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Original Article

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The HEPHAISTOS study: compliance and adherence with a novel orthotic device for calf muscle unloading

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Abstract

The present manuscript seeks to discuss methodological aspects regarding the application of the novel unloading orthosis 'HEPHAISTOS' that has been specifically developed to study physiological effects of muscular unloading without altering the impact of gravitational loading. The 'HEPHAISTOS' has been applied in an ambulatory clinical interventional study. During gait, the 'HEPHAISTOS' significantly reduces activation and force production of calf muscles while it completely retains body mass-related force on the tibia. Eleven healthy male subjects participated in the study and followed their normal everyday lives while wearing the orthosis. Several measurement sessions have been performed to investigate the time course of structural and functional adaptations during intervention and recovery. Follow-up measurements were performed for one year after the intervention. In consideration of the experiences of a unique ambulant unloading study, organizational and methodological recommendations are discussed in this manuscript. Activity monitoring data obtained with portable accelerometers reveal unchanged gait activities and good subject compliance throughout the intervention. Moreover, electromyography (EMG) and motion data investigating gait properties on reambulation day are illustrated. These data show that during the initial steps following removal of 'HEPHAISTOS', gait was significantly asynchronous indicating an acutely altered motor control in the unloaded lower leg muscles.

Keywords: Ambulant Unloading Intervention, Gravitational Loading, Muscular Unloading, Orthosis, Hephaistos

Introduction

It is well known that mechanical strain acts as a major driver for tissue adaptations, and the principles of mechanotransduction and mechano-adaptation have been described in various organ systems[4,8,11]. Of note, the mechanical forces vary within the human body, and musculoskeletal forces are a function of muscle contractions and mass acceleration, often in relation to gravity.

Bone particularly is thought to be sensitive to mechanical strain, and specific yet un-identified force regimens are needed to maintain bone size and strength[8]. Several strings of evidence suggest that larger strains, and also larger strain rates, are more effective to stimulate bone accrual than smaller strains and strain rates[13,18]. Biomechanical analyses suggest that muscle contractions are the origin of the greatest bone forces even in the leg[12], and it has been suggested that skeletal muscle drives skeletal development and maintenance[15]. However, there is currently no direct evidence to undermine the notion of bone being a slave of the musculature. Similar arguments may be applied to other tissues that follow the principle of mechanotransduction[9,19]. For example, arteries adapt to blood flow-driven shear forces that act on the endothelial layer[2,10] and mechanical signals are thought to play a key role in muscle growth[4]. In all of these examples, our knowledge of the specific regimens that drive the adaptive processes *in vivo* is very limited. This is mostly because, origin, magnitude and frequency of mechanical signals are very difficult to assess in the human body, given the interplay of muscle forces and gravity induced forces as major sources for mechanical strain in our one 'G' environment.

The authors have no conflict of interest.

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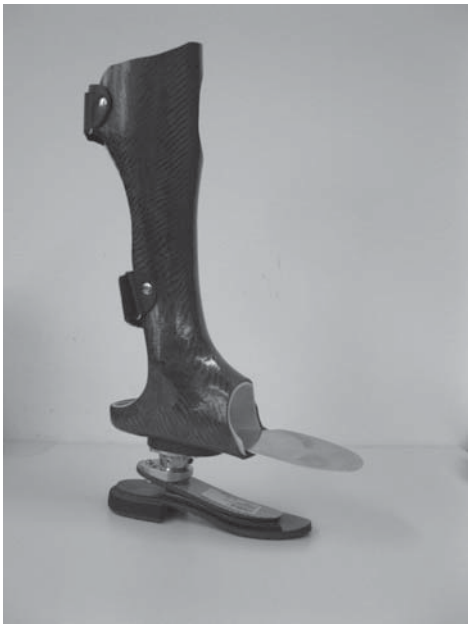

Figure 1. The HEPHAISTOS unloading orthosis.

Models like bed rest[14], limb immobilization[3] and space flight[21] allow the study of the physiology of gravitational unloading. Such studies demonstrate that gravitational unloading leads to a plethora of de-conditioning processes in various physiological systems[5,7,17]. Nonetheless, the above models are inconsistent with regards to the mechanisms by which forces with different origins act on the body, as none of these established models differentiates between muscle- and gravity-induced forces. Accordingly, the isolated role of gravity-induced forces as a precursor of physiological adaptations has to date not been addressed in a clinical study.

The aim of the present project was therefore to specifically investigate the effects of muscular unloading and thus the effects of greatly reduced muscular forces, whilst maintaining normal gravitational loading patterns, upon bone, muscles, tendon, blood vessels, cartilage and nervous system in the lower leg. Bone mineral content (BMC) was selected as primary response variable, thus testing the main hypothesis: 'Body weight bearing is insufficient to maintain bone mineral content in the distal human tibia'.

In order to test this hypothesis a novel unloading orthosis (HEPHAISTOS, Figure 1, patent application number: 102011082700.5) has been developed in the German Aerospace Center (DLR) and at the German Sport University. It has been designed to allow normal ambulation and thus body weight application to the skeleton whilst Achilles tendon force is significantly reduced (Ducos et al., manuscript in revision). Eleven healthy male subjects participated in an ambulant study and wore HEPHAISTOS for 56 days (Figure 1). During this time subjects were completely ambulant and followed their normal everyday activities.

The present article presents and discusses data related to design, subject compliance and adherence of and to the HEPHAISTOS study, respectively. It serves the scientific debate of a novel and unique study design, where a new unloading device has been applied. In light of the experiences made during this study, various aspects concerning subject recruitment, measurement protocols, subject compliance and application of the HEPHAISTOS are being discussed. In order to monitor subjects' activity profiles and compliance to the studies requirements, portable accelerometers were used and the results of the activity monitoring are presented in this manuscript. In addition, gait properties of the first steps following removal of the orthosis were carefully obtained and the results provide information on the safety and feasibility of reambulation after unilateral muscular unloading. We hypothesized that normal gait activities would be unchanged during the HEPHAISTOS intervention compared to walking with normal footwear. Furthermore, we expected to observe an asynchronous gait pattern during the initial steps of re-ambulation following 56 days of HEPHAISTOS utilization.

Methods

In the following a methodological overview of the study design is presented. This overview comprises important information on how the study was conducted, including details about sample size calculation, subject recruitment and the actual intervention. Thereafter, methods and technical information of reambulation and activity monitoring measurements are presented. The results of the latter measurements are presented in this manuscript.

Study characteristics: The HEPHAISTOS study (HEP-study) was conducted at the DLR in Cologne, Germany and is registered at www.clinicaltrials.gov (Identifier: NCT01576081). It was approved by the Ethics Committee of the Northern Rhine medical association (Ärztekammer Nordrhein, Düsseldorf, Germany). The time frame for the study was scheduled to 14 months, starting on the 19th of April 2011 with baseline data collection (BDC) and completing with the last measurement of the recovery phase on the 5th of July 2012.

Sample size calculation: a sample size calculation has been performed using BMC data from a previous bed rest study[16]. The sample size calculation formula has been chosen assuming that the mean sampling distribution of the variable of interest (BMC change) will follow a normal distribution. By estimating the value of the expected mean paired difference and standard deviation of BMC inherent to a period of bed rest of 56 days[16], it was possible to calculate the sample size for this study. For the present ambulant study, we hypothesized that half the loss of bed rest will be sufficient to verify the main hypothesis. Accordingly, the threshold was set to a loss of ≥1.7% of tibial BMC. The formula which allows the calculation of the sample size for the comparison of paired means was used:

$$\text{Subjects required} = \frac{(z_\alpha + z_{2\beta})^2 \times \sigma^2}{\delta^2}$$

Where:
z_α is the ordinate of the normal distribution for the first order error probability α
$z_{2\beta}$ is the ordinate of the normal distribution for the second order error probability β
σ^2 is the standard deviation of the paired difference between BDC and R+14 values (the within subject anticipated standard deviation)
δ^2 is the anticipated difference between the BMC values of BDC and R+14.

By accepting a risk of 5% to make an error of the first order (alpha=0.05) and a risk of 10 % to make an error of the second order (Beta=0.10; power: 1-beta=0.9), we obtained from the normal distribution ordinates table:
$z_{\alpha=0.05}$=1.96
$z_{2\beta=0.10}$=1.282

The expected difference between BDC and R+14 BMC values was set to 1.7%. Thus BMC loss was set to 1.7% (δ^2=0.017) and the intra subject variability was also set to 1.7% (σ^2=0.017) according to data from the literature[16].
The equation becomes then:

$$\text{Subjects required} = \frac{(1.96+1.282)^2 \times 0.017^2}{0.017^2}$$

Subjects required = **10.51**

Subject recruitment: Consequently, 11 male subjects were recruited for the study. The project was promoted by sending email information to previous subjects of the DLR database and to all DLR (Cologne) employees, distributing flyers at public places, hospitals and universities, broadcasting information in the local radio (Radio Köln), advertising the project in an online student job exchange (www.stellenwerk.de) and advertising it on the DLR-webpage. An initial cohort of 93 interested candidates underwent a strict recruitment process including telephone interviews, subject information presentations, medical check-ups, psychological questionnaires (FPI: Freiburger personality inventory), and psychological interviews to eventually identify the 11 suitable candiates. For inclusion into the study, subjects had to be psychologically suitable, medically healthy, aged between 20 and 45 years, in possession of a certificate of good conduct and body mass indexed between 20 and 30. Exclusion criteria were as follows: smoking, professional athletes, diabetes, muscle or joint disease, increased risk of thrombosis (checked via thrombophilia screening), bone fractures 12 months prior to the study, metal implants, any material of osteosynthesis, participation in another clinical intervention study 2 months prior to the study, bleeding disorder, anaesthetic intolerance, vascular disease, epilepsy, claustrophobia, herniated disk, pacemaker, alcohol or drug abuse, anti-inflammatory drug intake, hyperlipidaemia, kidney disease, hyperhomocysteinaemia, vitamin d deficit and chronic back pain. The number of all excluded candidates is listed in Table 1 for each recruitment stage.

Stage	Total number	Excluded	Invited	Did not attend
Telephone interview	93	15	78	-
Freiburger personality inventory	78	7	24	47
Medical check-up	24	4	20	-
Psychological interview	20	9	11	-

Table 1. Subject recruitment.

HEPHAISTOS intervention: A coin was tossed to randomly assign the intervention leg. The HEPHAISTOS was manufactured from an orthopaedic technics company (ORTEMA GmbH, Markgrönigen, Germany). The carbon-shaft of HEPHAISTOS was individually tailored for each subject, taking a plaster cast of the lower leg as anatomical template. Two weeks before study start, subjects were familiarized with HEPHAISTOS and final adjustments were made to facilitate a natural gait pattern. The following link leads to the webpage of the DLR with a video clip showing a subject walking with HEPHAISTOS (http://www.dlr.de/me/en/desktopdefault.aspx/tabid-7389/). The 11 subjects began the intervention in a staggered order, with three subjects per day over 4 days. Throughout the intervention subjects had to wear HEPHAISTOS during all locomotive activities that required loading of the legs. During this time subjects had to visit the DLR at least once a week for routine examinations, for measurements or for a weekly report. Table 2 depicts the time course of the entire study for one subject including all measurements and events.

Standardized food protocol: As some of the outcome parameters of the study are highly influenced by nutrition, a standardized diet was administered for 4 days on three occasions before taking blood and urine for the measurement of bone markers. Energy intake was calculated for each subject using the subject's body weight. Energy expenditure was calculated by summing the basal metabolic rate (BMR) according to the WHO equation[1] plus 40% of BMR for light physical activity, plus 10% of total energy expenditure (TEE) for energy expenditure associated with thermogenesis from food and beverages. The daily intake of protein (1.2 g/kg BW), fat (<30% of TEE), carbohydrates (50-55% of TEE), vitamins and minerals matched the German dietary recommended intakes[6]. Individual food packages were prepared and packed by a dietician for subjects to take home. Moreover, subjects had to fill out a nutrition questionnaire on six occasions before giving blood and 24H urine samples.

Blood and urine sampling: Blood and urine samples were collected under standardized conditions for bone marker assessment. Fasting morning blood was drawn in supine posture at 7:00 am for each of the sampling days. Once during the baseline collection period (BDC-7), 4 times during the intervention (HEP2, HEP14, HEP28, HEP56) and four times during the recovery period (R+5, R+14, R+28, R+92). Drawn blood samples were immediately centrifuged, aliquoted and stored in a freezer at -20 or -80° respectively their requirements for later analysis. Urine was collected as 24-h urine pools once

Study day	-52	-46	-34	BDC-14	BDC-8	BDC-7	BDC-3	HEP0	HEP1	HEP2	HEP7	HEP13	HEP14	HEP21	HEP27	HEP28	HEP36	HEP42	HEP49	HEP50	HEP55	HEP56	R+1	R+4	R+5	R+13	R+14	R+15	R+21	R+25	R+27	R+28	R+90	R+92	R+180	R+360
Diet (f=fastend, q=questionnaire, s=standardized, t=tailored)	f	0		s	t	f			t	f	f	q	f		q	f				s	t	f		q		q	f				q	f	q			
Medical check up	x																																			
Psychological interview		x																																		
Plaster cast			x																																	
pQCT				x		x					x		x			x						x			x		x					x		x	x	x
Xtreme CT							x																							x						
HEPHAISTOS adjustment, familiarization				x																																
Gait analysis				x							x		x	x		x	x	x		x		x														
Biopsies				x																x																
Blood samples						x				x			x			x						x			x		x					x		x		
24 hour urine					x				x			x			x						x		x		x					x			x			
Vascular ultrasound						x					x		x			x						x			x		x					x				
Muscle and cartilage MRI																			x									x								
Neuromuscular tests (fatigue and/or MVC)						x																x			x		x		x			x		x	x	x
H-reflex						x																x														
Achilles tendon stiffness								x															x													

Table 2. Study overview. Negative numbers refer to study days before intervention start. Days of baseline data collection=BDC; days of HEPHAISTOS intervention=HEP; recovery phase=R+.

during baseline collection period (BDC-8), 4 times during the intervention (HEP1, HEP13, HEP27, HEP55) and four times during the recovery period (R+4, R+13, R+27, R+90). Each void was kept dark and cold until final pooling to the 24-h urine pool. Aliquots were stored at -20 or -80°C respectively their requirements for later analysis.

Reambulation measurements

On reambulation day gait properties of the six initial steps without orthosis were investigated using electromyography (TeleMyo 2400 G2 Telemetry System, Noraxon U.S.A. Inc, Scottsdale, Arizona, USA) and a motion capture system (Vicon® Motion Systems Ltd., LA, USA). Both systems were synchronized using a custom-made trigger.

Calculation of step length: Reflective markers were placed on the skin using the standard marker set for the lower body (plug-in-gait skeleton template, Vicon® Motion Systems Ltd., LA, USA). The movement of the marker placed on the metatarsophalangeal joint of the middle toe was then analysed off-line and step length was calculated as the tracked distance between left and right middle toe markers during double limb support of the stance phase.

Electromyography: Surface electromyography (EMG) was recorded using a telemetric device. In order to detect side differences between intervention and contralateral legs, electrodes were placed on both legs applying the *Seniam* recommendations for surface electromyography (www.seniam.org) on following muscles: soleus (Sol), gastrocnemius medialis (GM), tibialis anterior (TA) and vastus lateralis (VL). Electromyographic recordings were obtained using a sampling frequency of 1500Hz. Data were off-line rectified and band-pass filtered (20Hz-500Hz) using MATLAB (Mathworks, Natick, MA, USA). Thereafter, stance phases were identified using the middle toe metatarsophalangeal joint reflective marker and RMS of the filtered signal was calculated for each stance phase.

Activity monitoring

Portable accelerometry: In order to quantify possible activity changes related to wearing HEPHAISTOS, accelerations (ACCs) of the intervention leg have been continuously recorded during the entire intervention using portable 3-axis digital accelerometers (X1-6A, Gulf Coast Data Concepts, Waveland, USA). Habitual acceleration profiles measured during the week from BDC-14 to BDC-7 were taken as reference. During this period, accelerometers were fixed to the shin using medical bandages (ORTEMA GmbH, Markgrönigen, Germany). During the HEP intervention they were attached to the shaft of the orthosis using Velcro® strips. The X-axis of the accelerometer was aligned with the body longitudinal axis, the Y-axis with the transverse axis and the Z-axis with the sagittal axis. Accelerometers were synchronized with a computer in-

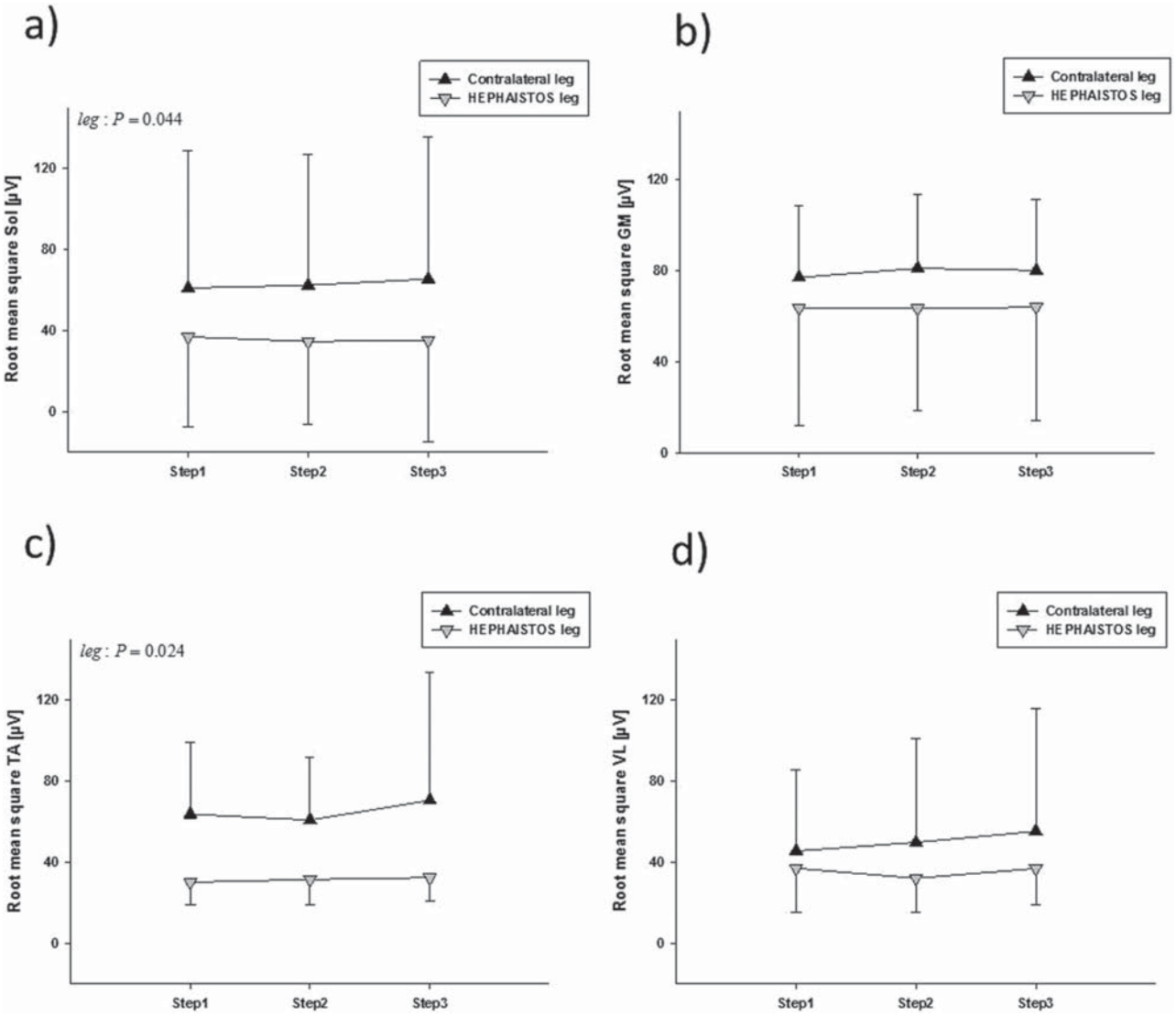

Figure 2. Reambulation electromyography. Electromyographic data of the initial six steps after removal of HEPHAISTOS were obtained for the soleus muscle (Sol), the gastrocnemius medialis muscle (GM), the tibialis anterior muscle (TA) and the vastus lateralis muscle (VL). Root mean square values were calculated for each stance phase and are expressed for the three initial steps on either side. Soleus muscle RMS (P=0.044) and TA RMS (P=0.024) were significantly lower on the HEPHAISTOS leg.

ternal clock and set up to automatically start and stop recording all 3-axis accelerations on a built-in SD card every day, from 5.00 am until 12.00 pm, at a 20 Hz sampling rate. A new data file was created for every two hours recording. The data were retrieved on hard disk during the subjects' weekly visit to the DLR using the USB connector the accelerometers were equipped with and the battery of the accelerometer was replaced on that occasion. Acceleration analysis was performed a posteriori using a custom made R program (http://www.r-project.org). Briefly, data were sorted per study week and within each weekly data set, files were pooled according to the time of the day (day^{time}) they were recorded. Thereafter, a moving window over 40 samples with 0-overlap was applied along the pooled data vector obtained for the X-axis, for each day^{time} of each week. For each iteration of the moving window, the standard deviation (SD) of the measured accelerations values was calculated. Each day^{time} acceleration data vector could then be represented by a daytime SD vector. Previously, task related mean SDs were identified for activities such as sitting (≈0.03 G), standing (≈0.01 G) walking (≈0.3 G), stair ascending (≈0.4 G) and descending (≈0.55 G) (unpublished observations). Therefore, the weekly proportion of activity during the HEP study was represented by the counts of SD vector samples equal or superior to 0.1 G in all day^{time} SD vectors in relation

to the total amount of SD samples. Moreover, the weekly activity was decomposed into light (ACT^{Light} with 0.1 G<SD<0.4 G) or heavy (ACT^{Heavy} with SD>0.4 G).

Statistical analyses: All statistical analyses were performed using using STATISTICA 10.0 for Windows (Statsoft, Tulsa, Oklahoma, USA, 1984-2008). Electromyography and Vicon® parameters were analysed applying a repeated measures ANOVA with *leg* (HEPHAISTOS vs. contralateral) and *step* (step1, step2, step3) as within effect. Accelerometer data were analysed applying the same test, testing effects for *time* for nine study weeks (BDC, W1, W2…W8). Tukey's test has been performed for post hoc analyses. All values are presented as means ± SD and the significance level was set at P≤0.05.

Results and relevant events during the study

HEPHAISTOS intervention: All eleven subjects completed the 56 intervention days. However, due to reasons which were not related to the study one subject could not attend the HEP56 measurements. All data of this subject which required pre and post comparisons, except the bone parameters, were discarded from further analysis. The EMG reambulation data of one subject had to be discarded from the analysis due to technical failure.

Reambulation: On the last day of the study, after the last HEP56 measurement, subjects made their first steps without HEPHAISTOS. Under controlled conditions subjects were asked to: (1) move the ankle while sitting, (2) stand on two feet, (3) sway, shifting body weight from foot to foot, (4) stand alternately on one foot, (5) sway, from heel stand to tiptoe stand, (6) if possible tiptoe stand on one leg, (7) squat, (8) jump carefully on the spot, (9) walk with assistance, (10) walk alone. Ground reaction forces and centre of gravity motions of movements 1-8 were measured using a force plate (Leonardo Mechanograph®, Novotec, Pforzheim, Germany). During the whole procedure EMG was recorded from soleus, gastrocnemius medialis, vastus lateralis and from tibialis anterior muscles using a telemetric EMG device. In addition gait properties were recorded using the Vicon® motion capturing system. The results of the gait trials (10) are presented below. On reambulation day a professional physiotherapist treated each subject for 60 minutes and checked the mobility of the unloaded ankle, which was for no subject considered as a serious counter indication for reambulation. Five days after reambulation one subject complained about pressure pain in the area of the intervention forefoot and at the dorsum of this foot. Morton's neuroma was diagnosed which was treated conservatively, and it was judged that this was likely to be facilitated by the subject having splay feet. The ailment vanished 12 weeks after reambulation. All ankle plantarflexor torque tests after HEP56 were cancelled for that subject.

Reambulation electromyography: During the initial six steps without orthosis, RMS of the soleus muscle was by 42.7% (SD=38.3%) significantly (leg: *P*=0.044) smaller in the HEPHAISTOS leg if compared to the contralateral leg. There was no difference of soleus muscle EMG between steps for either side (step: *P*=0.59). The RMS of the tibialis anterior muscle

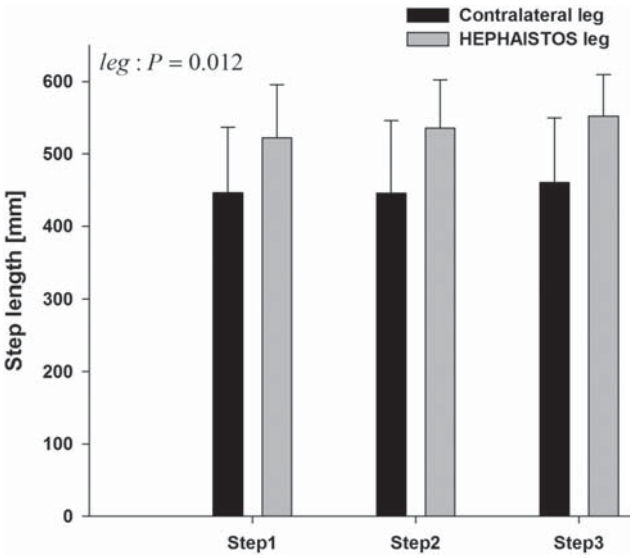

Figure 3. Reambulation step length. Step length was assessed using the Vicon® motion capturing system. One step was determined as the distance between left and right middle toe markers during double limb support of the stance phase. For the initial six steps after removal of HEPHAISTOS, steps were significantly (*P*=0.012) shorter on the contralateral side.

was also significantly (leg: *P*=0.024) reduced by 39.2% (SD=43.9%) in the HEPHAISTOS leg, with no differences between steps for either side (step: *P*=0.7). There were no leg or step specific differences between gastrocnemius medialis muscle (leg: *P*=0.38; step: *P*=0.87) and vastus lateralis muscle (leg: *P*=0.25; step: *P*=0.13) RMS (Figure 2).

Reambulation step length: The step length of the contralateral leg was by 16% (SD=16%) significantly (leg: *P*=0.012) shorter compared to the step length of the HEPHAISTOS leg. There was no length difference between steps (step: *P*=0.21) (Figure 3).

Accelerometer activity monitoring: The percentage value of the accelerometer recordings that have been assigned to activity (ACT^{Total}) has decreased significantly over time (*P*=0.023). Post hoc testing revealed a significant decrease of ACT^{Total} from 9.7% (SD=4.5%) at BDC to correspondingly 6.7% (SD=3%; *P*=0.04), 6.7% (SD=2.5%; *P*=0.04) and 6.4% (SD=2%; *P*=0.02) for intervention weeks five, six and seven. The percentage value of recordings that have been assigned to ACT^{Heavy} has also decreased over time (*P*<0.001). Post hoc testing revealed a significant decrease of ACT^{Heavy} (*P*<0.001) from 2.7% (SD=1.5%) at BDC to values below or equal to 1% for all intervention weeks (Figure 4). The percentage value of the recordings that have been assigned to light activities remained unaffected throughout the study (*P*=0.5).

CT-measurements: All CT-measurements were approved by the Federal Office for Radiation Protection, Berlin, Germany. Two x-ray devices were being applied in the present study. A

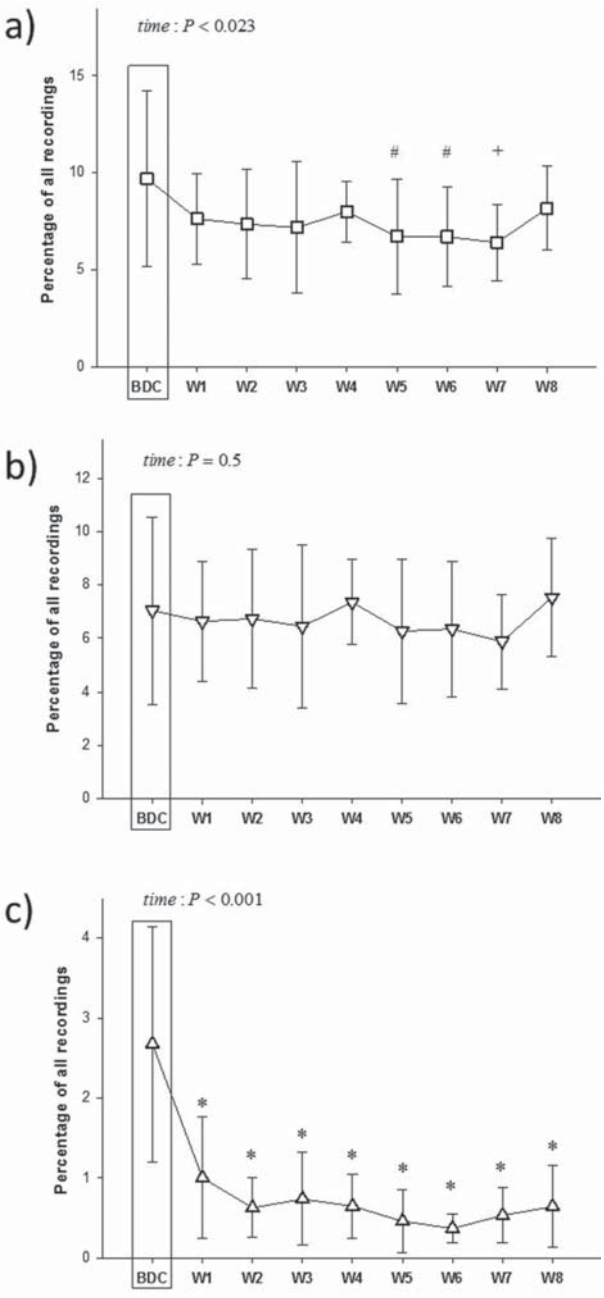

Figure 4. Activity monitoring. Subjects' activities were monitored throughout the entire study (BDC=baseline data collection; W1, W2…W8= intervention weeks) using portable accelerometers that were fixed to the lower leg. Panel (a) depicts the percentage value of all acceleration recordings that were assigned to general activity (ACT^{Total}). Panel (b) shows the percentage value of the recordings that were assigned to 'light' activities (ACT^{Light}, e.g. gait) and panel (c) depicts the percentage value of the recordings that were assigned to 'heavy' activities (ACT^{Heavy}, e.g. jumping, running). $^{\#}P$=0.04, ^{+}P=0.02, $^{*}P$<0.001.

high resolution Xtreme CT- scanner (Scanco, SCANCO Medical AG, Brüttisellen, Switzerland) for measurements at BDC-3 and R+25 and a peripheral quantitative tomographic scanner (XTC 3000, Stratec Medizintechnik), for all other bone density measurements. The Xtreme CT scan was conducted in the University clinic in Erlangen, Germany, which 3 subjects could not attend. The overall mean radiation dose for each subject and all measurements was 0,035356 mSv.

MRI-measurements: The cartilage measurements required administration of the contrast agent Gadopentetic acid (Magnevist®). Two subjects showed symptoms of intolerance and were excluded from this measurement. One subject refused the procedure.

Standardized nutrition, blood and urine sampling:

All samples could be collected as planned. The results of those measurements will be published together with the data for the long term bone adaptation (Ducos et al, in preparation).

Discussion

Scientific relevance: The significant reduction of mechanical stimuli as a consequence of gravitational unloading under conditions like bed rest, SCI or space flight is considered as a major source for bone loss[20]. For all of these conditions muscular unloading is combined with a change of gravity effects. Exercise interventions as applied for instance in bed rest, that try to compensate for the extensive reduction of muscle work, have only been partially efficient to counteract bone loss[17]. However, these interventions do not consider a potential role of gravitational acceleration. The investigation of the effects of the earth attraction force independently from the effects of muscle contraction forces is a logical complement to previous studies and the results of such a novel study approach greatly add to the current knowledge of bone adaptation (Ducos et al., in preparation). Moreover, the application of HEPHAISTOS offers a novel and unique possibility to study the specific effects of gravitational accelerations upon functional and structural adaptations of the other investigated organs[22].

HEPHAISTOS intervention: The intervention with the novel unloading device was very satisfactory. All eleven subjects completed the 56 intervention days without serious complications. Occasionally occurring pressure spots could be successfully counteracted using cushioning and re-adjusting the elastic foot of HEPHAISTOS (see Figure 1). The weak point of the HEPHAISTOS was the anti-slippery sole glued under the carbon prosthetic foot, which showed signs of premature wear during the second week of the intervention. However, selection of a superior material allowed the second sole to outlast the resting intervention time. Our experiences with the novel device made us confident enough to further pursue its application in upcoming clinical trials.

Ambulant study design and subject compliance: The major challenge of an ambulant study design is that subjects cannot be monitored for the biggest part of the intervention. To wear the HEPHAISTOS for 56 days during all daily activities required a great amount of motivation and mental strength from

the subjects. Several compliance strategies were developed to ensure that all subjects would use the HEPHAISTOS as stipulated and that all subjects would complete the 56 days: (a) Psychological questionnaires (FPIs) and the psychological interviews were applied to optimize our subject selection. The psychological assessment helped us to find reliable subjects who had a real interest in the topic and perceived the participation in our study as an interesting experience. The above combination of psychological subject characteristics was in our opinion crucial for a successful study. (b) The application of accelerometers to monitor subject activity throughout the entire study provided a certain control tool. The data obtained through accelerometry reveal that subjects wore the HEPHAISTOS orthosis throughout the entire study as the fraction of 'light' activities e.g. gait was comparable between BDC and all other intervention weeks. The finding that heavy activities were significantly reduced during the intervention can be basically attributed to the function of the HEPHAISTOS. Locomotive activities like jumping or running, leading to high acceleration profiles, are restricted with this device. (c) Social events before, during and after the study created a positive team spirit and a familiar atmosphere between subjects and investigators. They also helped to sustain a working relationship between subjects and investigators. (d) One of the project scientists participated as subject in the study. Thus, potential concerns regarding the intervention and measurement procedures have certainly been diminished. As an indication of good subject compliance the distinct artery adaptations shall be mentioned here, which could be detected for all of the eleven participants[22].

Reambulation

The gait trials on reambulation day clearly indicate that motor control of the HEPHAISTOS leg was acutely impaired for the initial steps without orthosis. When wearing HEPHAISTOS, the soleus muscle is primarily impacted (Ducos et al., manuscript in revision) and it therefore appears logical that muscle activation of this muscle is acutely impaired after 56 days HEPHAISTOS intervention. During the initial trials with HEPHAISTOS it seemed that muscle activation of the TA was less affected, however, reambulation data suggest that over the 8 intervention weeks, subjects learned to deactivate the dorsi flexor muscle as TA RMS on the HEPHAISTOS side was significantly decreased during the initial steps. Gastrocnemius medialis and vastus lateralis muscle activity seemed to be less impacted after the intervention, however, this is not unexpected as the HEPHAISTOS orthosis was developed to primarily reduce soleus plantar flexor torque production during gait. The apparent acute imbalance of muscle activation resulted then in an asynchronous gait pattern and it happened that step length of the control leg was significantly reduced. This altered gait pattern can most likely be attributed to a reduced stance time of the HEPHAISTOS leg. Nonetheless, gait properties normalized quickly and except for the subject suffering from Morton's neuroma, all subjects showed normal gait properties within a few days after reambulation.

Conclusion

In summary, one can conclude that the HEP-project was very successful. The present study adds important knowledge to our current understanding of physiological adaptations induced by muscle unloading. Although the asynchronous gait properties on the first day after removal of the orthosis, the application of HEPHAISTOS had no major side effects and all subjects recovered completely within the time frame of the study. Morton's neuroma, probably as a result of splay foot may be a complication during recovery from wearing the HEPHAISTOS orthosis, and it might also be expected after immobilisation with other models. Although there is no direct proof, activity monitoring data and several other strands of indirect evidence suggests that subjects' compliance with the protocol was very good, thus underlining the feasibility of ambulant immobilisation studies.

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