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Title: In vivo fascicle behaviour of the flexor hallucis longus muscle at different walking speeds
Running head: FHL mechanics in walking
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**ABSTRACT** 

Ankle plantar flexor muscles support and propel the body in the stance phase of locomotion.

Besides the triceps surae, flexor hallucis longus muscle (FHL) may also contribute to this role,

but very few in-vivo studies have examined FHL function during walking. Here we

investigated FHL fascicle behaviour at different walking speeds. Ten healthy males walked

overground at three different speeds while FHL fascicle length changes were recorded with

ultrasound and muscle activity was recorded with surface electromyography (EMG). Fascicle

length at heel strike, at toe off and at peak EMG activity did not change with speed. Range of

FHL fascicle length change (3.5-4.5 and 1.9-2.9 mm on average in stance and push-off phase,

respectively), as well as minimum (53.5-54.9 and 53.8-55.7 mm) and maximum (58-58.4 and

56.8-57.7 mm) fascicle length did not change with speed in the stance or push-off phase. Mean

fascicle velocity did not change in the stance phase, but increased significantly in the push-off

phase between slow and fast walking speeds (P=0.021). EMG activity increased significantly

in both phases from slow to preferred and preferred to fast speed (P<0.02 in all cases). FHL

muscle fascicles worked near-isometrically during the whole stance phase (at least during slow

walking) and operated at approximately the same length at different walking speeds. FHL and

medial gastrocnemius (MG) have similar fiber length to muscle belly length ratios and,

according to our results, also exhibit similar fascicle behaviour at different walking speeds.

**KEYWORDS**: flexor hallucis longus mechanics, human locomotion, ultrasonography

INTRODUCTION

During the stance phase of human walking, ankle plantar flexor muscles support the body, and

help to propel it during late stance (Winter, 1983; Kepple et al., 1997; Neptune et al., 2001).

Triceps surae muscle function in walking has been widely examined, but the deep plantar flexor muscles such as flexor hallucis longus (FHL) may also significantly contribute to this effort. Despite the relatively small anatomical cross-sectional area of FHL, its physiological cross-sectional area (PCSA), which is related to force production capacity (Blazevich et al., 2009), is significant, at around 70% of that of the lateral gastrocnemius (Fukunaga et al., 1992; Ward et al., 2009). FHL and flexor digitorum longus (FDL) together also constitute around 10% of the PCSA of all lower leg muscles (Wickiewicz et al., 1983), with around two times higher PCSA in FHL compared to FDL (Fukunaga et al., 1992). FHL contributes to load transmission from the shank to the ground during locomotion (Ferris et al., 1995), and its significant contribution to propulsion is reflected by the large forces acting along the FHL tendon during walking (52% body mass; (Jacob, 2001)).

One factor known to affect muscle function (and kinematics) in locomotion is the speed of movement (e.g. Lelas et al., 2003). Modelling studies have predicted that increased walking speed requires increased work from the plantar flexor muscles (Neptune et al., 2008). This is supported by in vivo measurements showing that increasing walking speed is related to higher ankle joint torque, increased muscle activity and altered fascicle behaviour of the soleus (SOL) (Lai et al., 2015) and medial gastrocnemius (MG) (Farris & Sawicki, 2012; Cronin et al., 2013a) muscles. Cronin et al. (2013a) found that shortening velocity in the push-off phase increases in MG but not in SOL muscle with increasing walking speed, changing the relative contribution of these muscles to propulsion. Similar to the calf muscles, FHL is also active in the stance phase of walking (Fujita, 1985; Perry, 1992), and exhibits increasing muscle activity and force under the big toe in the push-off phase with increasing walking speed (Péter et al., 2015). Thus, it might be expected that FHL also shows speed-dependent mechanical muscle

function, that is, fascicle length changes, which could have important implications for force production and economy.

According to the results of cadaver studies (Kirane et al., 2008; Hofmann et al., 2013), FHL functions near-isometrically during simulated walking, as is the case for MG and SOL muscles (Cronin & Finni, 2013; Cronin & Lichtwark, 2013). However, to date, no in vivo studies of FHL mechanical behaviour during locomotion have been conducted to the best of our knowledge. Thus, in this study we used ultrasound to investigate FHL fascicle length changes in vivo during overground walking at different speeds. It was hypothesized that the range of FHL fascicle length change would be relatively small in the stance phase of walking, and that increased walking speed would result in increased fascicle velocity.

#### **MATERIALS AND METHODS**

## **Participants**

Twenty healthy male individuals with no history of neuromuscular disorder or injury gave their written consent to participate in this study. Ultrasound data were obtained from ten participants (age 24.7±3.5 years; height 177.7±3.9 cm; body mass 71.9±4.9 kg) and included in this paper. The ethics committee of the University of Jyväskylä approved the study protocol. All procedures were in line with the Declaration of Helsinki.

# Study protocol

After some familiarization trials, participants walked at three different speeds over two 10 m custom-made force platforms (University of Jyväskylä, Finland; sampling frequency 1000 Hz) positioned parallel to each other. Force plate data were sampled to define step cycle phases and

photocells were placed at the beginning and end of the force platforms to determine walking speeds. Trials began 3 meters before and ended 2 meters after the force plates to ensure steady speed during the recording period. Firstly, participants performed 5 trials at their preferred speed. The slowest and fastest trials were discarded and the remaining three trials were averaged to yield preferred walking speed. Participants then performed faster and slower walking trials where the target times were 30% faster and 30% slower than at preferred speed, respectively. For each speed, at least 3 trials were obtained that were within ± 5% of the target time. Faster and slower walking trials were completed in a random order. During walking trials, FHL fascicle lengths were measured with ultrasound and muscle activity was recorded with surface electromyography (EMG). Ankle angle was measured with a custom-made electrogoniometer (University of Jyväskylä, Finland) for visualization purposes (Figure 2 and 3). For all participants, the right leg was tested.

## Methodology and analysis

#### Ultrasound

A personal computer-based portable ultrasound system (EchoBlaster 128; Telemed, Vilnius, Lithuania) was used with a 96-element linear probe (B-mode; 7 MHz, 60 mm transducer field width, 80 Hz sampling frequency), which was placed over the posterior site of the right leg to estimate FHL fascicle length change during walking. The ultrasound probe was firmly fixed over the mid-belly of the FHL muscle (Figure 1A), placed so that the probe was aligned with the FHL muscle fascicles to minimize perspective and parallax measurement errors. A flat-shaped probe was used to minimize probe rotation. Before starting the measurements, image quality was tested during big toe flexion and calf raises during standing. The probe was connected to a portable ultrasound unit (5 kg), which was carried by an assistant during walking. The assistant walked next to the force plate and approximately one meter behind the

participant to avoid influencing their performance. Participants reported no discomfort or disturbance due to the ultrasound probe or the cable during the measurements. To synchronize data collection with other sources, a digital output signal was sent from the ultrasound system to Spike2 software (Cambridge Electronic Design, Cambridge, UK), where force and EMG signals were also recorded.

A previously validated automated fascicle tracking algorithm using Lucas-Kanade optical flow and affine optic flow extension was used to determine FHL muscle fascicle length change during walking tasks (Cronin et al., 2011; Gillett et al., 2013; Farris & Lichtwark, 2016). The region of interest (Fig 1B) was between the deep and middle aponeuroses of the muscle since it was thicker than the more superficial part, as reported previously (Mickle et al., 2013). Thus, analysis error may also be relatively small in this region due to the longer fascicles. The initial and end points of a straight line from the deep to the middle aponeurosis were assigned parallel to the lines of collagenous tissue visible on the appointed ultrasound frame, and used to represent fascicle length. A typical example of the raw fascicle length changes at different walking speeds is shown in Figure 2.

Fascicle length data were smoothed with a 20-Hz fourth-order Butterworth low-pass filter in MATLAB (MathWorks Inc, Natick, MA, US). A total of 244 steps were analysed: 94, 70 and 80 for slow, preferred and fast walking trials, respectively. The stance phase of each cycle was analysed since FHL is active in this phase of the step cycle, but not in swing (Perry, 1992; Zelik et al., 2015). The push-off phase was also separately analysed, since plantar flexor muscles play a significant role in body propulsion in this phase (Neptune et al., 2001).

Initial fascicle length was defined as the fascicle length at heel strike. Minimum and maximum fascicle length were calculated in the stance and push-off phases separately, and range of fascicle length change was calculated as the difference between the minimum and maximum lengths. Fascicle length at toe-off and at the time of peak EMG activity were also calculated. Mean fascicle velocity was calculated by averaging the first derivation of fascicle length change data within stance and push-off separately. Values within a participant and walking speed were averaged and included in the statistical analysis.

## **EMG**

After shaving and abrading the skin and wiping with alcohol, bipolar surface EMG silver-silver chloride Ambu BlueSensor N electrodes (Ambu A/S, Ballerup, Denmark) with an interelectrode distance of 16 mm (Masood et al., 2014) were located over the FHL muscle belly on the medial side of the leg between the soleus insertion and the FHL muscle-tendon junction, where only the FHL muscle belly lies (Fukunaga et al., 1992). Proper placement was determined using ultrasonography (detailed description in (Péter et al., 2015)). Signal quality was confirmed during isolated ankle and big toe plantar flexion. A wireless EMG system (Noraxon Inc. Scottsdale, AZ, USA) was used to sample data at a frequency of 1500Hz. Data were sent to an A/D converter (Cambridge Electronic Design, Cambridge, UK) and recorded in Spike2 software.

Raw EMG data were band-pass filtered between 20 and 450 Hz using a fourth-order zero lag Butterworth filter in MATLAB. Root mean square (RMS) values were calculated for the stance and push-off phases for each step, and averaged within a participant and task. The peak dynamic method was used to normalize EMG (Burden, 2010) to the peak activity of preferred-speed walking (Cronin et al., 2015) in order to decrease inter-individual variability. Time of

peak activity relative to stance phase duration was calculated based on the smoothing procedure used with the peak dynamic method.

Statistical analysis

Statistical analysis was executed using SPSS software (IBM, New York, NY, USA). Shapiro-Wilk's W test was applied to determine the normality of data distribution. One-way repeated-measures ANOVA was used to test differences between variables across speeds. Where the assumption of sphericity – determined by Mauchly's test – was violated, Greenhouse-Geisser adjustment was applied. Where significant main effects were found, Bonferroni post hoc test was performed to define the location of differences (e.g. between slow and fast, SF; slow and preferred, SP). For all tests, p<.05 was set as a minimum level of statistical significance.

#### **RESULTS**

The number of analysed steps and their characteristics are shown in Table 1. Figure 3 shows the group mean FHL fascicle behaviour and EMG activity at the three different speeds of walking.

Fascicle length at heel strike, at toe off and at peak EMG activity did not change with speed. The timing of peak activity relative to stance phase duration did not change (72.7±3.3%, 72.7±3.1% and 70.4±3% at slow, preferred and fast walking speeds, respectively).

Stance phase

The range of fascicle length change, as well as minimum and maximum lengths, and mean fascicle velocity did not change significantly with increasing speed (Table 2).

Flexor hallucis longus RMS EMG activity increased significantly with increasing walking speed, with mean values of  $31\pm6\%$ ,  $44\pm8\%$  and  $77\pm22\%$  at slow, preferred and fast walking speeds, respectively (F=43.622, P<sub>SP</sub>=.003, P<sub>SF</sub>=.001, P<sub>PF</sub>=.001).

## Push-off phase

Range of fascicle length change, minimum and maximum length did not change with increasing speed. Mean fascicle velocity increased, with a significant difference between slow and fast walking speeds (F=9.038, P<sub>SF</sub>=.021) (Table 2).

Flexor hallucis longus RMS activity increased significantly with increasing walking speed in the push-off phase, with mean values of  $41\pm1\%$ ,  $60\pm16\%$  and  $105\pm39\%$  at slow, preferred and fast walking speeds, respectively (F=28.752, P<sub>SP</sub>=.011, P<sub>SF</sub>=.001, P<sub>PF</sub>=.002).

### **DISCUSSION**

In this study we investigated FHL fascicle behaviour in vivo at different speeds of overground walking. Concurring with our hypotheses, we found that the range of FHL fascicle length change was relatively small in the stance phase of walking, and that FHL fascicle velocity increased with increasing walking speed. Moreover, we observed a relatively constant FHL operating range across all speeds, whereas FHL EMG activity increased significantly with speed in both the stance and push-off phases. These findings suggest that FHL exhibits similar neuromechanical behaviour to the MG muscle during walking.

Flexor hallucis longus muscle arises from the distal 2/3 of the fibula and the membrana interossea and inserts on the distal phalanx of the big toe, while MG originates from the medial condyle of the femur and inserts on the posterior surface of the calcaneus (Gray, 1980). Although MG originates above the knee and FHL below the knee and they insert on different

places, both plantar flexor muscles cross more than one joint. The muscle belly length of FHL has been reported to be 26.9±3.6 cm, compared with 26.9±4.7 cm in MG (Ward et al., 2009). FHL and MG also have similar fibre lengths, 5.3±1.3 cm and 5.1±1 cm respectively (Ward et al., 2009). Thus, the fibre length to muscle belly length ratio is small in both muscles (0.20 and 0.19, respectively; (Ward et al., 2009)). This small ratio predicts near-isometric fascicle function, which is commonly reported in MG in the early stance phase of locomotion (e.g. Cronin & Finni, 2013; Fukunaga et al., 2001), and which we also observed in FHL in this study. In the push-off phase, MG fascicles typically shorten (Krishnaswamy et al., 2011; Farris & Sawicki, 2012), whereas in this study, FHL fascicles maintained a near-constant length during the entire stance phase of walking at the slow speed, while exhibiting a trend towards shortening at preferred and fast walking speeds.

It has been found that the kinematics of gait and muscle force requirements change with increasing speed (Lelas et al., 2003; Dubbeldam et al., 2010). For example, modelling studies predict that increasing walking speed requires increased work from the plantar flexor muscles (Neptune et al., 2008), and some in vivo mechanical data support this suggestion. With increasing walking speed (0.7, 1.4, 2.0 m/s), Lai et al. (2015) found higher ankle joint torques, increased SOL muscle activity and reduced SOL fascicle length. In MG, Farris & Sawicki (2012) examined fascicle length changes at four different walking speeds (0.75, 1.25, 1.75 and 2.0 m/s), and found relatively similar length changes at all speeds, but shortening velocity increased with increasing speed. Similarly, in our study of FHL fascicle behaviour, we found no changes in fascicle length at heel strike or in the range of FHL lengths with increasing walking speed, but an increase in mean fascicle velocity in the push-off phase with increasing walking speed. As FHL fascicle behaviour has not been examined so far, mean fascicle velocity was calculated to provide an overall picture about its mechanical behaviour. However, this limits the capability to compare our results to previous findings where shortening velocity was

calculated separately. Although a significant increase in mean fascicle velocity in the direction of shortening implies a similar response to increased walking speed in the FHL in this study as MG and SOL (Farris & Sawicki, 2012; Lai et al., 2015), the results are not quantitatively comparable. We also found that FHL EMG activity increased significantly with increasing speed, which is in agreement with the results of previous studies of plantar flexor muscles (Neptune et al., 2008; Lai et al., 2015; Péter et al., 2015; Maharaj et al. 2016). These speed-dependent findings collectively highlight further similarities between the function of FHL and triceps surae muscles.

Previous cadaver studies (Kirane et al., 2008; Hofmann et al., 2013) examined FHL tendon excursion and found it to be small (7.2 and 6.6 mm on average, respectively) relative to muscle fibre length (52.7 mm; (Ward et al., 2009)) during simulated walking. We have previously shown in vivo that movement of the FHL muscle-tendon junction is minimal during simulated push-off tasks (see Péter et al., 2015 supplementary video). In the present study we found a relatively small range of FHL fascicle length change both in stance and push-off, and a constant operating length across walking speeds. Although these findings collectively imply nearisometric function of the muscle fascicles/belly, we are lack of information regarding the behaviour of the long distal tendon of FHL. During the push-off phase of locomotion as the heel leaves the ground, the ankle joint plantarflexes and the first metatarsophalangeal joint dorsiflexes (e.g. Leardini et al., 1999). These opposing joint movements predict near-isometric function of the FHL muscle-tendon unit. However, it is well known that joint displacement parameters do not reflect the contraction mode of the muscle itself, especially for muscles with long tendons (e.g. Cronin et al., 2013b), as is the case for FHL. Furthermore, length change of the foot during walking (Stolwijk et al., 2014) and FHL lever arm at the crossed joints also affect muscle-tendon length change in response to joint angular displacements. Future studies are needed to examine fascicle and muscle-tendon unit length changes, taking all relevant variables into account, to determine whether muscle-tendon decoupling is similar to the triceps surae, or FHL tendon rather serves as a stiff force transducer in walking.

In conclusion, the results of this in vivo study show that FHL muscle fascicles work at a near-constant length during the whole stance phase at slow walking speeds, with a trend towards shortening with increasing walking speeds. Furthermore, mean fascicle velocity increased significantly with increasing speed, as did EMG activity. Thus, these results suggest that FHL and MG muscles exhibit similar neuromechanical behaviour with increasing walking speed.

## **PERSPECTIVES**

In this study, FHL fascicle behavior during walking was determined with ultrasonography in healthy people. We found a relatively small range of FHL fascicle length changes in the whole stance phase of walking as well as increased fascicle velocity and EMG activity with increasing walking speed. The possibility of measuring FHL fascicle behaviour with this method may make it feasible to examine in vivo the mechanical consequences of altered FHL morphology, for example in flat-footed people (Angin et al., 2014), a disorder that increases the risk of overuse injuries in the lower leg. It may also be possible to study changes in the mechanics of FHL muscle in people with Achilles tendon rupture (Finni et al., 2006, Masood et al. 2014), contributing to understand load sharing mechanisms between plantar flexor muscles.

## **GRANTS**

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#### CONFLICTS OF INTEREST

None of the authors have any conflicts of interest.

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# TABLES, FIGURE LEGENDS AND FIGURES

Figure 1. Ultrasound probe placement. Panel A represents ultrasound probe position. The probe was fixed with tape and an elastic bandage to minimize its movement relative to the skin. Proper placement was determined as the orientation in which the probe was parallel to the flexor hallucis longus (FHL) fascicles, based on scanning before fixing the probe. Panel B shows an ultrasound image of FHL fascicles during standing. The region of interest and one FHL fascicle are marked with a dashed line and solid line, respectively. FHL-S, FHL superficial region; FHL-D, FHL deep region

Figure 2. Typical example of flexor hallucis longus (FHL) fascicle and electromyography (EMG) behaviour from one participant. Each curve within a subfigure represents one step. Curves begin with heel contact (HC). Dotted lines represent the start of the push-off phase and dashed lines the time of toe off (TO). For better visualization, EMG data were smoothed with a 100-ms root-mean-square moving window, and then consequent right shifts of the curves were corrected. Ankle angle represents the sagittal plane rotation of the ankle joint based on electro-goniometer data, where 0° represents the angle in standing, positive indicates plantarflexion, and negative indicates dorsiflexion. GRF, ground reaction force

Figure 3. Flexor hallucis longus (FHL) fascicle and electromyography (EMG) behaviour of all participants. Group mean and standard deviation data (N=10). Fascicle length and ankle angle are normalized to the length and angle at heel contact, respectively. FHL EMG activity is expressed as a percentage of peak activity in preferred-speed walking (%PWpeak). Note that for FHL activity, error bars do not only represent individual differences because of the normalization method that was used. For better visualization, EMG data were smoothed with a 100-ms root-mean-square moving window, and then consequent right shifts of the curves were corrected. Ground reaction forces (GRF) are normalized to body mass. Ankle angle represents the sagittal plane rotation of the ankle joint based on electro-goniometer data. Curves begin with heel contact (HC). Dotted lines represent the start of the push-off phase and dashed lines the time of toe off (TO).

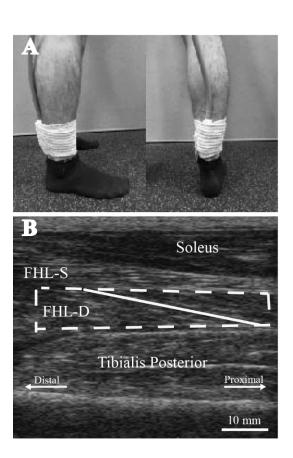


Figure 1

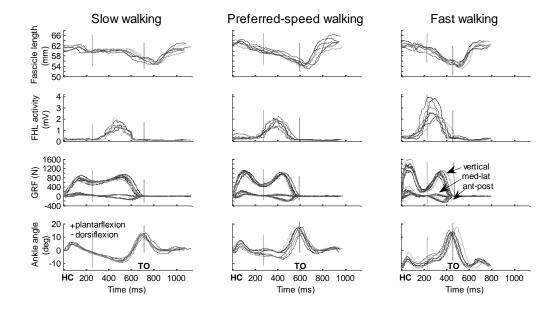


Figure 2

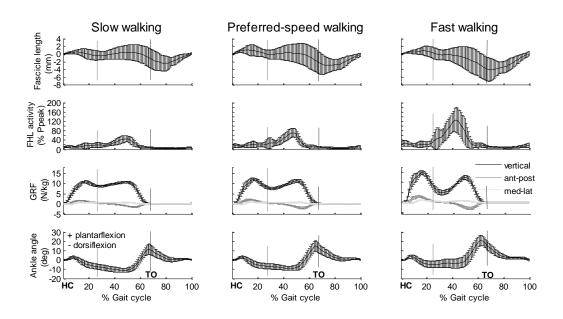


Figure 3

Table 1. Characteristics of the analysed steps

	Slow	Preferred	Fast		
Steps (n)	9 ± 4	7 ± 5	8 ± 2		
Speed (m/s)	$1.06 \hspace{0.2cm} \pm \hspace{0.2cm} 0.08$	$1.37 \pm 0.1$	$1.97 \pm 0.19$		
Step cycle duration (s)	$1.22 \pm 0.07*P,F$	$1.07 \pm 0.05$ *S,F	$0.91 \pm 0.05*S,P$		
Stance phase duration (s)	$0.73 \pm 0.04*P,F$	$0.63 \pm 0.03*S,F$	$0.52 \pm 0.04*S,P$		
Swing phase duration (s)	$0.49 \pm 0.04*P,F$	$0.44 \pm 0.02*S,F$	$0.39 \pm 0.02*S,P$		
Push-off phase duration (s)	$0.30 \pm 0.03*P,F$	$0.26 \pm 0.02*S,F$	$0.22 \pm 0.03*S,P$		
Stance phase (% of step cycle)	$60 \pm 2*F$	59 ± 1*F	57 ± 1*S,P		
Swing phase (% of step cycle)	$40 \pm 2*F$	41 ± 1*F	$43 \pm 1*S,P$		
Push-off phase (% of step cycle)	$25 \pm 3$	$25 \pm 2$	$24 \pm 2$		

Number of included steps is expressed as median  $\pm$  interquartile range. Other values are mean  $\pm$  SD.

<sup>\*</sup> different from preferred (P), fast (F) or slow (S) walking speed (p<.05).

Table 2. Characteristics of flexor hallucis longus fascicle behaviour in walking

		Slow		Preferred			Fast	
	Fascicle length at heel strike (mm)	56.7 ±	5.7	56	±	6.1	56.5 ±	7.7
	Fascicle length at toe off (mm)	56.2 ±	5.7	55.2	$\pm$	6.9	55.6 ±	8.1
	Fascicle length at peak EMG activity (mm)	57.1 ±	6.1	56.1	±	6	56.2 ±	7.8
Stance	Minimum fascicle length (mm)	54.9 ±	5.1	54	±	5.9	53.5 ±	6.9
	Maximum fascicle length (mm)	58.4 ±	6.1	58	$\pm$	6.4	58 ±	7.7
	Range of fascicle length change (mm)	3.5 ±	1.3	3.9	±	1.4	4.5 ±	1.8
	Mean fascicle length change velocity (mm/s)	-0.2 ±	3.2	-1.2	±	4.9	-4.4 ±	6.6
Push- off	Minimum fascicle length (mm)	55.7 ±	5.5	54.6	±	5.6	53.8 ±	7
	Maximum fascicle length (mm)	57.7 ±	6.2	57	±	6.1	56.8 ±	7.7
	Range of fascicle length change (mm)	1.9 ±	1.2	2.3	±	0.9	2.9 ±	1.4
	Mean fascicle length change velocity (mm/s)	0.3 ±	5.7 *F	-2.8	±	8.1	-9.4 ±	11.9 *S

F and S denote fast and slow walking speeds, respectively. Values are mean  $\pm$  SD.

<sup>\*</sup> p<.05.