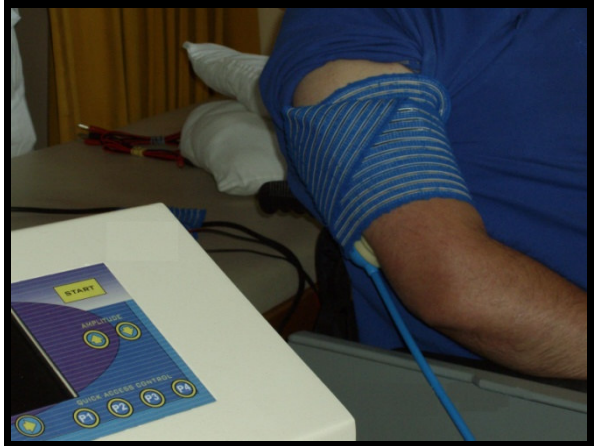
COMPUTER GENERATED SIMPLE RANDOM SAMPLING

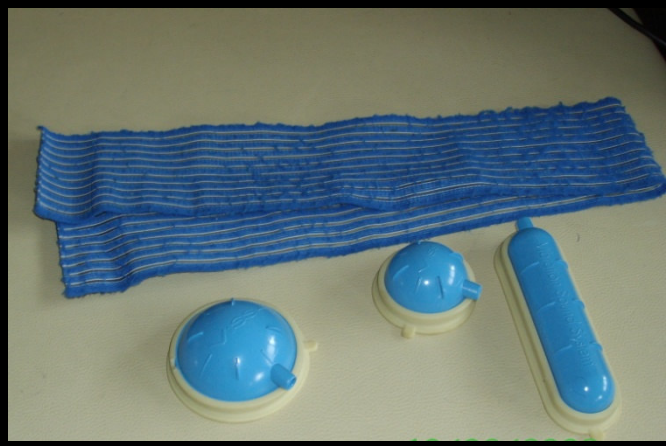
GROUP A: THERAPEUTIC EXERCISES FOCUSED ON UPPER LIMB SPASTICITY REDUCTION

GROUP B: THERAPEUTIC EXERCISES + VIBRATIONS GENERATED ENERGY (100 HZ/ 15 MIN)

10 SESSIONS / 45 MIN EACH

VIBRATIONS GENERATED ENERGY





100 HZ/ 15 Min
AMPLITUDE 2 mm
MEAN PRESSURE 250 mBar

OUTCOME MEASURES

FIM

MODIFIED ASHWORTH SCALE

ELBOW

WRIST

TIMING:

T_1 : BEGINNING OF THE TREATMENT

T_2 : 5° SESSION

T_3 : END OF THE TREATMENT

STATISTICAL ANALYSIS

DESCRIPTIVE ANALYSIS WAS PERFORMED TO SUMMARIZE THE INFORMATION COLLECTED

NON PARAMETRIC ANALYSIS WAS PERFORMED TO TEST THE DIFFERENCE IN EFFICACY BETWEEN THE EXPERIMENTAL AND THE STANDARD TREATMENTS

FUNCTIONAL INDIPENDENCE MEASURE

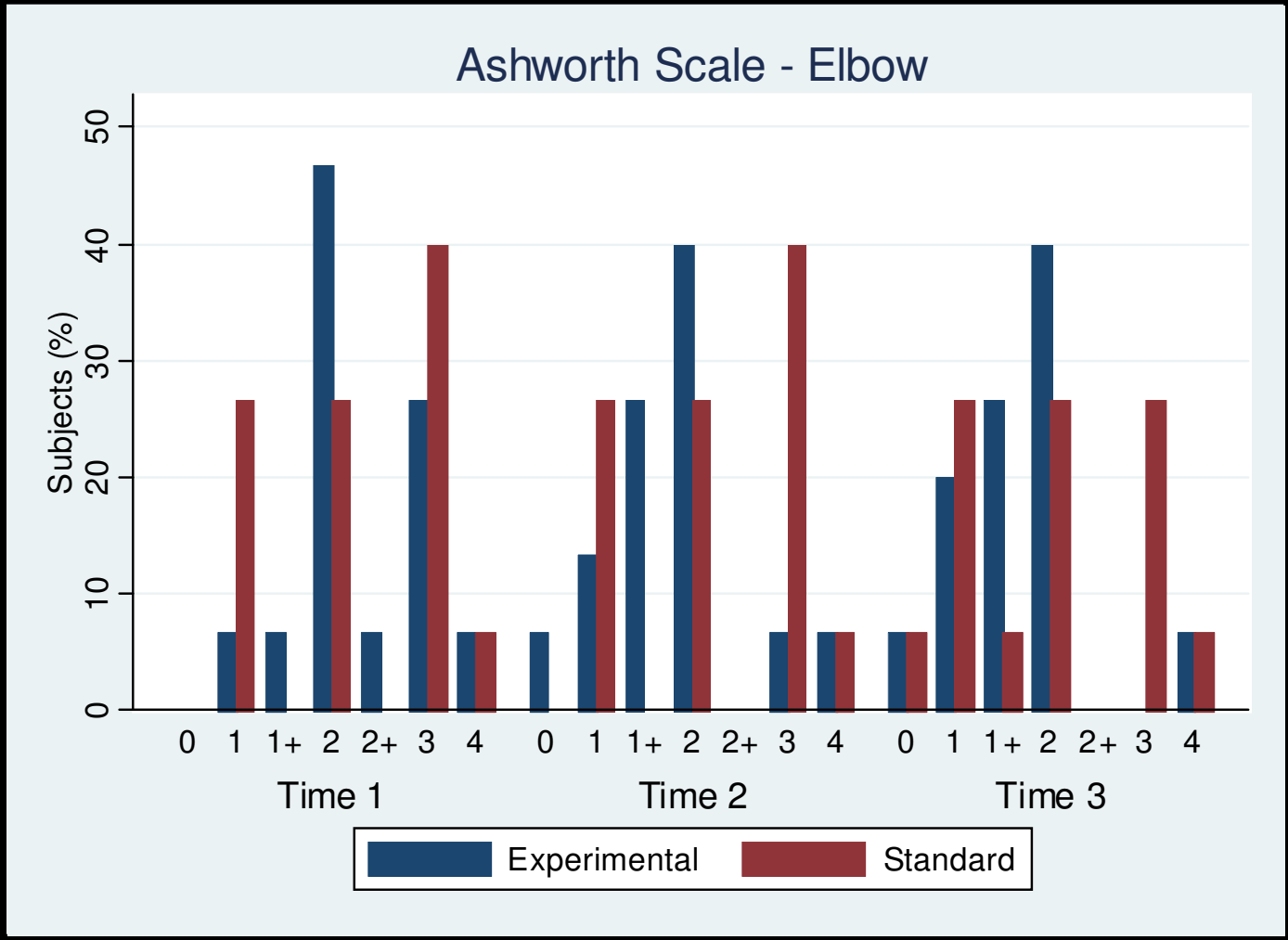
	Mean	Standard Deviation
Time 1	70.9	21.6
Time 2	72.4	21.6
Time 3	74.5	21.3

NOTE: FIM SCORES REMAIN STABLE OVER TIME

FREQUENCIES MODIFIED ASHWORTH

		Experimental			Standard		
	Score	Time1	Time2	Time3	Time1	Time2	Time3
Ashworth - Elbow	0	**0.00%**	**6.67%**	**6.67%**	**0.00%**	**0.00%**	**6.67%**
	1	**6.67%**	**13.33%**	**20.00%**	**26.67%**	**26.67%**	**26.67%**
	1+	6.67%	26.67%	26.67%	0.00%	0.00%	6.67%
	2	**46.67%**	**40.00%**	**40.00%**	**26.67%**	**26.67%**	**26.67%**
	2+	6.67%	0.00%	0.00%	0.00%	0.00%	0.00%
	3	26.67%	6.67%	0.00%	**40.00%**	**40.00%**	**26.67%**
	3+	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	4	**6.67%**	**6.67%**	**6.67%**	**6.67%**	**6.67%**	**6.67%**
	4+	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Ashworth - Wrist	0	**0.00%**	**13.33%**	**13.33%**	**0.00%**	**13.33%**	**13.33%**
	1	**20.00%**	**13.33%**	**33.33%**	**26.67%**	**20.00%**	**26.67%**
	1+	13.33%	20.00%	0.00%	6.67%	0.00%	0.00%
	2	**33.33%**	**40.00%**	**46.67%**	13.33%	13.33%	20.00%
	2+	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	3	26.67%	13.33%	6.67%	**33.33%**	**40.00%**	**33.33%**
	3+	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	4	**6.67%**	**0.00%**	**0.00%**	**20.00%**	**13.33%**	**6.67%**
	4+	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

DESCRIPTIVE ANALYSIS



DESCRIPTIVE ANALYSIS (AS ELBOW)

PERCENTAGE OF SUBJECTS WHO SCORED 4 ON EITHER TREATMENT WAS 7% AND STAYED CONSTANT OVER TIME

REDUCTION OF SUBJECTS ON EXP. TREAT. SCORED 2 POINTS:FROM 47% TO 40%

REDUCTION OF SUBJECTS ON ST. TREAT. SCORED 3 POINTS FROM 40% TO 27%

AN INCREASE OF SUBJECTS ON EXP. TREAT. WHO SCORED 1 FROM 7% TO 20%

PERCENTAGE OF SUBJECTS ON ST. TREAT. WHO SCORED 1 AND THOSE WHO SCORED 2 WAS 27% AT ALL TIMES OF MEASUREMENT.

AN INCREASE OF EXP. TREAT. GROUP WHO SCORED 0 FROM 0% TO 7% BY THE 2ND TIME POINT IN THE EXP. TREAT. , WHEREAS THIS PERCENTAGE WAS REACHED BY THE END OF THE TREATMENT PERIOD IN THE ST. TREAT. GROUP

DESCRIPTIVE ANALYSIS (Ctd1)

DESCRIPTIVE ANALYSIS (AS WRIST)

REDUCTION OF SUBJECTS ON EXP. TREAT WHO SCORED 4 FROM 7% TO 0%

A REDUCTION OF SUBJECTS ON STANDARD TREATMENT WHO SCORED 4 FROM 20% TO 7%

AN INCREASE OF SUBJECTS ON EXP. TREAT. SCORED 2 FROM 33% TO 47%

PERCENTAGE OF SUBJECTS ON EXP TREAT WHO SCORED 1 CHANGED FROM 20% TO 33%.

PERCENTAGE OF SUBJECTS ON ST. TREAT. WHO SCORED 1 CHANGED FROM 20% TO 27%.

PERCENTAGE OF SUBJECTS WHO SCORED 0 INCREASED FROM 0% TO 13% BY THE 2ND TIME POINT IN BOTH TREATMENT GROUPS.

MANN-WHITNEY TEST

Treatment Comparison	Spasticity		z	p
Therapeutic Exercises - Therapeutic Exercises and Vibrations Generated Energy	Ashworth – Elbow	Time1	-0.087	0.9307
		Time2	1.326	0.1849
		Time3	0.790	0.4296
	Ashworth – Wrist	Time1	0.683	0.4949
		Time2	1.380	0.1677
		Time3	1.142	0.2535

WILCOXON SIGNED-RANK TEST

Spasticity	Comparison	z	p
	Time1 – Time2	3.297	0.0010
Ashworth – Elbow	Time1 – Time3	4.157	< 0.0001
	Time2 – Time3	2.821	0.0048
	Time1 – Time2	3.297	0.0010
Ashworth – Wrist	Time1 – Time3	4.068	< 0.0001
	Time2 – Time3	2.821	0.0048

RESULTS

THE MANN-WHITNEY TEST COULD NOT SHOW EVIDENCE OF A SIGNIFICANT DIFFERENCE BETWEEN THE TWO TREATMENTS AT ANY TIME-MEASUREMENT.

THE WILCOXON SIGNED-RANK TEST FOR PAIRED DATA HIGHLIGHTED A SIGNIFICANT REDUCTION OF SPASTICITY OVER TIME.

DISCUSSION

BOTH TREATMENTS WERE EFFECTIVE IN REDUCING SPASTICITY OF UPPER LIMB OVER TIME STILL AFTER 10 SESSIONS.

SO FAR NO EVIDENCE OF DIFFERENCES BETWEEN TREATMENTS WAS FOUND.

THESE FINDINGS WERE ALSO CONFIRMED BY THE DESCRIPTIVE STATISTICS.

DISCUSSION (Ctd)

THE TRIAL CONFIRMS THE VALIDITY OF THE STANDARD THERAPEUTICAL APPROACH SO FAR ADOPTED.

THE TRAIL CONFIRM THE VALIDITY OF THE STANDARD APPROACH ADDED WITH THE VIBRATIONS GENERATED ENERGY

HOWEVER, THE EFFICACY OF THE STANDARD TREATMENT MIGHT HAVE OVERLAID WITH THE EFFICACY OF THE VIBRATIONS GENERATED ENERGY.

DISCUSSION (Ctd 1)

IN THE PATIENTS TREATED WITH THE MECHANO-ACOUSTIC VIBRATIONS ENERGY THE SPASTICITY SEEMS TO BE REDUCED BEFORE.

IT COULD BE CONSIDERED MORE SIGNIFICANT THE SPASTICITY DECREASE AFTER THE MECHANO-ACOUSTIC TREATMENT EVEN IF IT IS NOT GREATLY CONFIRMED BY THE STATISTIC RESULTS.

DISCUSSION (Ctd 2)

IN CONSIDERATION OF:

LOW COST/EFFECTIVENESS OF VIBRATIONS METHODS

NON OPERATOR DEPENDENT

POSSIBILITY TO TREAT MORE PATIENTS IN THE SAME TIME (12 CHANNELS)

DISCUSSION (Ctd 2)

FURTHER RESEARCH IS NEEDED TO EXPLORE THE SHORT TERM AND LONG TERM EFFECTS OF THE VIBRATIONS GENERATED ENERGY IN THE REDUCTION OF SPASTICITY.

A MORE POWERFUL AND BETTER STRUCTURED TRIAL SHOULD BE DESIGNED IN ORDER TO INVESTIGATE THE EFFICACY OF THE VIBRATIONS' ENERGY ALONE.

THE EFFECTS OF LOCAL MECHANO-ACOUSTIC VIBRATIONS ON UPPER LIMB SPASTICITY

T. Sciarra MD,R. Casale MD**, M. Castellana Vitaterna PT***,*
C. Foti MD FP, C. Damiani MD****

****San Raffaele Portuense Rehabilitation Hospital - Rome*
**Tor Vergata University of Rome Physical and Rehabilitation Medicine*
***Foundation Salvatore Maugeri IRCCS Montescano Pavia*

Effects of local vibrations on skeletal muscle trophism in elderly people: Mechanical, cellular, and molecular events

TIZIANA PIETRANGELO[1], ROSA MANCINELLI[1], LUANA TONIOLO[2], LINA CANCELLARA[2], ANTONIO PAOLI[2], CRISTINA PUGLIELLI[1], PIERPAOLO IODICE[1], CHRISTIAN DORIA[1], GERARDO BOSCO[1], LUIGI D'AMELIO[1], GUGLIELMO DI TANO[1], STEFANIA FULLE[1], RAOUL SAGGINI[1], GIORGIO FANO[1] and CARLO REGGIANI[2]

[1]Dipartimento Scienze Mediche di Base ed Applicate, Istituto Interuniversitario di Miologia, Centro di Studi sull'Invecchiamento, University G. d'Annunzio Chieti-Pescara, via dei Vestini 29, I-66013 Chieti; [2]Dipartimento Anatomia e Fisiologia, Università di Padova, via Marzolo 3, I-35131 Padova, Italy

DOI: 10.3892/ijmm_00000000

Abstract. Several studies have examined the effects of vibrations on muscle mass and performance in young healthy people. We studied the effects of vibrations on muscles of elderly male and female volunteers (65-85 years of age) diagnosed with sarcopenia. We applied mechanical vibrations locally (local vibrational training) to the thigh muscles at 300 Hz for a period of 12 weeks, starting with a session of 15 min stimulation once a week and increasing to three sessions of 15 min per week. Treated muscles displayed enhanced maximal isometric strength and increased content of fast MyHC-2X myosin. Single muscle fiber analysis did not show any change in cross-sectional area or in specific tension. Analysis of transcriptional profiles by microarray revealed changes in gene expression after 12 weeks of local vibrational training. In particular, pathways related with energy metabolism, sarcomeric protein balance and oxidative stress response were affected. We conclude that vibration treatment is effective in counteracting the loss of muscular strength associated with sarcopenia and the mode of action of vibration is based on cellular and molecular changes which do not include increase in fiber or muscle size.

Introduction

Aging is associated with progressive loss of neuromuscular function. The term sarcopenia is commonly used to describe the loss of skeletal muscle mass and strength that occurs in connection with biological aging in the elderly. The onset of sarcopenia is generally assumed to occur around 60 years of age, with atrophy being an important symptom. The progression of sarcopenia is influenced by several factors, including genetic components, lifestyle, age-related diseases, decreases in the levels of hormones (GH, testosterone, IGF-1), loss of motor units, and decreased regenerative capacity of skeletal muscle stem cells (1,2). Atrophic conditions in the elderly are exacerbated when diseases force them to bed. The main countermeasure is regular and moderate exercise, but, besides general advice to stay active, there are no definitive indications for optimal training and/or treatment of sarcopenia (3,4).

In recent years, mechanical muscle vibration received considerable attention as a useful method of muscle stimulation in clinical therapy and sports training, but the results remain controversial. Only a few studies described specific vibrational training protocols, and this lack of information generates uncertainties regarding the most effective vibration intensities, frequencies, and application protocols. When frequency is considered, the main question is whether vibrations are applied to the whole body or to specific muscles. The body tolerates a vibration frequency in the range 20-50 Hz, whereas the level for local application to specific skeletal muscles is in the range of 300-500 Hz.

In view of the diversity of applied treatments, a precise comparison of results reported in scientific literature is difficult, and for this reason we report data on different vibrational protocols without extensive discussion. For instance, Bosco and coworkers showed positive effects of passive whole-body vibration under different conditions, reporting significant increases in muscle strength and power (5), elevations in plasma concentrations of testosterone and growth hormone (6,7), and improvement in neuromuscular properties (8). Another study showed that maximal isometric voluntary contraction increased (in young men) only when vibration was combined with squat training (7). De Ruiter and colleagues studied the effects on young men over a period of 11 weeks of whole-body vibration at 30 Hz and found that neither the strength nor the contractile properties of the knee extensor muscle improved (9). It is also worth remembering that negative effects of whole-body vibration on health were reported. Hand-arm vibration syndrome and

Correspondence to: Dr Tiziana Pietrangelo, Dipartimento Scienze Mediche di Base ed Applicate, University 'G. d'Annunzio' Chieti-Pescara, Via dei Vestini 29, I-66013 Chieti, Italy
E-mail: tiziana@unich.it

Key words: vibrations, skeletal muscle, single fibers, gene expression

Table I. Anthropometric parameters of study participants.

	Age years	Height cm	Weight pre-training kg	Weight post-training kg	BMI pre-training kgm^{-2}	BMI post-training kgm^{-2}
Male	75.3±6.9	163.0±5.0	76.9±6.7	75.3± 6.2	29.1±3.5	29.0±3.3
Female	71.0±5.7	159.0±5.0	71.6±18.8	66.4 ±15	28.3±8.1	28.2±7.6

Data reported in the Table are the anthropometric measurements of male and female elderly volunteers. The values are means ±SD.

vascular disorders (10,11), low back pain (12) and spinal health risks (13), were reported in vibration-exposed workers.

The possible involvement of the nervous system in modifications of muscle performance induced by vibration has been the focus of several studies (14). It was hypothesized that mechanical stimuli are transmitted to sensory receptors of muscles, most likely the spindles, and that receptor activation results in the reflex stimulation of motor units, as seen in the tonic vibration reflex (15). However, the tonic vibration stretch reflex was originally described as the result of a brief exposure to high-frequency stimulation applied directly to a tendon (16,17). Any activation prolonged for tens of seconds could induce a reduction in muscle spindle firing frequency and, as a consequence, a decrease in muscle activation (15). Moreover, muscle spindle firing induced by vibration excites not only motor neurons but also interneurons in the spinal cord, which reciprocally inhibit the motor neurons of antagonist muscles (18). Brunetti and coworkers studied posture stability after vibratory stimulation following anterior cruciate ligament reconstruction. These authors concluded that vibration leads to a faster and more complete recovery of equilibrium, confirming a role for vibration in proprioceptive stimulation (19). In conclusion, it remains difficult to find a rationale that is logically used to design vibration treatment and to predict treatment outcomes.

In this study, we aimed to determine whether a training program of passive muscle stimulation, in which local mechanical vibrations at high frequency (300 Hz) were applied to the lower limbs in the absence of any voluntary muscle contraction, induces an increase in muscle mass and strength in elderly subjects showing signs of sarcopenia. Isometric strength developed in maximal voluntary contractions by knee extensor muscles, and thigh circumference, were the parameters selected to evaluate the impact of local muscle mechanical vibrations in elderly male and female volunteers, and thigh circumference was used as an indicator of structural modifications. Additionally, using small fragments of tissue from needle biopsies of the vastus lateralis muscle (pre- and post-training), some cellular features (fiber types, fiber cross-sectional area, single fiber tension development) and gene expression profiles were analyzed.

Materials and methods

Subjects. The study involved nine elderly people (four males and five females) with diagnoses of sarcopenia according to the criteria of the Centers for Disease Control and Prevention (CDCP). Anthropometric characteristics are summarized in Table I. The study was approved by the local ethics committee, and was performed in accordance with the 1964 Declaration of Helsinki. All individuals provided written informed consent before participating in the study.

The inclusion criteria were as follows: diagnosis of sarcopenia; normal ECG and blood pressure; and absence of bone/joint, metabolic, or cardiovascular diseases. Exclusion criteria were the presence of metabolic and/or cardiovascular diseases, evidence of hereditary or acquired muscular disease, or diagnosis of respiratory or psychiatric disorders. No subject was under treatment with testosterone or other pharmacological interventions known to influence muscle mass.

Training protocol and experimental design. The conditioning protocol consisted of application of local high-intensity vibrations on the lower limbs using the VISS apparatus. The VISS device (Vissman, Rome, Italy) is a tool capable of producing acoustic waves of different frequencies without affecting the set width. The device is not an acoustic wave generator, but rather a flux modulator, and has two components. These are a compressor delivering pressure in the range 0-400 millibar and a modulator producing an oscillatory air flux to create acoustic waves through a two-way rotating valve. The transducer develops a time-modulated sinusoidal wave (300 Hz). During application of vibration, subjects were invited to avoid isometric contractions of the treated muscle. The experimental protocol required that local mechano-acoustic vibratory stimulation was applied on the skin of the distal part of the quadriceps close to the tendon insertions of the intermedius femoris, rectus femoris, vastus medialis, and vastus lateralis muscles. The entire treatment lasted 12 weeks. The duration of each application was 15 min and the frequency chosen was 300 Hz. From weeks 1 to 8 the subjects received one application per week, whereas from weeks 9 to 12 they received three applications per week. We refer to this type of stimulation protocol as 'local high-intensity vibrational training'.

Two weeks before the training period, enrolled subjects were familiarized with the test session protocol. Isometric tests were performed a week before (pre-session) and a week after the conditioning period (post-session), to measure the bilateral maximal isometric strength of the lower limbs, the body mass index (BMI), and thigh circumference, assessed with a measuring tape at two levels on the dominant leg of each standing subject. The circumferences measured were the maximal circumference at one-third of the distance between the trochanter and the tibial-femoral joint space, and the minimum circumference above the knee. Skinfold measurements were next recorded at the anterior mid-thigh using a skinfold caliper.

Sixteen weeks after the end of the conditioning period of local high-intensity vibrational training, the isometric test was repeated to monitor lower limb strength level.

Isometric strength measurement. Bilateral isometric torque developed by the knee extensor muscles was measured during maximal voluntary contractions using a Leg Extension machine (Panatta Sport; Apiro [MC], Italy) equipped with a load cell (Globus Italia, Codognè [TV], Italy). Participants were seated with the trunk-thigh and the knee joint angles at 90˚. Subjects performed maximal voluntary isometric contractions of the knee extensors three times. Isometric contractions lasted for 5 sec, and were separated by 2 min rest intervals. The highest value of torque attained was taken as the isometric contraction strength. In each subject, the variation of knee extension isometric force was expressed as a percentage of the pre-training value.

Cellular and molecular analysis of muscle biopsies. Muscle biopsies were obtained using a semi-automatic needle (Precisa 13 Gauge; Hospital Service, Rome, Italy) from the vastus lateralis muscle at a level corresponding to one-third of the distance from the upper margin of the patella to the anterior superior iliac spine, after local anesthesia with lidocaine (0.5%, w/v). In each subject, several samples were collected from the same needle insertion and were divided into three groups: (i) samples immersed in skinning solution (see below) were used for dissection of single muscle fibers; (ii) samples immersed in Laemmli buffer (see below) were used for gel electrophoresis; and, (iii) samples immersed in Trizol reagent (Invitrogen, Paisley, UK) were used for extraction of RNA for analysis of transcriptional profiles.

Muscle fibers, mechanical characterization. Muscle biopsy fragments for single fiber dissection were immersed in ice-cold skinning solution with 50% (v/v) glycerol. Skinning solution is a high-potassium, high-EGTA solution which depolarizes membranes, removes calcium, and induces a rigor state, thus ensuring optimal conditions for fiber preservation. The fragments were stored at -20˚C and analyzed within 2 weeks of sampling. On the day of the experiment, the skinning-glycerol mixture was washed off and replaced with ice-cold skinning solution containing ATP, to induce fiber relaxation. Single fibers were manually dissected under a stereo-microscope (x10-60 magnification). Following dissection, fibers were bathed for 30 min in skinning solution containing 1% (v/v) Triton X-100 to ensure complete membrane solubilization. Fiber segments 1-2 mm in length were cut, and light aluminum clips were applied at both ends. Skinning, relaxing, pre-activating, and activating solutions employed for single fiber experiments were prepared as described previously (20). Fiber segments were transferred to the experimental apparatus, and cross-sectional area and tension development during maximal calcium-activated isometric contractions at 12˚C were measured according to a previously described procedure (21).

Electrophoretic separation and quantification of myosin heavy chain (MyHC) isoforms. MyHC isoform composition was determined in biopsy samples. Muscle biopsy fragments were stored in Laemmli solution (Tris 62.5 mM, Glycerol 10% [v/v], SDS 2.3% [w/v], ß-mercaptoethanol 5% [v/v], with E-64 0.1% [w/v] and leupeptin 0.1% [w/v] as anti-proteolytic factors; pH 6.8). After heating for 5 min at 80˚C appropriate amounts of the protein suspension were loaded onto poly-acrylamide gels (~1 μg of total protein/lane). Separation of MyHC isoforms was achieved on 8% (w/v) gels (18 cm x 16 cm x 1 mm) at 70 V for 1.5 h and at 230 V for a further time according to the guidelines of Talmadge and Roy (22). After silver staining, three separate bands were detected in the 200-kDa region, corresponding to MyHC-1, -2A, and -2X, in order of migration from fastest to slowest. To quantify myosin isoform distribution, densitometric analyses of silver-stained bands were performed on at least two independent electrophoretic runs of each biopsy sample fragment. The mean values represent the measurements presented. Gel patterns were digitized with an EPSON 1650 scanner at a resolution of 1,200 dpi. Each band was characterized by a value of the Brightness-Area Product (BAP), using a constant threshold after black/white inversion employing Adobe Photoshop software. From each gel, BAP values for the bands identified as MyHC isoforms were summed and the BAP value for each isoform was expressed as a percentage of the total. The reproducibility of the procedure was confirmed by calculating isoform ratios of selected samples from gels loaded with different amounts of such samples.

Gene expression profile. A high-density oligonucleotide microarray technique was used to identify variations in gene expression induced by mechanical vibration. This technique involves RNA isolation, amplification, and labeling. The human oligonucleotide gene set consisting of 21,329 (70-mer) oligonucleotides (Operon version 2.0), designed on the basis of the Human Unigene clusters, was employed. Microarray co-hybridization involved simultaneous hybridization with pre- and post-training samples. Expression levels of all spot replicates were normalized (23). RNA was extracted from the biopsy samples of three different elderly subjects before and at the end of the vibrational training period. Very pure RNA samples from each subject were amplified and reverse-transcribed into cDNA, and dye-swap duplicated microarrays were analyzed for each subject. Arrays were scanned, and recorded fluorescence intensities were subjected to Lowess normalization. The expression of each gene was defined as the log base-2 of the ratio between the intensity of cyanine-coupled aaRNA from post-training samples and that of cyanine-coupled aaRNA from pre-training samples ($\log_2 I_{post\text{-}training}/I_{pre\text{-}training}$). Differentially expressed genes were selected using a permutation test procedure known as the 'Significance Analysis of Microarrays' (SAM), which defines genes with a computed score larger than the threshold value as showing significant variation. The false discovery rate (FDR) associated with the given threshold was additionally calculated from permutation data.

Statistical analysis. Data were expressed as means ±SE (Figs.) or SD (Tables). Statistical significance was set at $p<0.05$ and was calculated using the unpaired Student's t-test, with Welch's correction. Prism5 GraphPad software (Abacus Concepts GraphPad Software, San Diego, CA) was employed for statistical analyses. Gene expression analysis was based on SAM data and variations are expressed as means with SD.

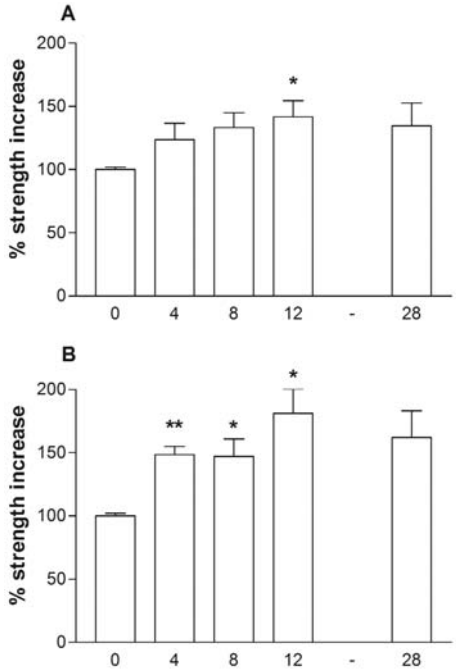


Figure 1. Variations in bilateral isometric strength of leg extensor muscles treated with local muscle vibration at 300 Hz in nine elderly male (A) and female (B) subjects. The histograms show the percentage increases in bilateral isometric strength, based on leg extension, at different times during the period of stimulation. 0 week, pre-training; 4, 8, and 12 weeks, intervals during the stimulation period when strength was measured. During the first 8 weeks, treatment was based on a single vibration application (300 Hz) of 15 min per week. From weeks 8 to 12, treatment was increased to three applications of 15 min per week (300 Hz). Treatment was interrupted after 12 weeks, and strength was measured again at week 28. Isometric strength measurements from the pre-training sessions are taken as 100%. Significant variations, $^{*}p<0.05$, $^{**}p<0.01$.

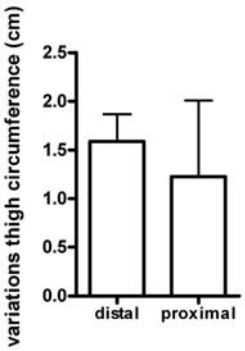


Figure 2. Variations in thigh circumference. Two thigh circumferences, one distal (just above the knee) and one proximal (at 2/3 of the knee-trochanter distance, to avoid the adductor muscles) were measured before the beginning of treatment (pre-training) and after 12 weeks of treatment (post-training). Changes are expressed in cm. As average, the distal and proximal circumferences showed small and insignificant increases after vibrational training in both male and female subjects.

Results

Maximal isometric strength of knee extensor muscles. Isometric strength of leg extensor muscles was measured before, and after 4, 8, and 12 weeks of vibrational training. The application of local vibration to the lower part of the thighs improved isometric strength (Fig. 1). In both male and female subjects (anthropometric characteristic in Table I), the increase was appreciable, beginning at 4 weeks (females) and 8 weeks (males) of vibration training. Importantly, during the first 8 weeks of the procedure, stimulation was scheduled only once a week. This increase in strength remained at a plateau upto week 12, even though training frequency was increased to three times per week from week 8 (see Training protocol). In general, the response was better in female than in male subjects. In male subjects, isometric strength increased by ~25% at weeks 4 and 8, with a significant increase at week 12 of training (141.7±12.7%, n=4, $p<0.05$). Follow-up measurements after 28 weeks revealed that the increase in strength was consistently maintained (134.5±18.0%) (Fig. 1A). In female subjects, the increase in bilateral isometric limb strength was higher than in males, commencing at week 4 (148.5±6.5%, n=5, $p\leq0.001$), and remained significant until the end of the training period (181.2±19.3%, n=4, $p\leq0.001$). The increase in strength at 12 weeks of stimulation was not significantly different between female and male subjects.

Variations of leg extensor muscle mass were evaluated from the measurements of two thigh circumferences, one distal (just above the knee), and one proximal (at 2/3 of the knee-trochanter distance), to avoid the influence of adductor muscles. After vibrational training the distal circumferences showed small and insignificant increases (0.5-3 cm) in both male and female subjects, whereas, with proximal circum-ferences, variations ranged from a slight decrease in two female subjects to an increase (0.5-6 cm) in other subjects (Fig. 2). Moreover, skinfold measurements at the end of the training period were not significantly different from those measured before training (data not shown). In conclusion, no significant variations in muscle mass accompanied increases in muscle strength.

Muscle fiber cross-sectional area and specific tension. Single muscle fibers were isolated from biopsy fragments of the vastus lateralis muscle obtained before and after vibrational training. From each biopsy, 8-9 fibers were analyzed, to yield data on a total of 80 fibers both before and after training. The average cross-sectional area (CSA) was 3667.0±310.7 μm^2 in pre-training samples, and 4238.0±357.4 μm^2 in post-training samples, thus, vibrational training did not significantly modify the CSA of single fibers (Fig. 3A). In specific tension measurements, the isometric strength per unit of fiber area was 177.0±14.6 mN/mm^2 in pre-training samples, and 164.0±17.4 mN/mm^2 in post-training specimens. These values were not significantly different (Fig. 3B).

Myosin isoform composition of biopsy samples. The fiber type composition of the vastus lateralis muscle was determined by analyzing the proportion of slow (MyHC-1) and fast (MyHC-2A and -2X) myosin heavy chain isoforms in biopsy samples taken before and after vibrational training. Myosin isoforms can be considered as molecular markers of fiber types (24). The average values for myosin isoform distribution in males (Fig. 4A) and females (Fig. 4B) are shown. In both groups, fast MyHC-2X levels were increased after vibration training, whereas slow MyHC-1 proportions were significantly lower in post-training compared to pre-training samples. The shift in myosin isoform expression might indicate either fiber

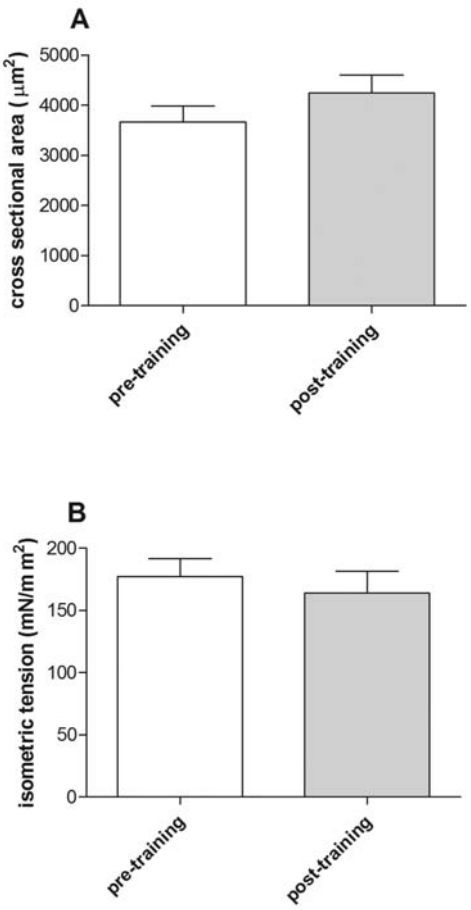


Figure 3. Single-fiber analysis, cross sectional area (A) and specific isometric tension (B). (A) Cross-sectional area (CSA) of single muscle fibers isolated from the biopsy samples from the vastus lateralis muscle before and after vibrational training of 12 weeks. (B) Measurements of specific isometric tension (force/CSA) developed in maximal calcium-activated contraction by the same fibers. In both cases, no significant difference was detected between pre- and post-training samples. For each measurement, a total of 80 fibers were analyzed.

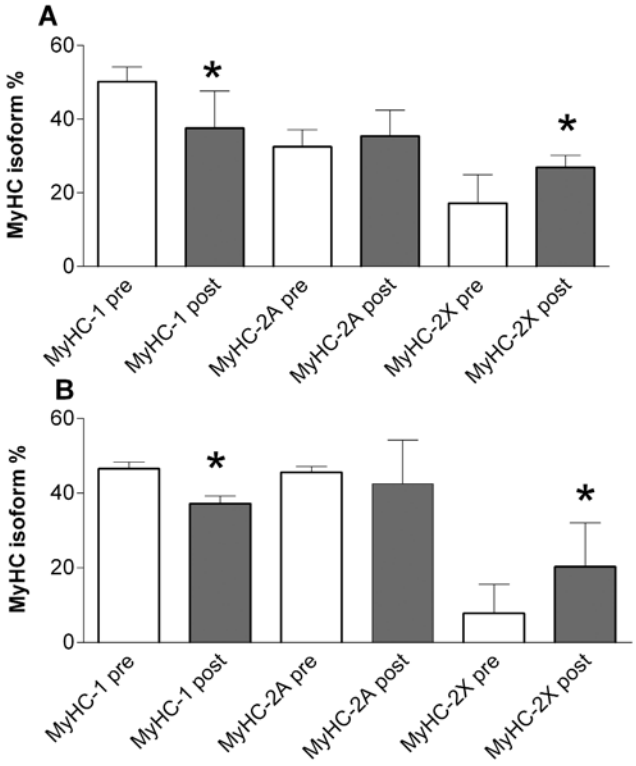


Figure 4. MyHC isoform distribution in biopsy samples collected before and after 12 weeks of vibrational training in nine elderly subjects. MyHC isoform distribution was determined by electrophoretic separation and densitometric analysis of proteins of biopsy samples from the vastus lateralis muscle. A and B show the percentage distributions of MyHC isoforms (1, 2A, and 2X) in male and female subjects, respectively. The grey columns represent the percentages of the MyHC isoform distribution post-training, and the white columns represent pre-training percentages. (A) The mean percentage of MyHC-1 decreased by 13%, whereas that of MyHC-2X increased by 12%, in elderly male subjects. (B) The mean percentage of MyHC-1 decreased by 11%, whereas that of MyHC-2X increased by 12%, in female subjects. $^{*}p \leq 0.05$.

transition (from slow to fast; 2X) or differential growth (a specific increase in the size of 2X fibers). The lack of any significant increase in fiber thickness after vibrational training (see Fig. 3) suggests that the change in myosin isoform distribution are caused by a fiber-type transition.

Gene expression profiles. Gene expression profiles from oligonucleotide microarrays were obtained from biopsy samples of three subjects before and after vibrational training. We listed the genes found significantly regulated after the vibrational training on each subject and we assessed possible changes in gene transcription analyzing these regulated genes. Genes that appeared to be significantly affected were further evaluated to elucidate the mechanisms by which vibration-trained muscles showed performance enhancement. Some genes with well-known muscle activities received particular attention, and were classified according to function. Such categories included, specifically, (i) genes of energy metabolism (Table II); (ii) genes involved in sarcomeric protein synthesis, protein degradation, and calcium homeostasis (Table III); and, (iii) genes dealing with oxidative stress (Table IV).

Genes involved in energy metabolism. Several enzymes involved in glucose and glycogen metabolism were upregulated after vibration therapy (Table II). We observed increased expression of the *phosphoinositide-3-kinase*, *regulatory subunit*, *polypeptide 3* (PIK3R3) gene that encodes a binding protein in the insulin-dependent pathway leading to inhibition of glycogen synthase kinase-3 (GSK-3), and that thus mediates net dephosphorylation of glycogen synthase (GS), with concomitant activation of the glycogen pathway (25). It is important to remember that PI3kinase functions upstream to Akt, a signaling factor very relevant to muscle hypertrophy mechanisms (26). Moreover, *protein phosphatase regulatory subunits 3A* and *3C* (PPP1R 3A and 3C), genes encoding key enzymes for glycogen conservation in muscle (25), were upregulated. Another gene showing enhanced expression was *dihydrolipoamide dehydrogenase*, *E3 complex of pyruvate dehydrogenase complex* (DLD), a protein of the pyruvate dehydrogenase complex that transforms pyruvate into acetyl-coenzyme A. In particular, the E3 component reduces NAD^{+} to NADH (27). Yet another up-regulated gene of interest was *fatty acid CoA ligase* that is important for fatty acid activation in the cytoplasm prior to ß-oxidation. *Glucan* (*1,4-α-*), *branching enzyme 1* (*glycogen branching enzyme*, [relevant

Table II. Changes in energy metabolism genes.

Subject	Gene name	Mean $\log_2 I_{post\text{-}training}/I_{pre\text{-}training}$ SD	GB accession	UniGene ID	Gene symbol
1	Phosphoinositide-3-kinase, regulatory subunit, polypeptide 3 (p55, γ)	0.86±0.39	NM_003629	88051	PIK3R3
2	Protein phosphatase 1, regulatory (inhibitor) subunit 3A (glycogen and sarcoplasmic reticulum binding	1.08±0.13	NM_002711	127614	PPP1R3A
2	Protein phosphatase 1, regulatory (inhibitor) subunit 3C	0.99±0.17	BC012625	303090	PPP1R3C
2	Dihydrolipoamide dehydrogenase (E3 component of pyruvate dehydrogenase complex, 2-oxo-glutarate complex)	0.85±0.16	NM_000108	74635	DLD
2	Fatty-acid-coenzyme A ligase, long-chain 2	0.91±0.09	NM_021122	154890	FACL2
1	Solute carrier family 16 (monocarboxylic acid transporters), member 4	0.84±0.32	NM_004696	23590	SLC16A4
1	Solute carrier family 21 (organic anion transporter), member 11	0.65±0.12	NM_013272	14805	SLC21A11
1	Solute carrier family 6 (neurotransmitter transporter), member 14	0.80±0.32	NM_007231	162211	SLC6A14
1	Ubiquinol-cytochrome c reductase core protein II	0.69±0.16	NM_003366	173554	UQCRC2
5	Glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme, Andersen disease, glycogen storage)	-1.16±0.15	NM_000158	1691	GBE1

The first column specifies in which of the three different elderly subjects (individuals 1, 2, or 5) the change in expression was detected. The second column lists the common names of the different genes up- or down-regulated in the vastus lateralis muscle, when post- and pre-training expression levels were compared. The third column reports changes in expression levels of genes as means of the log base-2 of the ratios ($\log_2 I_{post\text{-}training}/I_{pre\text{-}training}$), with SD. The fourth column lists Gene Bank Accession numbers, the fifth the UniGene Identification numbers, and the sixth specific gene symbols. Genes involved in energy metabolism were classified as upregulated (positive values) or downregulated (negative values).

to Andersen's disease and glycogen storage disease]) (GBE1), which participates in glycogen synthesis, was downregulated.

Expression of specific genes encoding several transporters was enhanced, including genes encoding *solute carrier family 16* (*monocarboxylic acid transporters*), *member 4* (SLC16A4) (28), a transport system implicated in both intracellular pH regulation during muscle contraction and lactate removal. The gene *solute carrier family 21* (*organic anion transporter*), *member 11* (SLC21A11), functioning in the transport of organic anions and thyroid hormones, and in modulation of glucose phosphorylation (29), was also up-regulated. Moreover, the expression of *solute carrier family 6* (*neurotransmitter transporter*), *member 14* (SLC6A14), a gene encoding an amino acid transporter implicated in the utilization of non-glucidic substrates for plastic cellular reorganization and as an energy source (30), was increased. Notably, we observed elevated expression of *ubiquinol-cytochrome c reductase core protein II* (UQCRC2), encoding a cytochrome reductase of the mitochondrial complex III (31).

Genes involved in sarcomeric protein synthesis and degradation, and calcium homeostasis. Several genes encoding sarcomeric proteins were upregulated (Table III). These included *actin α2* (ACTA2), one of the six actin isoforms of thin filament proteins, *destrin* (DSTN), a key enzyme in actin polymerization (32), and *myosin*, *light polypeptide*, *regulatory*, *non-sarcomeric* (MLCB), together with *chaperonin ADP-ribosylation factor-like 5* (ARL5A), and *short coiled-coil protein* (SCOCO), linked to myosin folding and myofibrillogenesis (33). In the context of protein turnover, we observed upregulation of *ubiquitin-specific protease 15* (USP15), involved in the removal of damaged proteins, and down-regulation of *ubiquitin-conjugating enzyme E2G 1* (UBE2G1), encoding a protease, and an enzyme that conjugates target proteins with ubiquitin, respectively. Moreover, expression of *calpain 3* (p94), specifically expressed in skeletal muscle, was enhanced. Calpain 3 mediates muscle remodeling by cleavage and release of myofibrillar proteins, targeting these proteins for ubiquitination and proteasomal degradation (34,35). The genes *α-actinin-2-associated LIM protein* (ALP), and *Leman coiled-coil protein* or *angiomotin-like protein 2* (LCCP), encoding proteins important for sarcomeric structure (in particular, the Z line), and *Titin immunoglobulin domain protein* (TTID), encoding a big, elastic protein that stabilizes sarcomeric filaments (36-38), were additionally upregulated. Another interesting gene displaying increased expression was *Popeye protein 3* (POP3). POP genes are expressed in cardiac and skeletal muscles (39), and participate in the synthesis and stabilization of mRNA encoding tubulin (40). Genes encoding *ryanodine type 3* (RyR3), a receptor of the sarcoplasmic reticulum, and *calcium homeostasis endoplasmic reticulum protein* (CHERP), an intracellular calcium-mobilizing agent (41), were additionally upregulated.

Table III. Genes encoding sarcomeric proteins.

Subject	Gene ID	Mean $\log_2 I_{post\text{-}training}/I_{pre\text{-}training}$ M2 VisS	GB accession	UniGene ID	Gene symbol
2	Actin, α 2, smooth muscle, aorta	1.29±0.37	NM_001613	195851	ACTA2
2	Destrin (actin depolymerizing factor)	0.83±0.13	NM_006870	82306	DSTN
1	Myosin, light polypeptide, regulatory, non-sarcomeric (20 kD)	0.80±0.32	NM_006471	233936	MLCB
2	ADP-ribosylation factor-like 5	1.09±0.20	NM_012097	342849	ARL5
2	Short coiled-coil protein	1.16±0.65	NM_032547	286013	HRIHFB2072
2	Titin immunoglobulin domain protein (myotilin)	1.07±0.26	NM_006790	84665	TTID
2	α-actinin-2-associated LIM protein	1.35±0.70	BC001017	135281	ALP
2	Leman coiled-coil protein	1.05±0.25	NM_016201	92186	LCCP
2	Ryanodine receptor 3	1.40±0.63	NM_001036	9349	RYR3
1	Calcium homeostasis endoplasmic reticulum protein	1.04±0.64	NM_006387	6430	CHERP
1	Popeye protein 3	0.89±0.18	AK055600	303154	POP3
2	Ubiquitin-specific protease 15	0.92±0.18	NM_006313	23168	USP15
5	Ubiquitin-conjugating enzyme E2G 1 (UBC7 homolog, C. elegans)	-2.23±0.41	NM_003342	78563	UBE2G1
1	Calpain 3, (p94)	0.84±0.22	NM_000070	40300	CAPN3

The first column specifies in which of the three different elderly subjects (individuals 1, 2, or 5) the change in expression was detected. The second column lists the common names of the different genes up- or down-regulated in the vastus lateralis muscle, when post- and pre-training expression levels were compared. The third column reports changes in expression levels of genes as means of the log base-2 of the ratios ($\log_2 I_{post\text{-}training}/I_{pre\text{-}training}$) with SD. The fourth column lists Gene Bank Accession numbers, the fifth lists the UniGene Identification numbers, and the sixth, specific gene symbols. Genes involved in energy metabolism were classified as upregulated (positive values) or downregulated (negative values).

Table IV. Genes involved in oxidative stress.

Subject	Gene ID	Mean $\log_2 I_{post\text{-}training}/I_{post\text{-}training}$ M2 VisS	GB accession	UniGene ID	Gene symbol
2	Nitric oxide synthase 1 (neuronal)	-1.18±0.53	NM_000620	46752	NOS1
2	DNA polymerase epsilon p12 subunit	-1.14±0.31	NM_019896	19980	P12
2	Peroxiredoxin 3	-1.12±0.35	NM_006793	75454	PRDX3
2	Thioredoxin	-1.11±0.47	NM_003329	76136	TXN
2	Methionine sulfoxide reductase A	-0.93±0.47	NM_014772	64096	MSRA

The first column specifies in which of the three different elderly subjects (individuals 1, 2, or 5) the change in expression was detected. The second column lists the common names of the different genes up- or downregulated in the vastus lateralis muscle, when post- and pre-training expression levels were compared. The third column reports changes in expression levels of genes as means of the log base-2 of the ratios ($\log_2 I_{post\text{-}training}/I_{post\text{-}training}$), with standard deviations (SDs). The fourth column lists Gene Bank Accession numbers, the fifth the UniGene Identification numbers, and the sixth specific gene symbols. Genes involved in energy metabolism were classified as upregulated (positive values) or downregulated (negative values).

Genes involved in oxidative stress. Oxidation of biological substrates, such as DNA, proteins, and lipids, in the elderly, is well-recognized, as oxidants are generated in the mitochondrial respiratory chain, and the deleterious function of such oxidants is particularly important in muscle fibers (1). Vibrational training caused down-regulation of the polymerase ε (P12) gene, encoding a low-affinity enzyme involved in DNA duplication and repair (42) and cell cycle control (Table IV). This enzyme, an important contributor to DNA repair, is involved in the rescue of oxidized DNA, although enzyme activity is less efficient than that of other polymerases. Furthermore, expression of the *peroxiredoxin* (PRDX3) gene was decreased. PRDX3 is a specific antioxidant enzyme able to remove endogenous cytokine-induced

H_2O_2 (43), using electrons donated by thioredoxin (TXN), the gene for which was also downregulated. *PRDX3* encodes a protein that acts as an antioxidant by facilitating the reduction of other proteins via cysteine thioldisulfide exchange. Another gene displaying diminished expression was methionine *sulfoxide reductase* (MSRA), the protein product of which repairs oxidized methionine (44), one of the most important targets of protein oxidation. Moreover, downregulation of the *constitutive nitric oxide synthase* (NOS1) was also observed. This gene encodes a constitutive form of the enzyme that synthesizes nitric oxide from L-arginine in the presence of NADPH and O_2, and downregulation may be linked to an altered nitric oxide signaling mechanism in aged skeletal muscle (45).

Discussion

Muscle isometric strength is defined as the maximum force exerted against resistance, and decreases significantly in humans with age (46). Healthy elderly subjects typically display diminished muscle mass, reduced movement velocity and skeletal muscle strength, combining to form the diagnostic characteristics of the condition termed sarcopenia (1).

In recent years, novel 'passive training' methods were proposed for clinical treatment of muscle atrophy and in sport training, consisting of mechanical vibrations applied to specific muscles or over the entire body. A number of investigators showed that such treatments enhance muscle strength and power (8,47), plasma concentrations of testosterone and growth hormone (5), and neuromuscular functions (6). Other studies, however, have drawn opposite conclusions, claiming that no improvement of muscle performance was obtained after vibration treatment (9). In addition, negative effects of whole-body vibration on health were documented. Workers exposed to daily vibration display vascular disorders (10), increased levels of lumbar prolapse, and lower back pain (12).

In this study, the measurements of knee extensor muscle isometric torque, obtained during pre-, during (weeks 4 and 8), and at the end of training, confirmed that local high-intensity vibration increases overall muscle strength in elderly male and female subjects. Specifically, isometric strength was elevated by the fourth week of stimulation, remained high during the training period, and also for at least several weeks after cessation of training. Indeed, follow-up measurements at 16 weeks after the end of training revealed consistently high values of muscle strength, similar to the levels recorded at the end of the vibrational protocol, for both female and male volunteers, although some inter-individual variability was noted.

The sustained increase in lower limb strength suggests modifications in the properties of skeletal muscle. Measurements of thigh circumferences and skinfolds showed that the force increase was not accompanied by an increase in muscle mass. We therefore examined the mechanical properties of single fibers to determine whether the changes can be explained at the cellular level. After 12 weeks of local high-intensity vibration, neither the cross-sectional area nor the specific tension of the vastus lateralis muscle fibers was changed, compared to pre-training data. Thus, the change in force of the whole extensor muscle group is not based on an alteration in the force-developing ability of single fibers. Previous studies have shown that muscle disuse in the elderly causes a decrease in specific tension (48), whereas resistance training in body builders is associated with increased specific tension (49). Apparently, the improved strength of leg extensor muscles after vibrational training is not related to an increase in muscle fiber-specific tension. Biopsy examination, however, yielded a valuable and unexpected result. The fiber phenotype distribution of the skeletal muscle was altered. The proportion of the fast MyHC-2X isoform was increased, and the proportion of the slow MyHC-1 isoform was significantly lower, in post-training biopsies compared to pre-training biopsies. This shift in myosin isoform expression is a clear indication of changes in fiber type distribution (49). Fiber type transition from slow to 2X, as suggested by the shift in myosin isoforms, should correspond to changes in the proportions of oxidative and glycolytic fibers, because, in human muscles, slow fibers are mainly oxidative whereas 2X fibers are principally glycolytic (24). Importantly, expression profile analysis validated this metabolic feature. In fact, indications for changes in fiber type distribution were seen in the transcriptional profile. In particular, changes in two of three functional categories (metabolic genes and those encoding sarcomeric proteins) of differentially regulated genes were identified in all screened elderly subjects. Glycolytic and glycogen-dependent metabolism appeared to be stimulated in trained subjects. In this study, personal transcriptional profiles, including data on specific differentially regulated genes, were generated for each of three subjects. Initially, the results appeared inconsistent, but, when the experimental plan was considered, and, particularly, when the long-term nature of the treatment was properly weighted, the transcriptional profiles of the three elderly subjects can be best regarded as specific temporal windows on genes functioning after consolidated or stabilized metabolic (in the wide sense) modifications. We employed a microarray technique to simultaneously screen all pathways activated at a given time-point, and we were able to detect compensatory pathways activated in the cells. Metabolic genes, either up- or down-regulated, were indeed distinct between subjects, but converged into enhancement of the glycogenosynthesis/glycolysis pathway. Glycogenosynthesis is considered as a compensator for increased glucose consumption during training. This hypothesis is consistent with the increased proportion of (specifically) fast MyHC-2X fibers as these fibers employ glycolytic metabolism.

The upregulation of genes, such as actin, destrin, titin and angiomotin, encoding sarcomeric proteins, is consistent with an increase in regeneration that recruits satellite cells to proliferate and fuse into new differentiated myotubes, that are comparable to *in vivo* primary fibers. We hypothesize that new fibers are generated during the first 4 weeks of local high-intensity vibrational stimulation, based on data showing that at week 4, the bilateral isometric strength of lower limbs was significantly increased. We used a biopsy fragment from one subject to collect and grow satellite cells (50), to study whether myogenesis properties *in vitro* are influenced by local high-intensity vibrational training. We induced the differentiation program in these cells, and, after 7 days of differentiation, we used an antibody MF20 against myosins

to measure the proportion of myotubes expressing the myosin heavy chain proteins. The preliminary data reveal that, after vibrational training, the proportion of MF20-positive myotubes increased by 11.8% (from 41.3% pre-training to 53.1% post-training). These data further support the hypothesis that novel fibers stimulated by local high-intensity vibration are induced to differentiate into new, mature, fast MyHC-2X fibers.

Sarcopenic skeletal muscle shows reduced metabolic power because of several factors, such as decreased blood perfusion, increased fibrosis, and development of the atrophic state. Skeletal muscle tissue undergoes constant oxidation, and the main source of reactive oxygen species is the mitochondrion. A previous study by our group showed consistent oxidative damage in aged skeletal muscle tissue (1). Altered metabolism impairs the balance between oxidative stress and scavenger activity. In fact, several antioxidant genes were downregulated in the elderly, suggesting that ROS scavenger activity is insufficient. Peroxiredoxin is an important anti-oxidant enzyme that counteracts lipid per-oxidation, protecting cells by removing H_2O_2 (51). Thioredoxin plays a key role in oxidoreduction reactions (43). Methionine sulfoxide reductase A counteracts methionine oxidation of proteins (44), whereas polymerase ε is able to repair oxidized DNA, albeit at low efficiency (42). The downregulated constitutive NO synthase (NOS) could be responsible for lowered nitric oxide production and may disrupt nitric oxide signaling in skeletal muscle arterioles, thus impairing vasodilatation in old age (45). Also, in a study of atrophy induction by horizontal bed rest in young males, it was observed that sarcolemmal NOS1 immuno-fluorescence in the vastus lateralis muscle increased (52). These opposite effects may be explained by age-related variations in muscle conditioning. In fact, during aging, the bioavailability of substrates and cofactors important for NOS activity decreases (45), and this was not reversed by high intensity vibrational training applied to elderly subjects.

Only one elderly subject (subject 2, Table IV) showed downregulation of antioxidant genes mentioned above. This observation suggests that local high-intensity vibration exacerbate existing physiological and personal gene regulation conditions.

Overall, the results of our study indicate that vibrational training was accompanied by several changes at the muscle molecular level. However, the lack of correlation between marked increases in the force developed during maximal voluntary contraction on the one hand, and muscle mass, single fiber thickness, or specific tension, on the other, suggests that nervous control of contraction must also be considered as possibly influenced by our training protocol. Improved recruitment or better activation of motor units might also be invoked to explain the observed increases in muscle force. A recent study by Fattorini *et al* (14) provided evidence of long-lasting changes in proprioceptive motor control after vibrational training at 100 Hz, and such changes may be partly responsible for the improved contractile performance observed here.

In conclusion, our data validate the effectiveness of the VISS training procedure to counteract sarcopenia. The technique is easy to use, requires little patient commitment or time, and can be employed on patients with joint and/or neuro-muscular disorders.

Acknowledgements

We thank Dr Bruno Loffredo for cardiological consultations and Professor Enzo Ballone for assistance with statistical analysis. This study was supported by research grants awarded to Professors R. Saggini, G. Fanò, and S. Fulle from the University ‘G. d'Annunzio’ of Chieti-Pescara.

References

1. Fulle S, Protasi F, Di Tano G, *et al*: The contribution of reactive oxygen species to sarcopenia and muscle ageing. Exp Geront 39: 17-24, 2004.
2. Argiles JM, Busquets S, Felipe A and Lopez-Soriano FJ: Muscle wasting in cancer and ageing: cachexia versus sarcopenia. Adv Gerontol 18: 39-54, 2006.
3. Avila-Funes JA and García-Mayo EJ: The benefits of doing excercise in the elderly. Gac Med Mex 140: 431-436, 2004.
4. Mühlberg W and Sieber C: Sarcopenia and frailty in geriatric patients: implications for training and prevention. Gerontol Geriatr 37: 2-8, 2004.
5. Bosco C, Cardinale M and Tsarpela O: Influence of vibration on mechanical power and electromyogram activity in human arm flexor muscles. Eur J Appl Physiol Occup Physiol 79: 306-311, 1999.
6. Bosco C, Iacovelli M, Tsarpela O, *et al*: Hormonal responses to whole-body vibration in men. Eur J Appl Physiol 81: 449-454, 2000.
7. Kvorning T, Bagger M, Caserotti P and Madsen K: Effects of vibration and resistance training on neuromuscular and hormonal measures. Eur J Appl Physiol 96: 615-625, 2006.
8. Bosco C, Colli R, Introini E, *et al*: Adaptive responses of human skeletal muscle to vibration exposure. Clin Physiol 19: 183-187, 1999.
9. De Ruiter CJ, Van Raak SM, Schilperoort JV, Hollander AP and de Hann A: The effects of 11 weeks whole body vibration training on jump height, contractile and activation of human knee extensors. Eur J Appl Physiol 90: 595-600, 2003.
10. Bovenzi M: Exposure-response relationship in the hand-arm vibration syndrome: an overview of current epidemiology research. Int Arch Occup Environ Health 71: 509-519, 1998.
11. Bovenzi M, D'Agostin F, Rui F, Ambrosi L and Zefferino R: Salivary endothelin and vascular disorders in vibration-exposed workers. Scand J Work Environ Health 34: 133-141, 2008.
12. Lings S and Leboeuf-Yde C: Whole-body vibration and low back pain: a systematic, critical review of the epidemiological literature 1992-1999. Int Arch Occup Environ Health 73: 290-297, 2000.
13. Seidel H: On the relationship between whole-body vibration exposure and spinal health risk. Ind Health 43: 361-377, 2005.
14. Fattorini L, Ferraresi A, Rodio A, Azzena GB and Filippi GM: Motor performance changes induced by muscle vibration. Eur J Appl Physiol 98: 79-87, 2006.
15. Ribot-Ciscar E, Rossi-Durand C and Roll JP: Muscle spindle activity following muscle tendon vibration in man. Neurosci Lett 258: 147-150, 1998.
16. Eklund G and Hagbarth KE: Normal variability of tonic vibration reflexes in man. Exp Neurol 16: 80-92, 1966.
17. Brown MC, Enberg I and Matthews PB: The use of vibration as a selective repetitive stimulus for Ia afferent fibers. J Physiol 191: 31-32, 1967.
18. Crone C and Nielsen J: Central control of disynaptic reciprocal inhibition in humans. Acta Physiol Scand 152: 351-363, 1994.
19. Brunetti O, Filippi GM, Lorenzini M, *et al*: Improvement of posture stability by vibratory stimulation following anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc 14: 1180-1187, 2006.
20. Bottinelli R, Canepari M, Pellegrino MA, *et al*: Force-velocity properties of human skeletal muscle fibers: myosin heavy chain isoform and temperature dependence. J Physiol 495: 573-586, 1996.
21. Toniolo L, Maccatrozzo L, Patruno M, *et al*: Fiber types in canine muscles: myosin isoform expression and functional characterization. Am J Physiol 292: C1915-C1926, 2007.
22. Talmadge RJ and Roy RR: Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. J Appl Physiol 75: 2337-2340, 1993.
23. Jonnalagadda S, and Srinivasan R: Principal components analysis based methodology to identify differentially expressed genes in time-course microarray data. BMC Bioinformatics 9: 267, 2008.

24. Schiaffino S and Reggiani C: Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiol Rev 76: 371-423, 1996.
25. Delibegovic M, Armstrong CG, Dobbie L, Watt PW, Smith AJ and Cohen PT: Disruption of the striated muscle glycogen targeting subunit PPP1R3A of protein phosphatase 1 leads to increased weight gain, fat deposition, and development of insulin resistance. Diabetes 52: 596-604, 2003.
26. Schiaffino S, Sandri, M and Murgia M: Activity-dependent signaling pathways controlling muscle diversity and plasticity. Physiology 22: 269-278, 2007.
27. Smolle M, Prior AE, Brown AE, Cooper A, Byron O and Lindsay JG: A new level of architectural complexity in the human pyruvate dehydrogenase complex. J Biol Chem 281: 19772-19780, 2006.
28. Bishop D, Edge J, Thomas C and Mercier J: High-intensity exercise acutely decreases the membrane content of MCT1 and MCT4 and buffer capacity in human skeletal muscle. J Appl Physiol 102: 616-621, 2007.
29. Abe T, Kakyo M, Tokui T, *et al*: Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. J Biol Chem 274: 17159-17163, 1999.
30. Durand E, Boutin P, Meyre D, Charles MA, Clement K, Dina C and Froguel P: Polymorphisms in the amino acid transporter solute carrier family 6 (neurotransmitter transporter) member 14 gene contribute to polygenic obesity in French Caucasians. Diabetes 53: 2483-2486, 2004.
31. Hu WH, Hausmann ON, Yan MS, Walters WM, Wong PK and Bethea JR: Identification and characterization of a novel Nogo-interacting mitochondrial protein. J Neurochem 81: 36-45, 2002.
32. Estornes Y, Gay F, Gevrey JC, Navoizat S, *et al*: Differential involvement of destrin and cofilin-1 in the control of invasive properties of Isreco1 human colon cancer cells. Int J Cancer 121: 2162-2171, 2007.
33. Panic B, Whyte JR and Munro S: The ARF-like GTPases Arl1p and Arl3p act in a pathway that interacts with vesicle-tethering factors at the Golgi apparatus. Curr Biol 13: 405-410, 2003.
34. Sorimachi H, Toyama-Sorimachi N, Saido TC, *et al*: Muscle-specific calpain, p94, is degraded by autolysis immediately after translation, resulting in disappearance from muscle. J Biol Chem 268: 10593-10605, 1993.
35. Kramerova I, Kudryashova E, Venkatraman G and Spencer MJ: Calpain 3 participates in sarcomere remodeling by acting upstream of the ubiquitin-proteasome pathway. Hum Mol Genet 14: 2125-2134, 2005.
36. Tskhovrebova L and Trinick J: Titin: properties and family relationships. Nat Rev Mol Cell Biol 4: 679-689, 2003.
37. Labeit S, Lahmers S, Burkart C, *et al*: Expression of distinct classes of titin isoforms in striated and smooth muscles by alternative splicing, and their conserved interaction with filamins. J Mol Biol 362: 664-681, 2006.
38. Bratt A, Wilson WJ, Troyanovsky B, Aase K, Kessler R, Van Meir EG and Holmgren L: Angiomotin belongs to a novel protein family with conserved coiled-coil and PDZ binding domains. Gene 298: 69-77, 2002.
39. Andrée B, Hillemann T, Kessler-Icekson G, Schmitt-John T, Jockusch H, Arnold HH and Brand T: Isolation and characterization of the novel popeye gene family expressed in skeletal muscle and heart. Dev Biol 223: 371-382, 2000.
40. Ochotorena IL, Hirata D, Kominami K, *et al*: Conserved Wat1/Pop3 WD-repeat protein of fission yeast secures genome stability through microtubule integrity and may be involved in mRNA maturation. J Cell Sci 114: 2911-2920, 2001.
41. Laplante JM, O'Rourke F, Lu X, Fein A, Olsen A, and Feinstein MB: Cloning of human Ca^{2+} homoeostasis endoplasmic reticulum protein (CHERP): regulated expression of antisense cDNA depletes CHERP, inhibits intracellular Ca^{2+} mobilization and decreases cell proliferation. Biochem J 348: 189-199, 2000.
42. Sugino A, Ohara T, Sebastian J, Nakashima N and Araki H: DNA polymerase epsilon encoded by $cdc20^{+}$ is required for chromosomal DNA replication in the fission yeast schizo-saccharomices pombe. Genes Cells 3: 99-110, 1998.
43. Nordberg J and Arnér ES: Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med 31: 1287-1312, 2001.
44. Vattanaviboon P, Seeanukun C, Whangsuk W, Utamapongchai S and Mongkolsuk S: Important role for methionine sulfoxide reductase in the oxidative stress response of Xanthomonas campestris pv. Phaseoli J Bacteriol 187: 5831-5836, 2005.
45. Delp MD, Behnke BJ, Spier SA, Wu G and Muller-Delp JM: Ageing diminishes endothelium-dependent vasodilatation and tetrahydrobiopterin content in rat skeletal muscle arterioles. J Physiol 586: 1161-1168, 2008.
46. Doherty TJ: Invited review: Aging and sarcopenia. J Appl Physiol 95: 1717-1727, 2003.
47. Samuelson B, Jorfeldt L, Ahlborg B: Influence of vibration on endurance of maximal isometric contraction. Clin Physiol 9: 21-25, 1989.
48. D'Antona G, Pellegrino MA, Adami R, *et al*: The effect of ageing and immobilization on structure and function of human skeletal muscle fibers. J Physiol 552: 499-511, 2003.
49. D'Antona G, Lanfranconi F, Pellegrino MA, *et al*: Skeletal muscle hypertrophy and structure and function of skeletal muscle fibers in male body builders. J Physiol 570: 611-627, 2006.
50. Beccafico S, Puglielli C, Pietrangelo T, Bellomo R, Fanò G and Fulle S: Age-dependent effects on functional aspects in human satellite cells. Ann NY Acad Sci 1100: 345-352, 2007.
51. Rhee SG, Chae HZ and Kim K: Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signalling. Free Radic Biol Med 38: 1543-1552, 2005.
52. Rudnick J, Püttmann B, Tesch PA, *et al*: Differential expression of nitric oxide synthases (NOS 1-3) in human skeletal muscle following exercise countermeasure during 12 weeks of bed rest. FASEB J 18: 1228-1230, 2004.

NeuroRehabilitation 32 (2013) 855–860
DOI:10.3233/NRE-130909
IOS Press

Combined rehabilitation program for postural instability in progressive supranuclear palsy

L. Di Pancrazio[a], R.G. Bellomo[a], R. Franciotti[b], P. Iodice[b], V. Galati[a], A. D'Andreagiovanni[b], S. Bifolchetti[b], A. Thomas[b], M. Onofrj[b], L. Bonanni[b,1,*] and R. Saggini[b,1]
[a]*Department of Human Movement, "G. d'Annunzio" University, Chieti, Italy*
[b]*Department of Neuroscience and Imaging, "G. d'Annunzio" University, Chieti, Italy*

Abstract.
BACKGROUND: Progressive supranuclear palsy (PSP) is an atypical parkinsonism clinically characterized by prominent axial extrapyramidal motor symptoms with frequent falls. The clinical response to L-dopa is poor and there is strong need for alternative treatment strategies.
METHODS: We tested the efficacy of a rehabilitative program combining a dynamic antigravity postural system (SPAD) and a vibration sound system (ViSS) on postural instability of 10 patients affected by PSP. The patients underwent SPAD and VISS treatments with a 3 sessions/week schedule for 2 months. Patients were clinically examined at baseline, every week during the 2-months treatment, and at 1 month after the end of treatment for the following parameters: baropodometry static, baropodometry dynamic and stabilometry. PSP rating scale and PD36 quality of life scale were also administered.
RESULTS: The combined rehabilitative program produced improvement of all the parameters explored ($p = 0.01$–0.05) at the end of treatment as compared to baseline. Baropodometric dynamics improvement lasted until the end of follow-up.
CONCLUSION: Our results suggest that a specific rehabilitation program could improve postural instability in PSP patients. A more continuous treatment protocol would allow stabilizations of results.

Keywords: Progressive supranuclear palsy, rehabilitation, SPAD, VISS

1. Introduction

Progressive supranuclear palsy (PSP) is an atypical parkinsonism clinically characterized by prominent axial extrapyramidal motor symptoms, including rigidity, bradykinesia, impaired balance, gait difficulties which progress at a faster rate than in Parkinson's Disease (PD), with slow walking and the tendency to tumble forward or backward. The consequential frequent falls represent a major problem for PSP patients, causing head traumas and bone fractures.

Axial parkinsonian signs typically show a poor response to dopaminergic treatment (Litvan et al., 1996; Bonanni et al., 2007). Therefore there is a strong need of alternative treatment techniques to cope with the aforementioned problems.

Different rehabilitative strategies have been tentatively applied to PSP patients, especially on visual disturbances (Zampieri & Di Fabio, 2008), but more structured studies are needed to quantify the efficacy of specific physical treatments.

A dynamic antigravity postural system (SPAD) is available at the University center for Motor Sciences of the University G. d'Annunzio of Chieti-Pescara, Italy.

It is composed by a conveyor belt, surmounted by a lifting structure, which forces the patient to walk in a straight line on a treadmill; able to work at very low

[1]These two authors equally contributed to the work.

*Address for correspondence: Laura Bonanni, Department of Neuroscience and Imaging, University G.d'Annunzio of Chieti-Pescara, 66013 Chieti, Italia. Tel./Fax: +39 0871562019; E-mail: l.bonanni@unich.it.

speed (0.01 Km/h) and allowing small speed increases (Hesse, 2001, Saggini et al., 2004).

The patient is suspended through a mechanical antigravity vertical traction system; a pneumatic suspension system, including a self-leveling system, which allows to follow the patient center of gravity during its vertical excursions and a second mechanical lateral traction system which allows to stabilize the pelvis. The SPAD includes four anterior mobile arms which act on the rotation components of scapulohumeral and pelvic girdles and on antero-superior iliac spines; and two posterior blocking systems located on the back of the patient at the T3-T4 vertebral level and on sacral apex.

The system works with a dual action, mechanical, allowing neuromotor retraining with cortico-subcortical forms of learning to acquire new body schema in a balance that minimizes the energy consumption needed to maintain posture, and proprioceptive, acting maintaining the adaptations induced in time relative to walking.

SPAD has been demonstrated to be able to improve postural stability in patients with Spinal Cord Injury and Low-Back pain (Dobkin et al., 2006; Saggini et al., 2004). ViSS, vibration sound system, is a multi-frequency system which reaches 300 Hz and an amplitude of 200 mbar which uses focalized mechanic-acoustic vibrations (Saggini et al., 2006; Karnath et al., 2000).

Adaptive metabolic and mechanical responses of the human neuromuscular apparatus subjected to mechanical acoustic vibrations (MAV) are widely supported in the literature (Lundeberg et al., 1984; Seidel 1988; Tanaka et al., 2003; Saggini et al., 2006). These vibrations applied to muscle bellies and tendons cause the "vibration tonic reflex" characterized by an improvement in power contraction of the stimulated muscles. Adaptations caused by the vibration tonic reflex involve particularly the superior motor centers of the neuromuscular apparatus (Pasetti & Ferriero, 2008). These responses are characterized by an improvement in the neural stimulation that permits recruitment of a wider number of muscular fibers.

The high-intensity focused vibratory stimulation has been shown capable of increasing the strength and muscular endurance. The ability to reach frequencies of 300 Hz to induce a "long term potentiation" allows to stimulate different receptors muscle and skin type mechanical and proprioceptive (corpuscles of Pacini, Golgi tendon organs, mechanoreceptors of type III-IV and muscle spindle) with the ultimate goal to increase the contractile capacity, flexibility and recruitment of fibers of the skeletal muscle tissue.

In this pilot study, repeated sessions of a combination of SPAD and VISS treatments were administered to 10 PSP patients in the attempt to restore a correct posture and to reduce postural instability. A 1 month follow-up evaluation was performed to assess stabilization of effects.

2. Materials and methods

Ten PSP patients, 7 male, 3 female, were randomly selected among 38 PSP patients afferent to the Movement Disorder Center of University G. d'Annunzio of Chieti-Pescara, serving a population of 1,200,000 in Abruzzo Region, Italy.

Diagnosis of PSP was made according to International Criteria (Litvan et al., 1996).

To be admitted to the study the patients had to be able to stand alone and to be free from serious co-morbidities, including thoraco-abdominal aneurysms and heart disease which contraindicate SPAD treatment.

Postural instability was quantified by PSP Rating Scale (item 27: postural stability). A score of 1 to 3 was required to be admitted to the study.

All patients were under L-dopa treatment, at the best of their clinical response and their daily dosage had to be stable in the month preceding the admission to the study.

Exclusion criteria were: cardiovascular diseases; severe cerebrovascular disease; diseases of the endocrine system; respiratory diseases; food intolerances and drug allergies.

The patients underwent SPAD and VISS treatments with a 3 sessions/week schedule for 2 months.

Every single SPAD session lasted twenty minutes. The patient body weight was alleviated by 20 to 30%. Every subject was asked to walk on a treadmill composed and aligned, straight pull, looking in the mirror positioned in front of him, to walk with correct and long strides, and to properly roll his foot on the floor (heel-plant-toes).

Patients were clinically examined at baseline, every week during the 2-months treatment, and at 1 month after the end of treatment.

The study was approved by the ethical committee of the local university. All participants gave written informed consent.

The following tests were administered at each evaluation session:

– digital biometrics called D.B.I.S (Digital Biometry Images Scanning) (Diasu, Roma, Italy);

The use of digital biometric measurements allows the observation of different structural characteristics of the human body: morphological characteristics (baropodometry static), the motor function (baropodometry dynamic) and receptor activity (stabilometry). These body characteristics are studied on three projections: frontal (anterior and posterior), sagittal (right and left side) and transverse (from the top).

The Baropodometry Static allows to monitor the subject posture in the standing position. The patient is required to stand on the floor bare feet assuming a natural, relaxed position for five seconds. The total area (cm^2) covered by left and right foot separately is quantified. This indicator allows the assessment of latero-lateral asymmetries of support and therefore possible overload on a single lower limb.

The Baropodometry Dynamic examines the different steps of body support, with visualization of the center of pressure of each foot during walk. The analysed indices are walking speed (m/sec), semite length (cm; it represents the distance between the first point of support of a foot and the next first point of support of the other foot) and semistep width (cm; distance between the most protruding part of the heel and the line of progression of the path, which passes between the two feet).

Stabilometry is a method that reliably quantifies the position of the body's center of pressure (COP). COP is defined as the coordinates of the resultant force applied through the feet on the force plate. It is the point of the vertical ground reaction force vector. It represents the average of all the pressures over the surface that are in contact with the ground. If only one foot is on the ground, the COP lies within that foot. If both feet are in contact with the ground, the COP lies somewhere between the two feet, depending on the relative weight borne by each foot. This method has been used extensively in our rehabilitative unit in both normal subjects and different patients groups allowing to set a normality range.

– Myometry (Diagnostic Support, Roma, Italy) (Simons & Mense, 2003);

The myoton is a patented, portable (<0.5 kg), non-invasive method, to measure the mechanical properties of muscles: tone, elasticity and strength. The Tone is the mechanic tension in the muscle which cannot be diminished voluntarily. The Elasticity is the ability of the muscle to regain its initial shape after a mechanical alteration. It is an index of the health condition of the muscle. The Stiffness is the ability of the muscle to resist to changes in its shape by external forces, and it is linked to the resistance provoked by the antagonist muscles.

– Berg Balance Scale (BBS) (Berg, 1992), Progressive Supranuclear Palsy Rating Scale (PSP-RS) (Golbe, 2007) and PDQ39 scale (Jenkinson, 1997) were administered at each time point.

3. Statistical analysis

One-way analysis of variance (one-way ANOVA) was applied to each evaluation tests with evaluation session as factor (PRE, T1, T2, T3, POST, 15 day follow-up, 30 day follow-up). For the total load (%) and the total area (cm^2), two-ways ANOVAs were performed with evaluation session and side (left and right foot) as factors. Duncan's *post hoc* tests were calculated for the significant effects.

4. Results

Demographic and clinical data are presented in Table 1.

Stabilometry test after the treatment showed a significant improvement of the distribution of the load in percentage as compared to pre-treatment condition. Statistical results on the total load (%) and on the total area (cm^2) covered by the foots showed a significant interaction between the side (right and left foot) and the evaluation session ($p < 0.01$). Specifically, the total load and the total area covered by the left foot was larger

Table 1
Demographic and clinical data

	AGE (years)	69 ± 7
	Daily L-dopa dose (mg)	638 ± 226
	MMSE	29 ± 1
PSP-rating scale	History	6 ± 2
	Mentation	2 ± 1
	Bulbar	3 ± 1
	Ocular	11 ± 2
	Limb	7 ± 2
	Gait	11 ± 2
	Tot	39 ± 4

All values represent mean ± standard deviation across group. Mini Mental State Examination (MMSE) score was normal for all patients.

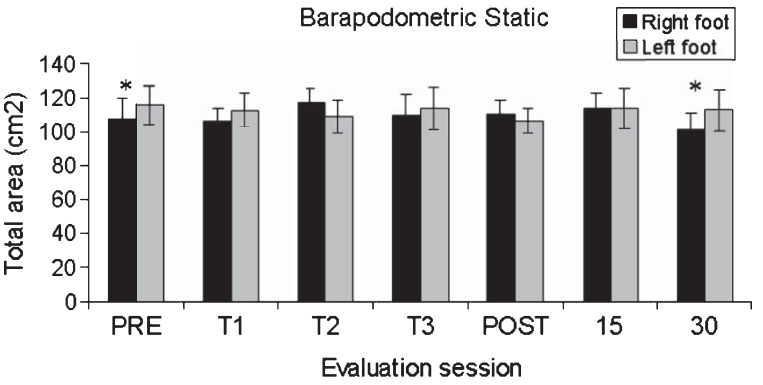


Fig. 1. Total area for left and right foot during the evaluation sessions (PRE treatment, during treatment, i.e. T1, T2, T3, POST treatment, 15 and 30 day follow-up). During the treatment and after 15 day follow-up, the distribution of the total area was not different between the left and the right foot, indicating a better distribution of the body weight between the two hemisomata. $^{*}=p<0.05$.

than the right foot before the treatment and after 30 day follow-up ($p=0.04$). Figure 1 shows the total area for the left and the right foot during the evaluation sessions. During the treatment and after 15 days follow-up, the distribution of the total load and the total area was not different between the left and the right foot, indicating a better distribution of the body weight between the two hemisomata.

The average pressure (gr/cm^2) improved, due to a more balanced redistribution of the load between the two hemisomata ($p<0,05$); as confirmed by the static baropodometry.

These data indicate more strength in the push of step, more security and stability while walking.

Baropodometric dynamics showed a reduction of the time of the foot contact to the ground, with a consequent raise of the walking speed at the end of treatment (POST) as compared to baseline ($p=0.02$), which persisted also in the follow-up phase ($p=0.05$). An increase of the semilength of the step (cm) during walking was evident by the end of treatment (POST) respect to the baseline ($p<0.001$) and persisted at one month after the treatment ($p<0.001$), highlighting a reduction of the base of support and consequently more stability.

The morphological analysis allowed us to evaluate and quantify the possible asymmetries in a patient compared with the position of reference. An improvement of the relation between cervical, thoracic and lumbar arrows was evidenced, producing a better postural alignment of the patient, and a reduction of the dysmetria between the two hemisomata. In fact, the reduction of arrows ($p<0.05$) showed a better postural alignment and a minor trunk anterior flexion, producing a better stability, thus contrasting the typical camptocormia (Litvan et al., 1996). This postural improvement persists also in the follow-up phase after 30 days ($p<0.05$).

Myometric analysis evidenced an Improvement of elasticity, stiffness and tone of examined muscles at the end of treatment ($p<0.05$).

The elasticity improved in all muscles, except for biceps femoris and tibialis anterior where no elasticity change was observed. The improved elasticity translates into a less energy requirement.

The stiffness, or the ability of the muscle to resist to an external force, went towards a reduction in all analyzed muscles. The tension reduction led to less efforts by the patient to win the resistance of the antagonist muscles, creating in this way a better fluidity and economy in the act of movement.

The muscular tone increased in rectus femoris, tibialis anterior and gluteus. The tone of biceps femoris decreased; this can be anyway considered positively, because the patient affected by Parkinsonism tends to use mainly flexor muscles of the leg (biceps femoris) to maintain the balance in standing position, contrarily to a healthy subject, who utilizes more the activation of extensor muscles of the leg (Dietz et al., 1993).

These results disappeared however after treatment.

PSP Rating Scale showed improvement of the motor score posture item ($p=0.01$), and in the motor score postural stability item ($p=0.01$), which confirms the data of the digital biometric analysis.

These data were however not confirmed at follow up evaluation.

Berg Balance Scale (BBS) score varied from a 37.7 ± 12.1 at the baseline to a score of $47,6\pm9.2$ at the end of treatment ($p=0.02$).

PDQ39 scale showed a significant improvement during the treatment which lasted until the end of follow-up ($p = 0.03$).

5. Discussion

The aim of our study was to create a rehabilitation program effective and able to influence in a positive way the quality of life of the patient affected by an atypical Parkinsonism, through the recovery of postural control, the use by the patient of a more stable scheme of walking and less demanding from the point of view of the energy necessary, a raise of the muscular strength and resistance and a raise of the mobility.

The research held in our center emphasized the existence of new techniques of rehabilitation to treat patients affected by atypical parkinsonism.

The postural re-programming obtained by the SPAD method allowed the observation of evident improvement of the values of important morphologic structural parameters. The rehabilitation program has also determined an improvement of balance of the patients while walking. The neurophysiological rationale is summarized in the stimulation of the plasticity of the system of the neuro-motor control, through a repetition of a motor task, functionally corrected, in an intensive/extensive form.

The alternating loading/unloading of the leg and hip while walking, are the main peripheral stimuli that activate the locomotor patterns central generators (Hausdorff et al., 1998). The improvement of walking could hypothetically suggest a re-learning process and a plastic re-adaptation of a walking generator which allows a more physiological and efficient gait cycle.

The stabilizing action on the body also causes beneficial effects on balance control through better synergy of anti-gravity muscles, a quicker response of the apparatus of support resulting in more rapid repositioning of the body center of gravity.

The SPAD treatment corrected the asymmetry of the two hemisomata and modified asymmetric adaptations of gait, using a vertical movement of the center of gravity of the subject.

The use of focalized mechanic-sound vibrations (ViSS) showed an improvement in the quality of muscle tissue, which has resulted in greater strength and security during the gait cycle.

In conclusion, our experience allowed us to state that a valid rehabilitative approach is necessary to maintain a good efficiency of voluntary movement and postural control in patients affected by parkinsonism. The rehabilitation treatment should complement the pharmacological treatment from the initial stage of the disease, and even when the motor and postural abnormalities become more important, it allows more rapid and lasting improvement in the quality of life of these patients.

The present study also shows that the synergy between Dynamic Antigravity Postural System (SPAD) and focused acoustic vibrations high capacity (ViSS) can be a valid rehabilitative approach to improve the performance of the gait cycle and reduce disability in ADL in patients with atypical parkinsonism.

The results of our study show that the rehabilitation program was efficient on posture and on walking quality. The patients showed an increase in walking speed, greater stability and a consequent reduction in the risk of falling.

A limitation of our study is represented by the concept that it is not yet established if the application of a body weight support systems for gait training has a positive additive effect to an overground treadmill training (Franceschini et al., 2009).

Further studies are forecasted to compare the body weight support training with an overground training.

On a final note we would like to underline that all patients reported an improvement of their quality of life, subjectively related not only to effective postural results, but also to the acquired notion of new treatment options for a nosological entity (PSP) lacking for effective pharmacological therapies.

References

Berg, K. O., Wood-Dauphinee, S. L., Williams, J. I., & Maki, B. (1992). Measuring balance in the elderly: Validation of an instrument. *Can J Public Health*, *83*(Suppl 2), S7-S11.

Bonanni, L., Thomas, A., Varanese, S., Scorrano, V., & Onofrj, M. (2007). Botulinum toxin treatment of lateral axial dystonia in Parkinsonism. *Mov Disord*, *22*, 2097-2103.

Dietz, V., Ziljstra, W., Assainte, C., Trippel, M., & Berger, W. (1993). Balance control in Parkinson's disease. *Gait Posture*, *1*, 77-84.

Dobkin, B., Apple, D., Barbeau, H., Basso, M., Behrman, A., Deforge, D., Ditunno, J., Dudley, G., Elashoff, R., Fugate, L., Harkema, S., Saulino, M., & Scott, M. (2006). Spinal Cord Injury Locomotor Trial Group. Weight-supported treadmill vs over-ground training for walking after acute incomplete SCI. *Neurology*, *66*, 484-493.

Franceschini, M., Carda, S., Agosti, M., Antenucci, R., Malgrati, D., & Cisari, C. (2009). Gruppo Italiano Studio Allevio Carico Ictus. Walking after stroke: What does treadmill training with body weight support add to overground gait training in patients

early after stroke? A single-blind, randomized, controlled trial. *Stroke*, *40*, 3079-3085.

Golbe, L. I., & Ohman-Strickland, P. A. (2007). A clinical rating scale for progressive supranuclear palsy. *Brain*, *130*, 1552-1565.

Hausdorff, J. M., Cudkowicz, M. E., Firtion, R., Wei, J. Y., & Goldberger, A. L. (1998). Gait variability and basal ganglia disorders: Stride-to-stride variations of gait cycle timing in Parkinson's disease and Huntington's disease. *Mov Disord*, *13*, 428-437.

Hesse, S. (2001). Locomotor therapy in neurorehabilitation. *Neurorehabilitation*, *16*, 133-139.

Jenkinson, C., Fitzpatrick, R., Peto, V., Greenhall, R., & Hyman, N. (1997). The Parkinson's Disease Questionnaire (PDQ-39): Development and validation of a Parkinson's disease summary index score. *Age Ageing*, *26*, 353-357.

Karnath, H. O., Konczak, J., & Dichgans, J. (2000). Effect of prolonged neck muscle vibration on lateral head tilt in severe spasmodic torticollis. *J Neurol Neurosurg Psychiatry*, *69*, 658-660.

Litvan, I., Agid, Y., Calne, D., Campbell, G., Dubois, B., Duvoisin, R. C., Goetz, C. G., Golbe, L. I., Grafman, J., Growdon, J. H., Hallett, M., Jankovic, J., Quinn, N. P., Tolosa, E., & Zee, D. S. (1996). Clinical research criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome): Report of the NINDS-SPSP international workshop. *Neurology*, *47*, 1-9.

Lundeberg, T., Nordemar, R., & Ottoson, D. (1984). Pain alleviation by vibratory stimulation. *Pain*, *20*, 25-44.

Pasetti, C., & Ferriero, G. (2008). Approccio Clinico e Riabilitativo alla malattia di Parkinson. In: G. N. Valobra, R. Gatto, & M. Monticone, Nuovo Trattato di Medicina Fisica e Riabilitazione. UTET Scienze Mediche, Torino.

Saggini, R., Scuderi, N., Bellomo, R. G., Dessy, R. A., Cancelli, F., & Iodice, P. (2006). Selective development of muscolar force in rehabilitative context. *Eur Med Phys*, *42*(suppl 1), 69-77.

Saggini, R., Vecchiet, J., Iezzi, S., Racciatti, D., Affaitati, G., Bellomo, R. G., Pizzigallo, E. (2006). Submaximal aerobic exercise with mechanical vibrations improves the functional status of patients with chronic fatigue syndrome. *Eura Medicophys*, *42*, 97-102.

Saggini, R., Cancelli, F., Di Bonaventura, V., Bellomo, R. G., Pezzatini, A., & Carniel, R. (2004). Efficacy of two microgravitational protocols to treat chronic low back pain associated with discal lesions: A randomized controlled trial. *Eur Med Phys*, *40*, 311-316.

Seidel, H. (1988). Myoelectric reactions to ultra-low frequency and low-frequency whole body vibration. *Eur J Appl Physiol Occup Physiol*, *57*(5), 558-562.

Simons, D. G., & Mense S. (2003). Diagnosis and therapy of myofascial trigger points. *Schmerz*, *17*(6), 419-424.

Tanaka, S. M., Li, J., Duncan, R. L., Yokota, H., Burr, D. B., & Turner, C. H. (2003). Effects of broad frequency vibration on cultured osteoblasts. *J Biomech*, *36*(1), 73-80.

Zampieri, C., & Di Fabio, R. P. (2008). Balance and eye movement training to improve gait in people with progressive supranuclear palsy: Quasi-randomized clinical trial. *Phys Ther*, *88*, 1460-1473.