

Abstract

1
2 Research addressing lower limb amputee gait and prosthetic design often focuses on men, despite
3 female lower limb amputees having different risk factors and lower success with their prosthetics
4 overall. It is widely agreed that sex differences exist in able-bodied gait, but research analyzing
5 sex differences in amputee gait is rare. This study compared male and female transtibial amputee
6 gait to ascertain potential sex differences. Forty-five transtibial amputees were asked to walk at
7 their self-selected speed and spatiotemporal gait data were obtained. Both the mean and variability
8 metric of parameters were analyzed for 10 male and 10 female participants. Within sexes,
9 amputated limbs had a shorter stance time, longer swing time, and larger step length. Females had
10 a 10% shorter stance time and 26% larger normalized step and stride length than males. Female
11 participants also walked over 20% faster than male participants. Finally, significant interactions
12 were found in the mean and variability metric of stride velocity, indicating greater variability in
13 women. These findings suggest that sex differences exist in transtibial amputee gait, offering
14 possible explanations for the different comorbidities experienced by female lower limb amputees.
15 These results have major implications for female amputees and for sex-specific research,
16 rehabilitation, and prosthetic design.

17

18 **Keywords:** sex-based research, female amputees, gait analysis, variability, prosthetic

19

20 **Word Count:** 3,489 words

21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42

Introduction

Lower limb amputees (LLAs) suffer from various comorbidities, including a higher incidence and risk of osteoarthritis (OA) in their intact limb as compared to age-matched non-amputees.^{1,2} Gait assessments of LLAs are often used to evaluate risk of comorbidities and assess aspects of prosthetic limb design.¹⁻⁴ While there is a great deal of research assessing gait in LLAs, the majority of the subject base is male amputee dominated.¹⁻⁴ This may be due to the fact that the majority (~70%) of LLAs in the U.S., Canada, and Australia are male.⁵⁻⁷ However, it may not be appropriate to apply research findings from male LLAs to the female LLA population, as able-bodied (AB) women have a 2-3x higher risk of developing knee OA than AB males.⁸ Furthermore, female LLAs experience a host of differing comorbidities compared to their male counterparts, including heightened risk of OA (15% per kg/m²), greater pain and lower functional abilities with their prosthetics, and they are ~6x more likely to experience a fall-related injury.^{5,9-12} This information brings into question what sex differences are contributing to the different comorbidities associated with female LLAs. As both the incidence of OA as well as falls have been associated with abnormalities in gait (e.g., asymmetry),^{8,9} a clear starting point would be to consider potential sex-based differences in gait.

In the AB population, it is well documented that men and women differ in spatiotemporal and kinematic gait parameters.^{8,13-16} Consistent differences in hip and ankle range of motion have been found and are believed to influence injury mechanisms in AB women.^{13,14,16} Sex differences in AB gait are often attributed to differences in gait control strategy, anatomy and muscular strength.^{13,15} However, despite these known differences in the AB population, greater prevalence of comorbidities in female LLAs, and the large population of female LLAs (~30% in the U.S.,

43 Canada, and Australia),⁵⁻⁷ there is very little research examining the biomechanics of female LLA
44 gait as well as if and how they differ from their male counterparts.

45 Sex-based research in general has shown to improve a large variety of medical science and
46 engineering outcomes.¹⁷ Thus, understanding sex differences in LLA gait not only has major
47 implications in prosthetic design and rehabilitation, but also in science and engineering research
48 as a whole. This presents a clear need to explore sex differences in LLA gait. Therefore, the
49 purpose of this descriptive study was to ascertain sex differences in transtibial amputee (TTA) gait
50 through the comparison of male and female TTA spatiotemporal gait parameters.

51

52

Methods

53 Anonymized gait data were obtained from a previously published study that included a
54 cohort of 45 unilateral TTAs (35 men, 78%; mean \pm standard deviation (SD): age = 61 ± 14 years;
55 height = 175 ± 10.0 cm; weight = 91.2 ± 19.1 kg; time since amputation = 16 ± 19 years) with
56 well-fitting prostheses as judged by the participant's prosthetist.¹⁸ A subsample of 10 male
57 participants was randomly selected from the 35 to have an equal sample size between groups for
58 statistical analyses. The characteristics of these 20 participants (10 men, 50%; mean \pm SD: age =
59 59 ± 16 years; height = 171 ± 12.0 cm; weight = 89.6 ± 20.7 kg; time since amputation = 15 ± 18
60 years) are given in Table 1. Ethics for the original study was approved by the XXXX Clinical
61 Human Research Ethics Committee with all participants providing written consent. For use of the
62 anonymized data in this study, further ethics approval was obtained by the XXXX Human
63 Research Ethics Board.

64 The data obtained were non-normalized spatiotemporal parameters, including gait velocity,
65 stride velocity, stance time, swing time, step length, stride length, and step time (Table 2). All

66 parameters, with the exception of gait velocity, were measured for both the amputated limb (AL)
67 and non-amputated limb (NAL) across 10 walking trials. Gait velocity was still measured across
68 10 trials but not for both limbs because it is a parameter of the body center and thus combined for
69 both limbs. The raw data shared from the previous study were participant characteristics and mean
70 parameter values for each limb (when available) at each trial. In the previous study, gait data were
71 obtained in a rehabilitation center using an instrumented GAITRite walkway system (CIR Systems
72 Inc., Sparta, NJ, USA) that captures footfalls at a sampling rate of 120 Hz across an area of 4.9 m
73 x 0.6 m.¹⁸ Participants were asked to walk at their self-selected speed across the GAITRite mat for
74 10 consecutive trials, starting and stopping 2 m before and after the walkway end.¹⁸

75 Scaling factors were used to normalize gait data by leg length (Table 2).¹⁹ However, only
76 height was measured in the previous study and not intact leg length, a more common normalization
77 parameter.¹⁹ Therefore, separate anthropometric scaling factors were used to determine leg length
78 of participants based on their height.^{20,21} Statistical analyses were run on both the normalized (non-
79 dimensional) and non-normalized gait parameters. These analyses are described as follows.
80 Normality of all parameters was confirmed using Shapiro-Wilk tests. The current study's statistical
81 design was developed to explore the effect of sex on spatiotemporal gait parameters while
82 considering potential interactions between sex, limb status, and trial number. Assessing parameter
83 means and variabilities across trials, rather than averaged over trials, provides a more
84 comprehensive investigation of participant gait and its variation trial-to-trial. Therefore, we were
85 interested in analyzing sex differences in both gait parameter means and gait parameter
86 variabilities across trials and across limbs. To do so, two separate statistical tests were run. First,
87 three-way mixed ANOVAs were run separately for the means and variability metrics (VMs) of all
88 gait parameters except gait velocity. In these tests, sex was the between subject's factor, split into

89 two levels (male, female). The two within subject's factors were trial number, split into seven
90 levels (trials 4-10), and limb status, split into two levels (AL, NAL). Only trials 4-10 were analyzed
91 as the first three trials demonstrated acclimatization effects (i.e., subjects were still adjusting to
92 gait analysis) and were thus considered practice trials. VM parameters for each limb were
93 calculated by subtracting the mean value of each trial by the average over the seven trials. For
94 example, to determine the VM of the amputated limb's stride velocity for trial 4, the following
95 calculation was performed:

$$strideV_{AL\ t4_{VM}} = strideV_{AL\ t4_{mean}} - avg(strideV_{AL\ t4_{mean}} : strideV_{AL\ t10_{mean}}) \quad (1)$$

96 As can be seen in the above equation, the VM data for a given parameter at a given trial is a form
97 of residual showing the difference of that data from the subject's own mean. However, when
98 considered across all trials, these VM data do indeed demonstrate the subject's gait variability for
99 that parameter. For example, if the VM of a parameter at every trial were zero, this would mean
100 there is no variability in that parameter across trials. Second, two-way mixed ANOVAs were run
101 separately for the mean and VM of gait velocity. In these tests, sex was the between subject's
102 factor, split into two levels (male, female) and the within subject's factor was trial number, split
103 into seven levels (trials 4-10). VMs were calculated the same way as previously described. The
104 need for a different statistical test for this parameter was because gait velocity was not measured
105 for each limb, therefore a within-subject's factor of limb could not be analyzed.

106 To assess demographic similarity between the male and female cohorts, characteristics
107 including age, stump length, height, weight, intact leg length, BMI, and time since amputation
108 were analyzed using independent-samples t-tests to determine if significant sex differences
109 existed.

110 To further interpret results, Bonferroni corrected post-hoc tests were conducted when
111 statistically significant interactions were found. All analyses were performed in SPSS (version
112 26.0, IBM Corp., Armonk, NY, USA) and significance was determined based on $p < .05$.

113

114

Results

115 Regarding participant characteristics, male participants were significantly taller ($179 \pm$
116 10.7 cm compared with 162 ± 6.64 cm, $p < .001$) and heavier (99.6 ± 18.9 kg compared with 79.6
117 ± 19.2 kg, $p = .031$) than female participants. Male participants also had a significantly longer
118 intact leg length than females (94.9 ± 5.66 cm compared with 84.9 ± 3.48 cm, $p < .001$). No
119 significant difference between sexes was found for participant BMI ($p = .754$), age ($p = .338$),
120 stump length ($p = .139$), or time since amputation ($p = .308$) (Table 1).

121 Considering gait parameter means and VMs, statistical findings for normalized (i.e., non-
122 dimensional) and non-normalized parameters were very similar. For any statistically significant
123 results found in both normalized and non-normalized variables, p -values are given for both.
124 Otherwise, differences in significant results are clearly noted. Only significant results
125 demonstrating a main effect of sex are shown in figures since sex differences were the main interest
126 of this exploratory study. If a significant main effect of sex was present for a variable when
127 normalized and non-normalized, figures are only given for the non-normalized case to ease
128 comprehension.

129 For gait parameter means, stride velocity demonstrated a statistically significant three-way
130 interaction between limb*trial*sex (normalized: $p = .008$; non-normalized: $p = .004$) (Figure 1A).
131 Through post-hoc analysis, a significant simple two-way interaction was found between trial*limb
132 for females (normalized: $p = .045$; non-normalized: $p = .044$). However, Bonferroni corrected post-

133 hoc tests did not demonstrate further statistically significant simple main effects of limb or trial on
134 stride velocity for females. There were no other statistically significant interactions. However,
135 statistically significant main effects of both limb and sex were found for parameter means. For all
136 participants, a statistically significant main effect of limb was found on stance time (Figure 2),
137 swing time, and step length (Figure 3A), with ALs having a shorter stance time (normalized: 2.31
138 ± 0.271 compared with 2.43 ± 0.258 , $p < .001$; non-normalized: 0.693 ± 0.079 s compared with
139 0.730 ± 0.077 s, $p < .001$), longer swing time (normalized: 1.34 ± 0.126 compared with $1.22 \pm$
140 0.121 , $p < .001$; non-normalized: 0.409 ± 0.037 s compared with 0.372 ± 0.033 s, $p < .001$), and
141 larger step length (normalized: 0.728 ± 0.176 compared with 0.693 ± 0.156 , $p = .026$; non-
142 normalized: 66.0 ± 13.2 cm compared with 63.1 ± 12.4 cm, $p = .020$). A statistically significant
143 main effect of sex was found on stride velocity (Figure 1A) and gait velocity (Figure 1B), with
144 females having a higher stride velocity (normalized: 0.452 ± 0.086 compared with 0.340 ± 0.082 ,
145 $p = 0.008$; non-normalized: 130 ± 24.8 cm/s compared with 103 ± 22.4 cm/s, $p = 0.019$) and a
146 higher gait velocity (normalized: 0.450 ± 0.087 compared with 0.339 ± 0.083 , $p = 0.009$; non-
147 normalized: 130 ± 25.1 cm/s compared with 103 ± 22.5 cm/s, $p = 0.020$). As well, females had a
148 significantly shorter non-normalized stance time than men (0.679 ± 0.067 s compared with 0.747
149 ± 0.074 s, $p = .002$) (Figure 2). To note, this result was not significant for normalized stance time
150 ($p = .065$). Finally, compared with male participants, females had a significantly longer normalized
151 step length (0.811 ± 0.143 compared with 0.598 ± 0.102 , $p = .002$) and normalized stride length
152 (1.63 ± 0.285 compared with 1.20 ± 0.202 , $p = .002$), see Figure 3A and Figure 3B respectively.
153 However, these results were not significant for non-normalized step length ($p = .137$) or stride
154 length ($p = .676$).

155 For gait parameter VMs, the stride velocity VM demonstrated a significant three-way
156 interaction between limb*trial*sex (normalized: $p = .015$; non-normalized: $p = .014$) (Figure 4).
157 Through post-hoc analysis, a significant simple two-way interaction was found between trial*limb
158 for females (normalized: $p = .045$; non-normalized: $p = .044$). Bonferroni corrected post-hoc tests
159 demonstrated a statistically significant simple main effect of limb on stride velocity VM for
160 females at trial 6 (normalized: AL: -0.00261 ± 0.00602 as compared to NAL: 0.00206 ± 0.00938 ,
161 $p = .041$; non-normalized: AL: -0.761 ± 1.74 cm/s as compared to NAL: 0.593 ± 2.70 cm/s, $p =$
162 $.040$). As well, a statistically significant two-way interaction between trial*sex was found for step
163 length VM (normalized: $p = .036$; non-normalized: $p = .033$). However, Bonferroni corrected post-
164 hoc tests failed to demonstrate statistically significant simple main effects of trial or sex on step
165 length.

166

167

Discussion

168 The purpose of this study was to analyze TTA gait parameters and ascertain potential sex
169 differences. With this objective in mind, there were three major findings. First, male and female
170 TTAs had common spatiotemporal gait differences between AL and NAL, with a shorter stance
171 time, longer swing time, and larger step length in their AL. This supports common kinematic
172 differences seen in literature as a result of prosthetic use.²²⁻²⁴ Although not new, the consistency
173 of this finding with other studies helps to confirm the validity of our results. Second, female TTAs
174 had a significantly higher gait and stride velocity, possibly achieved by their larger normalized
175 stride and step length or by their shorter non-normalized stance time. As many injury, fall-risk,
176 and other comorbidities are related to gait velocity,^{22,25} this result may help explain why female
177 TTAs have higher rates of these complications. Third, females demonstrated higher variability in

178 stride velocity through parameter mean and VM interactions. High variability in gait parameters
179 may contribute to fall-risk and future mobility impairments,²⁶⁻²⁸ suggesting these results offer
180 important female TTA rehabilitation considerations. Taken together, these results suggest that
181 while differences in AL and NAL gait parameters occur across both sexes, greater gait velocity
182 and variability in female TTAs may necessitate female LLA-specific research. The following
183 paragraphs further discuss potential implications of the three main findings.

184 As the first main finding, ALs were found to have a shorter stance time (Figure 2), longer
185 swing time, and larger step length (Figure 3A) across both sexes. Such differences were significant
186 when analyzing normalized or non-normalized gait parameters. These results align with the current
187 understanding of compensatory mechanisms in amputee gait, helping to confirm the validity of
188 our findings.^{3,22-24} More specifically, a shorter stance time on ALs indicates an amputee's distrust
189 or the discomfort experienced when loading their prosthetic limb.²² Therefore, amputees will
190 shorten the time they spend on their AL to mitigate these concerns. Limb strength asymmetries are
191 also believed to contribute to a greater reliance on their intact limb for compensation.³ A longer
192 AL swing time is believed to allude to variations between the AL and NAL in inertial and mass
193 properties.²² The AL's larger step length is thought to relate to compensating for poor push-off
194 power from the prosthetic limb.^{23,24}

195 The second major finding in this study was the higher stride and gait velocity seen in
196 females (Figure 1A and Figure 1B). It is acknowledged that velocity results are likely correlated
197 to those of other spatiotemporal parameters like stride length and step length or stance time. Sex
198 differences in these parameters may provide insights into female versus male LLA gait strategies.
199 For normalized parameters, it was found that in spite of their smaller stature, females took
200 significantly larger steps and strides than males (Figure 3A and Figure 3B). Therefore, it can be

201 postulated that females achieved their greater velocity by taking larger steps or strides. Considering
202 non-normalized parameters, the faster velocity observed in females may have also been achieved
203 in part by their statistically shorter stance time (Figure 2). Conversely, no difference is typically
204 found in gait speed of AB men and women, where AB women are found to increase their cadence
205 to achieve similar speeds as men.^{13,15} Together, these findings provide new insights to how female
206 LLAs mobilize compared to males. Greater walking speed has been associated with a higher
207 Medicare Functional Classification Level (K-level), which is a system commonly used to describe
208 walking potential in LLAs.²⁹ Despite this link suggesting successful mobility,²⁹ this result has
209 further implications for female amputee health as increased walking speed potentially presents a
210 greater fall-risk as well as OA in amputees.²⁵ Increased fall-risk has been linked with minimum
211 toe clearance (MTC) during forward swing, as it is the point where an individual's toes are closest
212 to the ground.²⁵ MTC increases with faster walking speed in AB individuals, thus no increased
213 fall-risk is presented.²⁵ However, a study found MTC is actually reduced on the AL of TTAs in
214 general and does not increase with increased speeds.²⁵ Therefore, TTAs may be presented with an
215 increased risk of falls at faster walking speeds.²⁵ Considering this relation between fall-risk and
216 walking speed, this study's finding of greater gait and stride velocity in females offers a possible
217 explanation for their increased fall-risk as well as suggests a potential need for female LLA-
218 specific rehabilitation and prosthetic design. Regarding rehabilitation, females could be cautioned
219 on walking speed, however, reducing walking speed below one's preferred pace has shown to
220 increase energetic cost.³⁰ Alternatively, designing a female prosthetic foot that dorsiflexes during
221 forward swing could reduce fall-risk as such designs have shown to increase MTC.^{31,32}

222 Studies have also shown that faster walking speeds in LLAs increases both vertical ground
223 reaction force (vGRF) and loading asymmetry, hypothesized to be from improper prosthetic

224 inertial properties and asymmetric placement of their center of gravity.²² Both increased intact
225 limb vGRF and loading asymmetries are linked to knee pain and risk of OA in LLAs.^{1-3,22} From
226 this relation between walking speed and risk of OA, this study's result of increased gait and stride
227 velocity in females presents implications for their health and quality of life. This link may provide
228 an explanation for the heightened risk of OA seen in female LLAs and indicate a need for sex-
229 specific prosthetics. More specifically, foot stiffness parameters in female prosthetics could be
230 altered to potentially reduce intact limb vGRF since such parameters have been shown to impact
231 intact limb loading as well as metabolic cost of gait.³³

232 Overall, the suggestion that a higher walking speed indicates greater mobility potential in
233 LLAs does not negate its links to comorbidities seen in females, including increased risk of falls
234 as well as OA. Future research needs to consider the implications these links have for females and
235 how they may suggest a need for sex-specific rehabilitation and prosthetic design.

236 The third major finding was the statistically significant interactions between limb*trial*sex
237 for the mean and VM of stride velocity. These interactions demonstrate greater trial-to-trial
238 variability in female participants (Figure 4). In male participants, parameter values remained more
239 consistent across trials and limbs while in females, parameter values varied greatly across trials
240 and limbs. In AB individuals, some gait variability and fluctuations are expected and not cause for
241 concern.²⁸ However, increased gait variability has been associated with greater fall-risk as well as
242 being a predictor for future mobility impairments.²⁶⁻²⁸ Gait variability is often viewed as the
243 neuromuscular control system's inability to consistently maintain a steady gait pattern, resulting
244 in instability and higher fall-risk.²⁸ Due to this link between fall-risk and gait variability, this
245 study's finding presents potential major ramifications for female LLAs and their rehabilitation,
246 offering a possible explanation for their greater risk of falls.

247 Limitations of this study include only involving TTAs, therefore the results do not extend
248 over all LLAs. However, transtibial amputation level is common in LLAs,^{6,7} and is a reasonable
249 starting point to assess sex differences without introducing confounding factors like amputation
250 level. As well, further information regarding participant prosthesis type or componentry could help
251 reinforce arguments regarding female LLA gait strategy and the potential links to their increased
252 risk factors. Despite the above limitations, the available data and research on female amputees is
253 lacking. Therefore, it is believed these data provide a useful initial and exploratory attempt to
254 understand and determine sex differences in LLAs.

255 The findings of this study offer preliminary information and insight into TTA sex
256 differences, potentially linking them to female comorbidities. These findings present implications
257 for female LLAs in the context of rehabilitation and prosthetic design. However, further research
258 is needed to better understand the implications of sex differences and how they can guide sex-
259 specific rehabilitation protocols and prosthetics. For example, research addressing how prosthetic
260 mass and stiffness properties can be optimized for women may give such insight into improving
261 their success with artificial limbs. As well, future work assessing sex differences in gait and joint
262 kinematics is needed to analyze their correspondence to the spatiotemporal sex differences found
263 here. As well, insight into female joint kinematics is needed for sex-specific prosthesis
264 development. In general, future research may indicate that sex-specific rehabilitation and
265 prosthetics are necessary to improve the outcomes and attempt to address the various comorbidities
266 of female LLAs.

267 To summarize, this study is the first to our knowledge to analyze sex differences in TTA
268 gait. A number of differences were found between sexes, including females having a shorter stance
269 time, greater normalized step and stride length, and faster gait and stride velocity. As well, females

270 had a greater stride velocity variability compared to males. Results from this study have been
271 associated with comorbidities present in female LLAs. Further research into the biomechanics of
272 female LLA gait is needed to corroborate the sex differences found and to help understand the
273 needs of female LLAs for rehabilitation and prosthetic design.

274

275

Acknowledgments

276 Graduate funding for XXXX was provided by the XXXX Graduate Scholarship (XXXX).

277 XXXX was funded by a National Health and Medical Research Council (NHMRC) fellowship

278 (1125054). These sources of funding had no input on the study design nor on the collection,

279 analysis and interpretation of data. They also had no input on the preparation of the manuscript

280 nor on the decision to submit the manuscript for publication.

281

282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312

References

1. Morgenroth DC, Roland M, Pruziner AL, Czerniecki JM. Transfemoral amputee intact limb loading and compensatory gait mechanics during down slope ambulation and the effect of prosthetic knee mechanisms. *Clin Biomech.* 2018;55:65-72. doi:10.1016/j.clinbiomech.2018.04.007
2. Morgenroth DC, Segal AD, Zelik KE, et al. The effect of prosthetic foot push-off on mechanical loading associated with knee osteoarthritis in lower extremity amputees. *Gait Posture.* 2011;34:502-507. doi:10.1016/j.gaitpost.2011.07.001
3. Lloyd CH, Stanhope SJ, Davis IS, Royer TD. Strength asymmetry and osteoarthritis risk factors in unilateral trans-tibial, amputee gait. *Gait Posture.* 2010;32(3):296-300. doi:10.1016/j.gaitpost.2010.05.003
4. Heitzmann DWW, Salami F, De Asha AR, et al. Benefits of an increased prosthetic ankle range of motion for individuals with a trans-tibial amputation walking with a new prosthetic foot. *Gait Posture.* 2018;64:174-180. doi:10.1016/j.gaitpost.2018.06.022
5. Randolph BJ, Nelson LM, Highsmith MJ. A Review of Unique Considerations for Female Veterans With Amputation. *Mil Med.* Published online 2016. doi:10.7205/milmed-d-16-00262
6. Imam B, Miller WC, Finlayson HC, Eng JJ, Jarus T. Incidence of lower limb amputation in Canada. *Can J Public Heal.* 2017;108(4):e374-e380. doi:10.17269/cjph.108.6093
7. Hordacre BG, Stevermuer T, Simmonds F, Crotty M, Eagar K. Lower-limb amputee rehabilitation in Australia: analysis of a national data set 2004 - 10. *Aust Heal Rev.* 2013;37(1):41. doi:10.1071/AH11138
8. Phinyomark A, Osis ST, Hettinga BA, Kobsar D, Ferber R. Gender differences in gait kinematics for patients with knee osteoarthritis. *BMC Musculoskelet Disord.* 2016;17(1):157. doi:10.1186/s12891-016-1013-z
9. Chihuri S, Wong CK. Factors associated with the likelihood of fall-related injury among people with lower limb loss. *Inj Epidemiol.* 2018;5(1):42. doi:10.1186/s40621-018-0171-x
10. Wong CK, Chihuri ST, Li G. Risk of fall-related injury in people with lower limb amputations: A prospective cohort study. *J Rehabil Med.* 2016;48(1):80-85. doi:10.2340/16501977-2042
11. Vareka, Varekova R, Janura M, Jindra M, Vanaskova E. Functional independence in the

- 313 lower limb amputees - The effect of gender and amputation level. *Ann Phys Rehabil Med.*
314 2018;61:e378-e379. doi:10.1016/j.rehab.2018.05.880
- 315 12. Davie-Smith F, Paul L, Nicholls N, Stuart WP, Kennon B. The impact of gender, level of
316 amputation and diabetes on prosthetic fit rates following major lower extremity
317 amputation. *Prosthet Orthot Int.* 2017;41(1):19-25. doi:10.1177/0309364616628341
- 318 13. Bruening DA, Frimenko RE, Goodyear CD, Bowden DR, Fullenkamp AM. Sex
319 differences in whole body gait kinematics at preferred speeds. *Gait Posture.*
320 2015;41(2):540-545. doi:10.1016/j.gaitpost.2014.12.011
- 321 14. Gabrielli AS, Maxim A, Gale T, LeVasseur C, Hogan M, Anderst W. Bilateral Symmetry
322 and Sex Differences in Ankle Kinematics During the Stance Phase of Gait. *Foot Ankle*
323 *Orthop.* 2019;4(4). doi:10.1177/2473011419S00179
- 324 15. Stansfield B, Hawkins K, Adams S, Bhatt H. A mixed linear modelling characterisation of
325 gender and speed related changes in spatiotemporal and kinematic characteristics of gait
326 across a wide speed range in healthy adults. *Med Eng Phys.* 2018;60:94-102.
327 doi:10.1016/j.medengphy.2018.07.015
- 328 16. Fukano M, Fukubayashi T, Banks SA. Sex differences in three-dimensional talocrural and
329 subtalar joint kinematics during stance phase in healthy young adults. *Hum Mov Sci.*
330 2018;61:117-125. doi:10.1016/j.humov.2018.06.003
- 331 17. Tannenbaum C, Ellis RP, Eyssel F, Zou J, Schiebinger L. Sex and gender analysis
332 improves science and engineering. *Nature.* 2019;575(7781):137-146. doi:10.1038/s41586-
333 019-1657-6
- 334 18. Hordacre BG, Barr C, Patrilli BL, Crotty M. Assessing gait variability in transtibial
335 amputee fallers based on spatial-temporal gait parameters normalized for walking speed.
336 *Arch Phys Med Rehabil.* 2015;96(6):1162-1165. doi:10.1016/j.apmr.2014.11.015
- 337 19. Müller B, Wolf SI. Handbook of Human Motion. *Handb Hum Motion.* 2018;1-3:1-2543.
338 doi:10.1007/978-3-319-14418-4
- 339 20. Winter DA. Biomechanics and Motor Control of Human Movement: Fourth Edition.
340 *Biomech Mot Control Hum Mov Fourth Ed.* Published online September 17, 2009:1-370.
341 doi:10.1002/9780470549148
- 342 21. Contini R. Body Segment Parameters, Part II. *Artif Limbs.* 1972;16(1):1-19.
343 http://www.oandplibrary.org/al/pdf/1972_01_001.pdf

- 344 22. Nolan L, Wit A, Dudziński K, Lees A, Lake M, Wychowański M. Adjustments in gait
345 symmetry with walking speed in trans-femoral and trans-tibial amputees. *Gait Posture*.
346 2003;17(2):142-151. doi:10.1016/S0966-6362(02)00066-8
- 347 23. Houdijk H, Lhak, Beek PJ, Van Dieën JH. Step length asymmetry in transtibial amputees:
348 A strategy to regulate gait stability? *Gait Posture*. 2014;39:S84.
349 doi:10.1016/j.gaitpost.2014.04.116
- 350 24. Adamczyk PG, Kuo AD. Mechanisms of gait asymmetry due to push-off deficiency in
351 unilateral amputees. *IEEE Trans Neural Syst Rehabil Eng*. 2015;23(5):776-785.
352 doi:10.1109/TNSRE.2014.2356722
- 353 25. de Asha AR, Buckley JG. The effects of walking speed on minimum toe clearance and on
354 the temporal relationship between minimum clearance and peak swing-foot velocity in
355 unilateral trans-tibial amputees. *Prosthet Orthot Int*. 2015;39(2):120-125.
356 doi:10.1177/0309364613515493
- 357 26. Keklicek H, Kirdi E, Yalcin A, et al. Comparison of gait variability and symmetry in
358 trained individuals with transtibial and transfemoral limb loss. *J Orthop Surg*.
359 2019;27(1):1-6. doi:10.1177/2309499019832665
- 360 27. Hausdorff JM, Rios DA, Edelberg HK. Gait variability and fall risk in community-living
361 older adults: A 1-year prospective study. *Arch Phys Med Rehabil*. 2001;82(8):1050-1056.
362 doi:10.1053/apmr.2001.24893
- 363 28. Hausdorff JM. Gait variability: Methods, modeling and meaning. *J Neuroeng Rehabil*.
364 2005;2(1):19. doi:10.1186/1743-0003-2-19
- 365 29. Batten HR, McPhail SM, Mandrusiak AM, Varghese PN, Kuys SS. Gait speed as an
366 indicator of prosthetic walking potential following lower limb amputation. *Prosthet*
367 *Orthot Int*. 2019;43(2):196-203. doi:10.1177/0309364618792723
- 368 30. Ralston HJ. Energy-speed relation and optimal speed during level walking. *Int Zeitschrift*
369 *für Angew Physiol Einschl Arbeitsphysiologie*. 1958;17(4):277-283.
370 doi:10.1007/BF00698754
- 371 31. Rosenblatt NJ, Bauer A, Rotter D, Grabiner MD. Active dorsiflexing prostheses may
372 reduce trip-related fall risk in people with transtibial amputation. *J Rehabil Res Dev*.
373 2014;51(8):1229-1242. doi:10.1682/JRRD.2014.01.0031
- 374 32. Johnson L, De Asha AR, Munjal R, Kulkarni J, Buckley JG. Toe clearance when walking

375 in people with unilateral transtibial amputation: Effects of passive hydraulic ankle. *J*
376 *Rehabil Res Dev.* 2014;51(3):429-438. doi:10.1682/JRRD.2013.05.0126
377 33. Fey NP, Klute GK, Neptune RR. Optimization of Prosthetic Foot Stiffness to Reduce
378 Metabolic Cost and Intact Knee Loading During Below-Knee Amputee Walking: A
379 Theoretical Study. Published online 2012. doi:10.1115/1.4007824
380
381

383 Table 1 Characteristics of the 10 male and 10 female TTA participants.

| Characteristic | Female (mean \pm SD) | Male (mean \pm SD) | <i>p</i> -Value |
|-------------------------------|------------------------|----------------------|-----------------|
| Age (years) | 63 \pm 21 | 56 \pm 10 | .338 |
| Stump length (cm) | 17.1 \pm 2.34 | 15.2 \pm 3.09 | .139 |
| Time since amputation (years) | 13 \pm 21 | 17 \pm 17 | .308 |
| Height (cm) | 162 \pm 6.64 | 179 \pm 10.7 | < .001* |
| Weight (kg) | 79.6 \pm 19.2 | 99.6 \pm 18.9 | .031* |
| BMI (kg/m ²) | 30.3 \pm 6.83 | 31.2 \pm 6.11 | .754 |
| Intact leg length (cm) | 84.9 \pm 3.48 | 94.9 \pm 5.66 | < .001* |
| Amputation pathology (%) | | | |
| Peripheral vascular | 40.0 | 30 | - |
| Trauma | 20.0 | 50 | - |
| Other | 40.0 | 20 | - |

384 Abbreviations: SD, standard deviation; TTA, transtibial amputee. * Denotes significant *p*-values.

386 Table 2 Gait parameter means and SDs for the 10 male and 10 female participants.

| (A) Non-normalized gait parameter | Female (mean ± SD) | Male (mean ± SD) |
|------------------------------------|--------------------|------------------|
| Gait velocity (cm/s) | 129.9 ± 25.1 | 102.8 ± 22.5 |
| Stance time (s) | | |
| AL | 0.660 ± 0.066 | 0.728 ± 0.080 |
| NAL | 0.697 ± 0.070 | 0.767 ± 0.069 |
| Swing time (s) | | |
| AL | 0.402 ± 0.030 | 0.416 ± 0.045 |
| NAL | 0.367 ± 0.031 | 0.377 ± 0.037 |
| Stride velocity (cm/s) | | |
| AL | 130.4 ± 24.5 | 103.1 ± 22.5 |
| NAL | 130.4 ± 25.0 | 103.3 ± 22.3 |
| Stride length (cm) | | |
| AL | 137.8 ± 23.2 | 120.8 ± 24.8 |
| NAL | 137.7 ± 23.2 | 121.2 ± 24.9 |
| Step time (s) | | |
| AL | 0.538 ± 0.039 | 0.581 ± 0.056 |
| NAL | 0.522 ± 0.011 | 0.566 ± 0.055 |
| Step length (cm) | | |
| AL | 70.8 ± 12.0 | 61.2 ± 13.2 |
| NAL | 66.8 ± 11.8 | 59.5 ± 12.4 |
| (B) Non-dimensional gait parameter | Female (mean ± SD) | Male (mean ± SD) |
| Gait velocity | 0.450 ± 0.087 | 0.339 ± 0.083 |
| Stance time | | |
| AL | 2.19 ± 0.149 | 2.42 ± 0.318 |
| NAL | 2.32 ± 0.186 | 2.53 ± 0.279 |
| Swing time | | |
| AL | 1.34 ± 0.077 | 1.35 ± 0.166 |
| NAL | 1.22 ± 0.101 | 1.22 ± 0.143 |
| Stride velocity | | |
| AL | 0.452 ± 0.085 | 0.340 ± 0.083 |
| NAL | 0.452 ± 0.087 | 0.340 ± 0.082 |
| Stride length | | |
| AL | 1.63 ± 0.285 | 1.20 ± 0.199 |
| NAL | 1.62 ± 0.285 | 1.20 ± 0.204 |
| Step time | | |
| AL | 1.83 ± 0.149 | 1.93 ± 0.259 |
| NAL | 1.77 ± 0.052 | 1.88 ± 0.245 |
| Step length | | |
| AL | 0.835 ± 0.153 | 0.608 ± 0.113 |
| NAL | 0.787 ± 0.139 | 0.589 ± 0.100 |

387 Abbreviations: SD, standard deviation; AL, amputated limb; NAL, non-amputated limb.

388

389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411

Figure Captions

Figure 1 – (A) Male and Female Stride Velocity Mean Interaction Graph. This graph demonstrates the statistically significant three-way interaction of limb*trial*sex on stride velocity for men and women. It also demonstrates the statistically significant main effect of sex on stride velocity. Although small, error bars are present and indicate the standard error. (B) Male and Female Gait Velocity Mean Interaction Graph. This graph demonstrates the two-way interaction of trial*sex on gait velocity for men and women. It also demonstrates the statistically significant main effect of sex on gait velocity. Although small, error bars are present and indicate the standard error.

Figure 2 – Male and Female Stance Time Mean Interaction Graph. This graph demonstrates the three-way interaction of limb*trial*sex on stance time for men and women. It also demonstrates the statistically significant main effect of limb and of sex on stance time. Although small, error bars are present and indicate the standard error.

Figure 3 – (A) Male and Female Normalized Step Length Mean Interaction Graph. This graph demonstrates the three-way interaction of limb*trial*sex on normalized step length for men and women. It also demonstrates the statistically significant main effect of limb and of sex on normalized step length. Although small, error bars are present and indicate the standard error. (B) Male and Female Normalized Stride Length Mean Interaction Graph. This graph demonstrates the three-way interaction of limb*trial*sex on normalized stride length for men and women. It also demonstrates the statistically significant main effect of sex on normalized stride length. Although small, error bars are present and indicate the standard error.

412 **Figure 4** – Male and Female Stride Velocity Variability Metric Interaction Graph. This graph
413 demonstrates the statistically significant three-way interaction of limb*trial*sex on stride velocity
414 variability metric for men (A) and women (B). The asterisks indicates at which trial the post-hoc
415 tests found the statistically significant simple main effect of limb. Error bars indicate the standard
416 error.