

Gastrocnemius medialis contractile behavior is preserved during 30% body weight supported gait training

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25 **Abstract**

26 Rehabilitative body weight supported gait training aims at restoring walking function as a key element
27 in activities of daily living. Studies demonstrated reductions in muscle and joint forces, while kinematic
28 gait patterns appear to be preserved with up to 30% weight support. However, the influence of body
29 weight support on muscle architecture, with respect to fascicle and series elastic element behavior is
30 unknown, despite this having potential clinical implications for gait retraining. Eight males (31.9 ± 4.7
31 yrs) walked at 75% of the speed at which they typically transition to running, with 0% and 30% body
32 weight support on a lower-body positive pressure treadmill. Gastrocnemius medialis fascicle lengths
33 and pennation angles were measured via ultrasonography. Additionally, joint kinematics were analyzed
34 to determine gastrocnemius medialis muscle–tendon unit lengths, consisting of the muscle’s contractile
35 and series elastic elements. Series elastic element length was assessed using a muscle–tendon unit
36 model. Depending on whether data were normally distributed, a paired t-test or Wilcoxon signed rank
37 test was performed to determine if body weight supported walking had any effects on joint kinematics
38 and fascicle–series elastic element behavior. Walking with 30% body weight support had no
39 statistically significant effect on joint kinematics and peak series elastic element length. Furthermore,
40 at the time when peak series elastic element length was achieved, and on average across the entire
41 stance phase, muscle–tendon unit length, fascicle length, pennation angle and fascicle velocity were
42 unchanged with respect to body weight support. In accordance with unchanged gait kinematics,
43 preservation of fascicle–series elastic element behavior was observed during walking with 30% body
44 weight support, which suggests transferability of gait patterns to subsequent unsupported walking.

45 Introduction

46 Orthopedic and neurological rehabilitation regimens often involve patients performing gait training
47 with body weight support (BWS) in an attempt to retrain ‘natural’ walking gait function. Whilst
48 overhead suspension systems are largely employed to promote gait rehabilitation from neurologic
49 disorders (Apte et al., 2018), lower-body positive pressure (LBPP) treadmills are frequently used
50 following orthopedic injuries to re-expose patients to walking whilst bearing progressively greater
51 proportions of their body weight (Quigley et al., 2000; Mishra, 2015). In order to restore gait function,
52 movement patterns should be as similar, and thus transferable to daily activities, as possible albeit with
53 a reduction of lower limb muscle and joint forces (Cutuk et al., 2006). Studies assessing LBPP have
54 demonstrated that whilst ground reaction forces are reduced (Eastlack et al., 2005; Cutuk et al., 2006;
55 Grabowski, 2010), gait kinematics are largely preserved (Apte et al., 2018).

56 During normal walking, mechanical energy is largely conserved due to the pendulum-like exchange
57 between potential and kinetic energy (Cavagna et al., 2000). Despite this, additional mechanical work
58 by the muscle–tendon unit (MTU) is required to sustain the movement of the body’s centre of mass.
59 However, walking with BWS reduces the total mechanical energy of the centre of mass, and thus
60 presumably requires less force and work from the MTU to vertically support and accelerate the body
61 (Cavagna et al., 2000; Pavei et al., 2015). In fact, significant reductions in the metabolic cost of
62 locomotion have been observed (Farley and McMahon, 1992; Grabowski et al., 2005; Pavei et al.,
63 2015). Furthermore, reductions of knee joint contact forces (Patil et al., 2013), ankle joint moments
64 (Lewek, 2011; Goldberg and Stanhope, 2013; Fischer and Wolf, 2015) ankle joint angular momentum
65 (McGowan et al., 2008; Barela et al., 2014) and ankle joint power (Lewek, 2011) have been reported
66 during unloading. Despite reduced kinetic and metabolic requirements for vertical body support and
67 forward acceleration, LBPP (unless BWS is $> 75\%$) has been reported to not induce significant
68 differences in spatio-temporal gait parameters such as cadence, stride duration (Grabowski, 2010; Patil
69 et al., 2013) and stride length (Quigley et al., 2000; Cutuk et al., 2006; Patil et al., 2013), nor range of
70 ankle (Quigley et al., 2000; Cutuk et al., 2006) and knee (Eastlack et al., 2005; Cutuk et al., 2006) joint
71 motion. In addition, whilst muscle activity patterns appear unchanged, lower limb muscle activity is
72 reduced during LBPP-treadmill walking (Quigley et al., 2000; Liebenberg et al., 2011; Fischer et al.,
73 2015) with the plantar flexor muscles being particularly susceptible to manipulations of body weight
74 (McGowan et al., 2008). This demonstrates their critical role in human locomotion by providing the
75 majority of the force necessary for vertical body weight support and horizontal propulsion (Neptune et
76 al., 2001; Anderson and Pandy, 2003; McGowan et al., 2008). To gain a better understanding of the
77 plantar flexor’s response to different locomotor tasks, ultrasound imaging is a convenient technique to
78 visualize architectural changes, which help to draw conclusions about muscle function.

79 Ultrasonic visualization of muscle fascicle behavior during locomotion without BWS has not only
80 demonstrated the importance of the storage and release of elastic energy in the Achilles tendon for
81 running and walking (Fukunaga, 2001; Lichtwark et al., 2007), but also that the plantar flexor muscles
82 modulate their behavior depending on gait type, and speed (Farris and Sawicki, 2012). In fact,
83 increased walking speeds have been shown to increase gastrocnemius medialis (GM) shortening
84 velocities (Farris and Sawicki, 2012), and to shorten soleus fascicles (Lai et al., 2015), thereby
85 impairing plantar flexor force generation due to shifting the force–length–velocity relationship towards
86 less favorable contractile conditions (Neptune and Sasaki, 2005; Arnold et al., 2013). However, it is
87 unknown whether walking with BWS modulates fascicle and series elastic element (SEE) behavior to
88 meet the reduced locomotor demands (Richter et al., 2017). Knowledge of any changes in GM’s muscle
89 architecture (primarily fascicle length and pennation angle) in addition to fascicle shortening velocity,
90 which affect the force–length–velocity relationships, would facilitate inference of the mechanisms

91 determining mechanical power generation when BWS is applied. Whereas preservation of fascicle
92 contraction behavior concurrent with preservation of gait kinematics would support the validity of
93 rehabilitative gait training with BWS.

94 30% BWS is typically recommended for rehabilitative re-introduction to walking and running, due to
95 the preservation of kinematic and spatio-temporal gait parameters (Fischer and Wolf, 2015; Apte et al.,
96 2018) in addition to muscle activation patterns (Neal et al., 2016; Hansen et al., 2017). As during early
97 postoperative rehabilitation patients usually start with recovering their walking function, the present
98 study focuses on walking with BWS. Increasing BWS is known to result in walk-to-run transitions
99 occurring at slower absolute walking speeds (Kram et al., 1997), but similar Froude number, a
100 dimensionless number embedding gait speed, leg lengths and gravitational acceleration (in the present
101 paper expressed as BWS) (Kram et al., 1997; Labini et al., 2011). Thus, to obtain mechanically
102 equivalent speeds (i.e., a similar walking speed relative to the preferred walk-to-run transition speed)
103 at different BWS levels, walking speeds should be adjusted to the same Froude number (Kram et al.,
104 1997; Minetti, 2001; Vaughan and O'Malley, 2005), which requires a reduction in absolute walking
105 speed.

106 Therefore, the aim of the present study was to determine via ultrasonography GM's fascicle–SEE
107 behavior during walking at mechanically equivalent speeds, namely 75% of the preferred walk-to-run
108 transition speed (PTS), on a LBPP treadmill, with, and without 30% BWS.

109 It was hypothesized that during walking with BWS (i.e., where forces acting on the SEE are reduced)
110 peak SEE length decreases and is compensated for by longer fascicles and/or smaller pennation angles,
111 rather than by a shorter MTU as ankle and knee joint kinematics are reported to be preserved.

112 **Materials and Methods**

113 **Participants**

114 Eight healthy male volunteers (mean \pm standard deviation: 31.9 ± 4.7 years, 178.4 ± 5.7 cm heights,
115 94 ± 6 cm leg lengths and 73.5 ± 7.3 kg body masses) with treadmill running experience provided
116 informed written consent to participate in this observational study, which received approval from the
117 'Ärzttekammer Nordrhein' Ethical Committee of Düsseldorf, Germany. The study was conducted in
118 the Physiology Laboratory of the Institute of Aerospace Medicine at the German Aerospace Center in
119 Cologne, where all participants underwent a standard medical examination. Exclusion criteria included
120 any cardiovascular, musculoskeletal or neurological disorders within the previous two years in addition
121 to any lower limb surgery that may affect MTU behavior.

122 **Study design and experimental protocol**

123 Participants attended the laboratory on a single occasion and walked on an Anti-Gravity Treadmill
124 (AlterG; AlterG®, M320, Fremont, USA; Figure 1), an LBPP treadmill, with 0% BWS and thereafter
125 with ~30% BWS (recommended load for rehabilitative gait training; Hesse, 2008; Fischer and Wolf,
126 2015). Before each trial, participants familiarized themselves until they have acclimatized to the BWS
127 level and the predefined walking speed (~ 4 min). After another 2 min accommodation time given to
128 produce reproducible gait kinematics (Karamanidis et al., 2003) and hence a total warm-up time of ~
129 6 min, which is further required for the Achilles tendon to achieve a relatively stable steady-state
130 behavior (Hawkins et al., 2009), data were collected for 30 s. Blinding of participants was not
131 applicable due to the nature of the experiment setup.

132 Walking speeds were defined as 75% of the preferred walk-to-run transition speed (PTS) expressed as
 133 a Froude number (PTS_{FR}). PTS_{FR} was estimated by fitting an exponential regression
 134 equation ($PTS_{FR}(a) = 1.183e^{-5.952a} + 0.4745$) with a least-squares method ($r^2 = 0.99$) to the
 135 experimental data of Kram and co-workers (Kram et al., 1997) using the resulting acceleration (a) as
 136 the independent variable. Hence, for $a = 0.7 g$ ($g = 9.81 m \cdot s^{-2}$), a PTS_{FR} value of 0.49 was obtained.
 137 By accounting for the participants' leg lengths (l), measured from the greater trochanter to the ground,
 138 the individual $PTS(a) = \sqrt{PTS_{FR}(a) \cdot a \cdot l}$ expressed in meters per second was determined resulting
 139 in walking speeds of $1.58 \pm 0.05 m \cdot s^{-1}$ at 0% BWS, and $1.34 \pm 0.04 m \cdot s^{-1}$ at 30% BWS.

140 The AlterG was enclosed within a sealed height-adjustable chamber, which allowed air pressure to
 141 increase inside the chamber and generated an additional vertical buoyant force to produce controlled
 142 and stable BWS levels. A seal between the participant and the chamber was created through a neoprene
 143 kayak-type skirt that could be zipped into the aperture of the chamber.

144 [Please insert Figure 1 here]

145 **Joint kinematics**

146 Knee and ankle joint angles were recorded using a twin-axis (Penny and Giles Biometrics Ltd.,
 147 Blackwood Gwent, UK) and a custom-made potentiometer based electrogoniometer, respectively. The
 148 end blocks of the knee electrogoniometer were placed along the leg from the greater trochanter to the
 149 lateral femur epicondyle and along the leg from the lateral epicondyle of the femur to the lateral
 150 malleolus. The end blocks of the ankle electrogoniometer were placed along the leg from the lateral
 151 femur epicondyle to the lateral malleolus and from the lateral malleolus to the most distal end of the
 152 fifth metatarsal. Before each walking trial, a reference measurement was taken in the anatomical neutral
 153 position to define the 0° joint angles. Data were sampled at a frequency of 1500 Hz via the TeleMyo
 154 2400 G2 Telemetry System (Noraxon USA., Inc., Scottsdale, USA) and MyoResearch XP software
 155 (Master Edition 1.08.16).

156 **Spatio-temporal parameters**

157 To determine gait cycle events and thereby define stance phases of the left leg, participant plantar
 158 pressure was measured (83 Hz) via insoles (Novel GmbH, loadsol® version 1.4.60, Munich,
 159 Germany). Touchdown and toe-off were automatically detected using a 20 N threshold for 0.1 s via a
 160 custom-made script (MATLAB R2018a, MathWorks, Inc., Natick, United States). Insole and
 161 electrogoniometer signals were time-synchronized via recording of a rectangular pulse generated by
 162 pressing on a custom-made pedal.

163 **GM muscle fascicle length and pennation angle**

164 Real-time B-mode ultrasound (Prosound $\alpha 7$, ALOKA, Tokyo, Japan) captured at 73 Hz using a T-
 165 shaped 6-cm linear array transducer (13 MHz) was performed over the midbelly of the left GM muscle.
 166 Transducer position was standardized by determining the intersection of the mediolateral and
 167 proximodistal midline of the GM and aligning the transducer longitudinally to the fascicles, while
 168 transducer movement was minimized by using a custom-made cast, which was secured with elastic
 169 Velcro. Ultrasound recordings and electrogoniometer signals were time-synchronized via a rectangular
 170 pulse generated by a hand switch, which was recorded synchronously through the electrocardiography
 171 channel of the ultrasound and the MyoResearch software. A semi-automatic tracking algorithm
 172 (UltraTrack Software, version 4.2; Farris and Lichtwark, 2016) was used to quantify muscle fascicle

173 length (distance between the insertion of the fascicles into the superficial and the deep aponeuroses)
174 and pennation angles (angle between the fascicle and the deep aponeurosis) during the stance phase.
175 Manual correction of the digitized fascicle and the deep aponeurosis, defined as a second fascicle, were
176 performed where appropriate. If the field of view of the transducer was not sufficiently wide to capture
177 the entire fascicle, the missing portion was estimated via manual extrapolation based on the assumption
178 that the fascicle and the aponeurosis extend linearly. Ultrasonography has been frequently used in
179 dynamic conditions (Cronin and Lichtwark, 2013) and has been demonstrated to provide reliable
180 measures of GM fascicle lengths and pennation angles (Aggeloussis et al., 2010; Van Hooren et al.,
181 2020).

182 **SEE and MTU lengths**

183 Series elastic element length was estimated using an MTU model by subtracting muscle fascicle lengths
184 multiplied by the cosine of their pennation angles from the MTU lengths (Fukunaga, 2001).
185 Muscle–tendon unit length was calculated via a linear regression equation (Hawkins and Hull, 1990),
186 using participant’s shank length data (the distance from the lateral malleolus to the lateral femur
187 epicondyle) in addition to recorded knee and ankle joint angles.

188 **Data processing**

189 For each participant, and each outcome, eight consecutive stance phases (touchdown to toe-off) of the
190 left foot per condition were analyzed and averaged using custom-made scripts (MATLAB R2018a,
191 MathWorks, Inc., Natick, United States). Prior to being resampled to 101 data points per stance phase
192 (to represent data as a percentage), fascicle lengths and pennation angles were smoothed with a five-
193 point moving average filter. Electrogoniometer signals were smoothed with a fifth-order Butterworth
194 low-pass filter, and a 10-Hz cut-off frequency. Fascicle velocity was calculated as the time derivative
195 of its length using the central difference method (Robertson et al., 2013).

196 Based on the ultrasound and joint-angle recordings SEE length, MTU length, fascicle length, pennation
197 angle and fascicle velocity were determined at the time when peak SEE length was achieved, and thus
198 force acting on the SEE is presumably at its greatest. Furthermore, average values across the stance
199 phase were determined. Overall fascicle shortening was calculated by subtracting the minimum from
200 maximum fascicle length. Knee and ankle joint range of motion were defined as the delta between their
201 respective minimum and maximum joint angles. Additionally, the difference in knee and ankle joint
202 angles between touchdown to the time of first local maximum and maximum dorsiflexion, were defined
203 as knee flexion and ankle dorsiflexion, respectively. Knee and Ankle joint angles at touchdown and
204 toe-off as well as ground-contact times were determined. To estimate the level of BWS achieved by
205 applying LBPP, average plantar forces over the stance phase were determined and expressed as
206 percentage of the average plantar forces when walking without BWS.

207 **Statistical analysis**

208 Distribution normality was assessed using the Shapiro–Wilk normality test. If normally distributed, a
209 two-tailed paired t-test was performed, whereas if not, a non-parametric Wilcoxon (matched-pairs)
210 signed rank test was used to compare conditions (30% vs. 0% BWS). All tests were performed in
211 GraphPad Prism (v 7.04) with a significance level of $\alpha = 0.05$. Effect Sizes (d_z) were calculated using
212 the G*Power software version 3.1.9.4 (Faul et al., 2007). Thresholds of 0.2, 0.5 and 0.8 were defined
213 as small, moderate and large effects between the two comparison groups (Cohen, 1988).

214 **Results**

215 Participants walking with 30% BWS generated significantly lower average plantar forces (-194 ± 32
 216 N, $P < 0.001$, $d_z = -6.07$) corresponding to $68 \pm 4\%$ of the average plantar forces when walking without
 217 BWS, which did not differ significantly from the target of 70% ($P = 0.223$). Ground-contact times were
 218 0.03 ± 0.03 s longer when walking with 30% BWS, however, the effect was statistically not significant
 219 ($P = 0.078$, $d_z = 0.80$) (Table 1).

220 Figure 2 presents the averages and standard errors of joint angles and muscle–SEE outcomes time
 221 normalized to a single stance phase for participants walking with 0% and 30% BWS.

222 No statistically significant differences in knee and ankle joint angles at touchdown ($P = 0.164$, $d_z =$
 223 -0.55 ; $P = 0.635$, $d_z = -0.18$), at toe-off ($P = 0.848$, $d_z = -0.07$; $P = 0.641$, $d_z < 0.01$) and at the time of
 224 the peak SEE length ($P = 0.461$, $d_z = 0.42$; $P = 0.742$, $d_z = 0.04$) were observed (Table 1). Furthermore,
 225 knee and ankle joint range of motion ($P = 0.860$, $d_z = 0.06$; $P = 0.844$, $d_z = -0.04$), knee flexion ($P =$
 226 0.347 , $d_z = -0.36$) and ankle dorsiflexion ($P = 0.204$, $d_z = 0.50$) were unaffected by walking with 30%
 227 BWS (Table 1).

228 [Please insert Table 1 here]

229 [Please insert Figure 2 here]

230 Walking with 30% BWS had no effect on peak SEE length ($P = 0.976$, $d_z = -0.01$) (Figure 3A).
 231 Furthermore, at the time when peak SEE length was reached, no statistically significant differences
 232 from 0% BWS were observed for MTU length ($P = 0.641$, $d_z = -0.04$), fascicle length ($P = 0.890$, $d_z =$
 233 -0.05), pennation angle ($P = 0.945$, $d_z = -0.03$) and fascicle velocity ($P = 0.576$, $d_z = -0.21$) (Figure
 234 3A-C).

235 [Please insert Figure 3 here]

236 No statistically significant differences of the average values across the entire stance phase were also
 237 observed for SEE length ($P = 0.945$, $d_z = 0.05$), MTU length ($P = 0.641$, $d_z = 0.01$), fascicle length (P
 238 $= 0.790$, $d_z = -0.10$), pennation angle ($P = 0.641$, $d_z = 0.16$) and fascicle velocity ($P = 0.148$, $d_z = 0.51$)
 239 between 0% and 30% BWS walking (Table 2). Furthermore, overall fascicle shortening did not differ
 240 between conditions ($P = 0.313$, $d_z = -0.43$) (Table 2).

241 [Please insert Table 2 here]

242 Discussion

243 Effects of walking with 30% BWS on contractile and series elastic element behavior

244 During the walking trials, participants were successfully unloaded by 30% of their body weight, as the
 245 average plantar forces actually achieved by inducing LBPP did not differ significantly from the target
 246 average plantar forces. The main findings were that walking with 30% BWS did not significantly affect
 247 joint kinematics. Furthermore, in contrast to the hypotheses, walking with 30% BWS induced no
 248 statistically significant differences from 0% BWS in peak SEE length as well as MTU length, fascicle
 249 length and pennation angle neither at the time of the peak SEE length, nor on average across the stance
 250 phase. Also, in contrast with the hypotheses, no statistically significant effect of 30% BWS was found
 251 on fascicle shortening velocity at the time of the peak SEE length, nor on average across stance, despite
 252 a reduction in absolute walking speed (albeit same Froude number). These findings are further
 253 supported by the overall small effect sizes.

254 Previous studies and simulation models have shown that the GM force-length-velocity behavior shifts
255 with gait type and speed to meet the varying locomotor demands (Farris and Sawicki, 2012; Arnold et
256 al., 2013). However, in the present study, fascicle length and pennation angle were unchanged when
257 walking with 30% BWS, which implies that the GM remains operating on a similar part of the
258 force–length relationship, thereby preserving its force generation ability (Arnold et al., 2013).
259 Moreover, GM fascicle velocity has been reported to decrease with decreasing walking speed, thereby
260 increasing GM’s force generation ability (Farris and Sawicki, 2012; Arnold et al., 2013). In fact, in the
261 present study average fascicle velocity was $5.0 \pm 10 \text{ mm} \cdot \text{s}^{-1}$ slower when walking at a slightly slower
262 speed at 30% vs. 0% BWS ($-0.24 \text{ m} \cdot \text{s}^{-1}$) reaching a moderate effect ($d_z = 0.51$), however, high
263 variability may have contributed to it failing to reach statistical significance.

264 It has been reported that the ankle plantarflexion moment decreases with increasing BWS (Lewek,
265 2011; Goldberg and Stanhope, 2013; Fischer and Wolf, 2015). In fact, the present results suggests a
266 reduction in average plantar force by almost 200 N whilst ankle joint kinematics were largely preserved
267 when walking with 30% BWS suggesting that ankle joint moment was reduced. Interestingly, this did
268 not affect peak SEE length, which incorporates the length of the free tendon and aponeurosis. As
269 aponeurosis stiffness varies upon contractile conditions (e.g. reduced muscle activity results in lower
270 orthogonal muscle expansion linked to lower transverse strain) (Azizi and Roberts, 2009), SEE length
271 can remain similar despite a reduction in ankle joint moment.

272 However, as in the present observational study MTU interaction was only modelled for the GM, which
273 accounts for a modest fraction ($\sim 17\%$) of the physiologic cross-sectional area of the plantar flexor
274 muscles (Ward et al., 2009), changes that influence the ankle joint moment might not be fully reflected.
275 Furthermore, joint moments were not determined and SEE length was not measured directly but
276 estimated using an MTU model. Thus, if tuning of the mechanical properties of the SEE actually causes
277 preserved SEE and fascicle kinematics warrants further study.

278 **Implications for rehabilitative body weight supported gait training**

279 Maintenance of joint kinematics and GM behavior may facilitate rehabilitative gait training by
280 preserving ‘natural’ movement patterns, despite joint loads and related pain being reduced (Eastlack et
281 al., 2005; Cutuk et al., 2006). Preserved fascicle’s operating range suggests that the stimuli exerted on
282 the muscle remain the same and thus help to maintain optimum fascicle length for force production,
283 which is key for locomotor recovery. Furthermore, the maintenance of SEE strain, as possibly achieved
284 by an increased aponeurosis strain, might help to prevent degeneration and maintain function of the
285 aponeurosis despite external unloading. Patients who may benefit from LBPP gait training during their
286 early postoperative rehabilitation include not only those with tendon, ligament and meniscus repairs
287 but also joint replacements or fractures (Eastlack et al., 2005). However, the increased aponeurosis
288 strain, which is required to compensate for the decreased free tendon strain (and thus to maintain SEE
289 strain), could pose a potential risk to patients after Achilles tendon rupture if the rupture does not
290 exclusively affect the free tendon. Therefore, BWS rehabilitation should be individualized to the
291 specific pathological characteristics of patients, depending on the impaired biological tissues that
292 require unloading, e.g. rehabilitation after total knee arthroplasty vs. ankle or Achilles tendon injury.
293 Based on the current findings, further studies including different patient groups are required.

294 The present data, are not only in agreement with a recent systematic review, which concluded that
295 spatio-temporal and kinematic gait parameters can be preserved with up to 30% BWS (Apte et al.,
296 2018), but extends this view to preserved muscle–SEE mechanics. In fact, healthy individuals appear
297 able to retain normal walking kinematics even when unloaded by up 50% BWS (Van Hedel et al.,

298 2006; Awai et al., 2017). The absence of any effects when BWS was increased from 0% to 30%
299 suggests that the modulation of fascicle–SEE behavior does not develop linearly with increasing BWS
300 but is determined by a certain threshold, however if this threshold is below or above 50% BWS remains
301 to be determined. Additionally, if non-LBPP BWS systems, such as overhead suspension harnesses,
302 therapist-assisted waist belts or robotic-assisted gait-training devices, are also able to preserve GM
303 behavior also warrants further study. Nevertheless, the present observational study supports the
304 recommendation (Fischer and Wolf, 2015) for LBPP-induced 30% BWS in rehabilitative gait training.
305 Finally, it should be noted that walking speed was intentionally reduced with increasing BWS via the
306 adjustment to the same Froude number to obtain mechanically equivalent walking speeds (Vaughan
307 and O'Malley, 2005). Thus, the observation that the neural system appears to largely preserve GM
308 overall contraction behavior in addition to joint kinematics suggests that the approach of producing
309 comparable gait patterns across the different walking conditions was successful and should be
310 considered for future gait rehabilitation.

311 **Conclusions**

312 This is the first study to examine in vivo GM fascicle–SEE behavior during walking at 30% BWS,
313 frequently employed in gait rehabilitation, at 75% PTS on an LBPP treadmill. The present findings
314 reveal that during walking with 30% BWS fascicle–SEE behavior was largely preserved, in contrast
315 to the hypothesis. Thus, the present study not only supports the contention made in previous studies
316 that walking with the recommended therapeutic dose of 30% BWS largely retains spatio-temporal and
317 joint kinematic characteristics but extends this to GM fascicle and SEE mechanics. This may be
318 advantageous during rehabilitative gait training with BWS as it indicates transferability of gait patterns
319 to subsequent unsupported walking.

320 **Abbreviations**

321	AlterG	AlterG® Anti-Gravity Treadmill™
322	BWS	Body weight support
323	g	Earth's gravitational acceleration
324	GM	Gastrocnemius medialis
325	LBPP	Lower-body positive pressure
326	MTU	Muscle–tendon unit
327	PTS	Preferred walk-to-run transition speed
328	SEE	Series elastic element

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In review

451 **Figure Captions**452 **Figure 1. Experimental set-up**

453 Participant walking on the lower-body positive pressure treadmill (the AlterG) with an ultrasound
 454 transducer attached to the midbelly of the gastrocnemius medialis muscle and electrogoniometers
 455 (added in green to accentuate placement) to measure ankle and knee joint angles.

456 **Figure 2. Sample average and standard error of plantar forces (A), knee joint angle (B), ankle**
 457 **joint angle (C), muscle–tendon unit length (D), series elastic element length (E), fascicle length**
 458 **(F), pennation angle (G) and fascicle velocity (H) for participants walking at 75% of their**
 459 **preferred walk-to-run transition speed with 0% body weight support (black line) and 30% body**
 460 **weight support (red line) during the entire stance phase.**

461 The solid lines represent the sample average, and the corresponding shaded areas represent the standard
 462 error of measurement.

463 **Figure 3. Series elastic element length (A), muscle–tendon unit length (A), fascicle length (A),**
 464 **pennation angle (B) and fascicle velocity (C) at the time of the peak series elastic element length**
 465 **as presented as boxplots for participants walking without body weight support (black box) and**
 466 **30% body weight support (red box)**

467 The lower and upper parts of the box represent the first and third quartile, respectively. The length of
 468 the whisker represent the minimum and maximum values. The horizontal line in the box represents the
 469 statistical median of the sample; + the mean of the sample; ○ individual data points

470 **Table 1. Means and standard deviations of kinematic outcome measures while participants walked at**
 471 **75% of their preferred walk-to-run transition speed with 0% and 30% body weight support**

	0% BWS	30% BWS	Differences	95% CI	P	Effect Size
Ground contact time [s]	0.59 ± 0.04	0.62 ± 0.04	0.02 ± 0.03	0.01 to 0.05	0.078 ^w	0.80
Ankle joint angle at touch-down [°]	-6.35 ± 3.26	-7.61 ± 7.74	-1.26 ± 7.17	-7.25 to 4.73	0.635 ^t	-0.18
Knee joint angle at touch-down [°]	3.07 ± 5.92	1.24 ± 5.21	-1.84 ± 3.34	-4.63 to 0.95	0.164 ^t	-0.55
Ankle joint angle at toe-off [°]	-17.47 ± 7.42	-17.45 ± 7.21	0.02 ± 10.69	-8.92 to 8.95	0.641 ^w	< 0.01
Knee joint angle at toe-off [°]	47.67 ± 11.38	46.94 ± 7.91	-0.73 ± 10.38	-9.41 to 7.94	0.848 ^t	-0.07
Ankle joint range of motion [°]	21.04 ± 5.47	20.88 ± 4.93	-0.16 ± 3.55	-3.12 to 2.81	0.844 ^w	-0.04
Knee joint range of motion [°]	45.21 ± 9.09	45.72 ± 3.77	0.51 ± 7.82	-6.03 to 7.04	0.860 ^t	0.06
Ankle dorsiflexion [°]	10.26 ± 3.04	11.82 ± 3.77	1.56 ± 3.15	-1.07 to 4.19	0.204 ^t	0.50
Knee flexion [°]	17.82 ± 4.42	15.81 ± 6.49	-2.01 ± 5.65	-6.73 to 2.71	0.347 ^t	-0.36
Ankle joint angle at peak SEE length [°]	2.41 ± 3.18	2.72 ± 6.06	0.31 ± 7.91	-6.30 to 6.92	0.742 ^w	0.04
Knee joint angle at peak SEE length [°]	9.19 ± 6.83	11.04 ± 4.75	1.85 ± 4.43	-1.86 to 5.55	0.461 ^w	0.42

472 BWS: body weight support; CI: confidence interval; P: result of the paired t test (°) or Wilcoxon matched-pairs signed rank
 473 test (°) indicating a statistically significant effect of body weight support ($\alpha = 0.05$). n = 8

474

475 **Table 2. Means and standard deviations of gastrocnemius medialis muscle and SEE outcome measures**
 476 **while participants walked at 75% of their preferred walk-to-run transition speed with 0% and 30% body**
 477 **weight support**

	0% BWS	30% BWS	Differences	95% CI	P	Effect Size
Average SEE length [mm]	404.19 ± 23.40	404.62 ± 20.74	0.42 ± 7.70	−6.02 to 6.86	0.945 ^w	0.05
Average MTU length [mm]	449.11 ± 19.80	449.18 ± 18.27	0.07 ± 6.24	−5.15 to 5.28	0.641 ^w	0.01
Average fascicle length [mm]	49.44 ± 6.48	49.18 ± 5.28	−0.25 ± 2.58	−2.41 to 1.90	0.790 ^t	−0.10
Average pennation angle [°]	24.92 ± 3.93	25.16 ± 3.84	0.24 ± 1.46	−0.98 to 1.46	0.641 ^w	0.16
Average fascicle velocity [mm·s ^{−1}]	−31.03 ± 16.18	−26.01 ± 8.72	5.02 ± 9.91	−3.27 to 13.31	0.148 ^w	0.51
Overall fascicle shortening [mm]	17.23 ± 7.54	15.12 ± 4.64	−2.10 ± 4.91	−6.20 to 2.00	0.313 ^w	−0.43

478 BWS: body weight support; CI: confidence interval; P result of the paired t test (^t) or Wilcoxon matched-pairs signed rank
 479 test (^w) indicating a statistically significant effect of body weight support ($\alpha = 0.05$). n = 8

Figure 1.JPEG



Figure 2.TIF

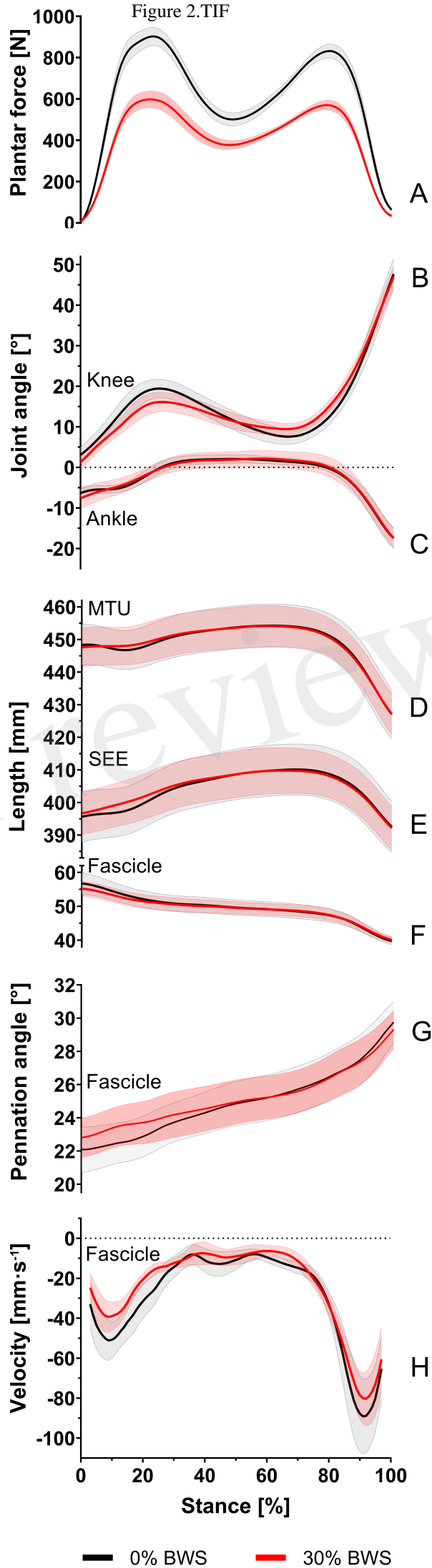


Figure 3.TIF

