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4   **Title**: Differences in contractile behaviour between the soleus and medial gastrocnemius muscles

5   during human walking

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8   **Running head**: Triceps surae behaviour in walking

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Summary

The functional roles of individual lower limb muscles during human walking may differ depending on walking speed or duration. In this study, eleven volunteers walked on a treadmill for 60 minutes at speeds corresponding to both optimal and 20% above optimal energetic cost of transport whilst oxygen consumption and medial gastrocnemius (MG) and soleus fascicle lengths were measured. Although energetic cost of transport was ~12% higher at the faster speed, it remained constant over 60 minutes at both speeds, suggesting that humans can walk for prolonged periods at a range of speeds without compromising energetic efficiency. The fascicles of both muscles exhibited rather 'isometric' behaviour during the early to mid stance phase of walking, which appears to be independent of walking speed or movement efficiency. However, several functional differences were observed between muscles: MG exhibited time- and speed-dependent decreases in operating length, and shortened faster during the pushoff phase at the faster walking speed. Conversely, soleus exhibited consistent contractile behaviour regardless of walking speed or duration, and always shortened slower than MG during pushoff. Soleus appears to play a more important functional role than MG during walking. This may be especially true when walking for prolonged periods or at speeds above the most energetically efficient, where the force potential and thus the functional importance of MG appears to decline.

Key words: muscle fascicle, human locomotion, efficiency, ultrasound, automatic tracking

List of abbreviations: COT, cost of transport; GG, Geisser-Greenhouse; MG, medial gastrocnemius; RQ, respiratory quotient; Vmax, maximal shortening velocity

Introduction

During human walking, movement efficiency is greatest at intermediate walking speeds, where the exchange between kinetic and potential energy is greatest and thus the total mechanical work requirement of the muscles is minimised (Cavagna and Kaneko, 1977). This is reflected in measurements of energetic cost of transport (COT), which is essentially a measure of the cumulative metabolic rates of the muscles that power locomotion, and is defined as the energetic cost of moving a unit mass a unit distance. In human walking, COT exhibits a U-shaped curve when plotted against speed; it is lowest at intermediate speeds of ~4.5 km/h and increases at speeds faster or slower than this optimum (Margaria, 1938).

62   Triceps surae muscle fascicles operate essentially isometrically during the early to mid stance phase

63   of walking (Cronin et al., 2011), thus minimising muscular work. During the subsequent pushoff

64   phase, the fascicles shorten at velocities concomitant with high efficiency (Farris and Sawicki,

65   2012; Krishnaswamy et al., 2011). However, as walking speed increases above the speed

66   corresponding to optimal efficiency (i.e. minimal COT), the fascicles of the medial gastrocnemius

67   (MG) muscle have been shown to shorten faster during pushoff in humans and cats, resulting in a

68   decrease in force production due to the force-velocity relationship of muscle (Farris and Sawicki,

69   2012; Prilutsky et al., 1996). Similar findings have been reported after prolonged walking for one

70   hour (Cronin et al., 2011). Decreased MG force production would in turn require the activation of a

71   larger proportion of MG motor units and/or an increase in activation of synergistic muscles (e.g.

72   soleus) to maintain sufficient ankle joint torque (Cronin et al., 2011; Lichtwark and Wilson, 2008).

73   As well as complicating motor control, such changes could affect movement efficiency.

74

75   It is currently unknown whether and to what extent soleus may be able to compensate for a decrease

76   in MG force production during human walking. This is due in part to the small number of studies

77   that have examined soleus fascicle behaviour during walking, which in turn can be attributed to the

78   relative difficulty of imaging soleus compared to the gastrocnemius muscles. Recent advances in

79   both imaging and analysis techniques enable the simultaneous study of MG and soleus fascicle

80   length changes during dynamic tasks like walking (Cronin and Lichtwark, 2012). Rubenson et al.

81   (2012) recently reported that the soleus muscle's operating length is highly conserved between

82   walking and running, and suggested that soleus is preferentially selected for stable, rather than

83   optimal, force output. It has also been suggested that soleus is more metabolically efficient and

84   shortens at a lower velocity than MG (Krishnaswamy et al., 2011). Thus, despite the fact that MG

85   and soleus are functionally synergistic, their roles and relative importance within this synergy may

86   differ.

87

88   Accordingly, we have previously hypothesised that a decrease in MG force output could be

89   compensated by an increase in soleus force in order to maintain appropriate ankle joint torque. This

90   hypothesis was based on data obtained during walking for one hour at 4.5 km/h (Cronin et al.,

91   2011). However, as the speed corresponding to optimal COT is individual-specific (Weyand et al.,

92   2010), and as MG operates less efficiently at speeds that do not correspond to optimal COT (Farris

93   and Sawicki, 2012), the use of a standardised speed is unlikely to have corresponded to optimal

94   efficiency for all subjects in our previous study. The present study was thus designed to examine

95   the effects of walking at individually determined energetically optimal and sub-optimal walking

96   speeds, as well as the effects of prolonged walking (up to 60 minutes), on fascicle behaviour of the

97   soleus and medial gastrocnemius muscles. These tasks were chosen since they are frequently

98   encountered by humans, and because they have both been found to induce disadvantageous changes

99   in MG fascicle behaviour and/or a decrease in MG force potential (Cronin et al., 2011; Farris and

100 Sawicki, 2012). It was thus expected that this paradigm could shed light on the functional

101 importance of the soleus muscle when contractile conditions are sub-optimal in the synergistic

102 gastrocnemius. Two hypotheses were tested: 1) walking at a faster than optimal speed would

103 increase muscle fascicle shortening amplitude and/or shortening velocity in MG but not soleus; 2)

104 time-dependent changes in muscle fascicle behaviour would also be more evident in MG than

105 soleus.

106

107   Methods

108

109 Participants

110

111 Eleven healthy volunteers (six males, five females; age 28±5 years; height 172±7 cm; body mass

112 67±8 kg) with no history of neurological disorder took part in the study. Participants were

113 instructed to abstain from caffeine consumption and food intake two hours prior to testing. All

114 experimental procedures had received prior approval by the local ethics committee and were

115 conducted in accordance with the Declaration of Helsinki. All volunteers provided written informed

116 consent.

117

118 Experimental protocol

119

120 Participants attended the laboratory on three separate occasions. In the first session, cost of

121 transport (COT) was measured while participants walked on a motorised treadmill (OJK-1;

122 Telineyhtymä, Kotka, Finland) at seven speeds between 3.5-6.5 km/h in a randomised order. Each

123 speed was maintained for four minutes and oxygen consumption was recorded from the fourth

124 minute. In the second session, which was at least 24 hours after the first, participants walked on the

125 treadmill for 60 minutes at the speed corresponding to their lowest COT in session one (hereafter

126 referred to as Optimal condition) whilst oxygen consumption and MG and soleus fascicle lengths

127 were measured. In the third session, which was always at least one week after the second, the

128 protocol was repeated but at a speed 20% faster than the individually determined optimal speed

129 (Fast condition). All sessions were performed at the same time of day within ± 2 hours.

130

131 Methodology and analysis

132

133 **Cost of transport (COT).** Breath-by-breath O_2 consumption was measured using open circuit

134 indirect calorimetry (Sensormedics vmax 229, Yorba Linda, CA, USA). Participants wore a face

135 mask through which air was inspired, and the gas content of inspired air was analysed using Vmax

136 vision software. COT was determined as the mass specific rate of O_2 consumption divided by

137 walking speed (ml O_2 kg^{-1} km^{-1}) and values were averaged over one minute intervals. Rate of O_2

138 consumption values recorded during quiet standing were subtracted from the values recorded during

139 walking to calculate the O_2 consumption attributable to walking.

140

141 **Ultrasound.** An ultrasonographic device (Alpha-10; Aloka, Tokyo, Japan) was used to measure

142 fascicle lengths in MG and soleus of the right leg during walking at a scanning frequency of 95 Hz.

143 The probe was positioned over the medio-lateral centre of MG to facilitate comparisons between

144 sessions and to allow soleus muscle fascicles to be visualised in the same image. The repeatability

145 of fascicle lengths measured on separate days has been found to be high using similar methodology

146 (Aggeloussis et al., 2010). The probe was secured over the skin surface with a custom-made support

147 device to prevent movement of the probe relative to the skin. MG and soleus muscle fascicle

148 lengths were determined throughout the step cycle using an automated fascicle tracking algorithm

149 validated previously (Cronin et al., 2011; Gillett et al., 2012). Data were sampled at 10 minute

150 intervals and analysed from 4-5 steps per interval for all participants and then averaged. Fascicle

151 velocities were determined by differentiating fascicle length with respect to time. The onset of

152 fascicle shortening in the pushoff phase was determined as the first incidence where five

153 consecutive data points exhibited a negative instantaneous velocity.

154

155 **Additional measures.** Foot–ground contact was detected by a force-sensitive resistor placed under

156 the right heel, and these signals were used to determine temporal stride parameters.

157

158 <u>Statistics</u>

159

160 Differences in COT between speeds in the first session were tested with repeated measures

161 ANOVA. Two-way repeated measures ANOVA was used to assess the effects of speed (Optimal

162 versus Fast) and measurement interval (0, 10, 20, 30, 40, 50 and 60 minutes) on all outcome

163 measures. Mauchly's test of sphericity was applied, and where the assumption of sphericity was

164 violated, Geisser-Greenhouse (GG) adjustments were used. Where significant main effects were

165 observed, post hoc bonferroni corrections were used to identify the location of differences. For all

166 tests, the minimum level of statistical significance was set at $p < 0.05$.

167

168 <div align="center">Results</div>

169

170 **Cardio-respiratory parameters**. Walking speed had a significant effect on COT in the first

171 session (F[GG] = 4.556, p = 0.034; Fig 1a). On average, COT was lowest at 4.5 km/h, although

172 three subjects showed optimal COT at 4, 5 or 5.5 km/h. In the 60 minute walking sessions, COT

173 was significantly different between speeds (F = 4.295, p = 0.042) but not measurement intervals (F

174 = 1.389, p = 0.230; Fig 1b). Respiratory quotient (RQ), calculated as the ratio of CO_2 production/O_2

175 consumption, was not significantly different between speeds (F = 2.649, p = 0.155), but was

176 influenced by measurement interval (F = 13.951, p < 0.001; Fig 1c).

177

178 **Muscle fascicle behaviour**. In soleus, there was no effect of speed (F = 0.001, p = 0.983) or

179 measurement interval (F = 0.371, p = 0.890) on fascicle length at the instant of ground contact,

180 whereas in MG, fascicle length at ground contact decreased between the first and last measurement

181 intervals at both speeds (F = 4.005, p = 0.01), as well as being shorter in the Fast condition at all

182 intervals (F = 2.576, p = 0.035). There was no effect of speed or measurement interval on the range

183 of fascicle length changes throughout the mean stride in soleus (F = 0.039, p = 0.851), but the range

184 of length changes decreased in MG at both speeds between the first and last measurement intervals

185 (F= 3.551, p = 0.048; Fig 2). The timing within the stance phase when fascicle shortening began

186 was unaffected by speed or measurement interval in both muscles (F = 0.087 – 0.608, p = 0.599 -

187 0.995). Soleus shortening velocity during the pushoff phase was unaltered by speed (F = 0.855, p =

188 0.539) or measurement interval (F = 0.620, p = 0.564). In MG, there was a main effect of speed (F

189 = 3.148, p = 0.033) but not measurement interval (F = 0.170, p = 0.983) on shortening velocity (Fig

190 3).

191

192 **Gait characteristics**. Stride duration was shorter at all intervals in the Fast condition (F = 12,165, p

193 = 0.018; grand mean: 1123 versus 1020 ms), and also increased at the Optimal speed between the

194 first and last measurement intervals from 1108 ± 17 to 1127 ± 17 ms (F = 3.553, p = 0.009).

195

196 <div align="center">Discussion</div>

197

198 This study demonstrates several novel findings. Firstly, even when walking at inefficient speeds,

199 COT remained constant over 60 minutes, highlighting the human capability to walk for prolonged

200 periods at a relatively wide range of speeds without a decline in energetic efficiency. In addition,

201 MG fascicle length decreased over 60 minutes at both walking speeds by ~5-6%, suggesting a

202 speed-independent increase in MG tendinous compliance, which was not evident in soleus at either

203 speed, despite the shared distal outer tendon of these muscles. At the faster speed, the MG fascicles

204 operated at an even shorter length and shortened ~20% faster during pushoff, neither of which

205 occurred in soleus. These results suggest that when walking for prolonged periods and/or at faster

206 than optimal speeds, the force potential of MG decreases due to exercise-induced changes in its

207 force-length and force-velocity relations. Due to the consistency of soleus behaviour across walking

208 speed and duration, we hypothesise that an increase in soleus activation may be one mechanism by

209 which decreased MG force is compensated in the conditions studied. As well as potentially

210 indicating a more important functional role of soleus in these conditions, the findings have

211 important implications for motor control, implying that the motor commands to MG and soleus

212 must be continuously and independently modulated to maintain appropriate joint torques, despite

213 the apparently synergistic function of these muscles. Interestingly, although COT is a measure of

214 the cumulative metabolic rates of the locomotor muscles, changes in MG fascicle behaviour (and

215 presumably also efficiency) did not affect COT values.

216

217 <u>The evolutionary origins of human walking</u>

218

219 In this study, COT remained constant over 60 minutes when walking at the most efficient speed. In

220 light of the long evolutionary history of human locomotion, it is perhaps unsurprising that humans

221 can maintain the optimal speed for an extended period of time without detectable changes in COT.

222 For example, oxygen consumption has also been found to remain constant when running for an

223 equivalent period of time (Finni et al., 2003). In addition, at walking speeds where COT is

224 minimised, Achilles tendon storage and return of elastic energy (Neptune et al., 2008) and muscular

225 efficiency (Cavagna and Kaneko, 1977) have been suggested to be maximal. Knee joint flexion in

226 the stance phase is also minimal around optimal COT, which is associated with decreased cost of

227 muscle work (Winter, 1983). It thus seems that humans are both biomechanically and metabolically

228 tuned to walk at intermediate speeds efficiently. Our results provide indirect evidence for some of

229 these claims, since the rate and amplitude of MG fascicle shortening increased at the faster, non-

230 optimal speed, implying decreased efficiency due to the force-velocity relation and increased

231 contractile work, respectively.

232

233 When walking at a speed faster than optimal, COT was approximately 12% higher on average (Fig

234 1a) but still remained constant over 60 minutes. The consistency of COT at both speeds cannot be

235 attributed to a training effect because humans tend to select speeds that coincide closely with those

236 that are most energetically efficient (Martin et al., 1992), and are thus unaccustomed to walking

237   20% faster than optimal for prolonged periods. The Fast speed was approximately 1 km/h faster

238   than optimal, which was sufficient to produce significantly higher COT values. However, the U-

239   shaped curve of COT versus speed for human walking is quite flat within the narrow range of

240   walking speeds that humans tend to naturally select, whereby large changes in speed result in

241   relatively small increases in COT (Fig 1a; see also Willis et al., 2005). Our data show that humans

242   are capable of walking for prolonged periods of time at a relatively wide range of speeds without a

243   decline in energetic efficiency, even when the initial COT is sub-optimal (see also Carrier et al.,

244   2011).

245

246   <u>Effects of prolonged walking on triceps surae contractile behaviour</u>

247

248   When the duration of walking exceeds approximately 30 minutes, the repeated MTU length

249   changes that occur with every step can lead to an increase in the compliance of the MG tendinous

250   tissues, but this effect is not evident in soleus during level walking (Cronin et al., 2011). In the

251   present study, MG fascicle length decreased over the course of 60 minutes at both walking speeds

252   by ~5-6%, supporting the notion of increased MG tendinous compliance, which appears to be

253   independent of walking speed and thus movement efficiency. This effect was not evident in soleus

254   at either speed, implying that changes in tendinous compliance can occur in individual muscles,

255   presumably in the aponeurotic tissues since MG and soleus share a distal outer tendon. Despite time

256   and speed-dependent changes in MG behaviour, both muscles exhibited rather 'isometric'

257   behaviour during the early to mid stance phase of walking. Our results suggest that this

258   phenomenon is independent of walking speed or movement efficiency.

259

260   <u>Consequences of changes in MG fascicle behaviour</u>

261

262   It has been suggested that MG operates primarily on the plateau region of the force-length relation

263   in walking (Fukunaga et al., 2001). Therefore, when the MG fascicles operate at a shorter length, as

264   observed here during prolonged walking and even more so at the faster speed (~4.3mm shorter

265   compared to the Optimal speed), MG force output may decrease due to a shift of the MG fibres

266   towards the ascending limb of the force-length relation. Furthermore, at the faster speed, our results

267   showed that the MG muscle also shortened ~20% faster in the pushoff phase, which would

268   theoretically further decrease MG force due to the force-velocity relation (Farris and Sawicki, 2012;

269   Prilutsky et al., 1996). Our results thus lend support to the suggestion that the MG muscle

270   experiences less favourable contractile conditions at faster walking speeds (Farris and Sawicki,

271 2012; Neptune and Sasaki, 2005; Prilutsky et al., 1996) and when walking for prolonged periods

272 (Cronin et al., 2011), which can decrease its ability to produce force.

273

274 Increases in walking speed within the range studied here are associated with similar peak Achilles

275 tendon forces but an increase in the rate of force development in the stance phase (Finni et al.,

276 1998). Therefore, a decrease in MG force must be compensated within the triceps surae group in

277 order to maintain the required Achilles tendon force level. Within the MG muscle, higher forces

278 could be achieved by activating a larger muscle volume, which would involve recruitment of faster

279 motor units, but this increases energy consumption (Roberts et al., 1998). Alternatively, activation

280 of synergistic muscles such as soleus may be increased (Cronin et al., 2011; Lichtwark and Wilson,

281 2008). Although this would still increase energy consumption, the additional cost is likely smaller

282 in soleus because it is more suited to relatively slow, prolonged movements compared to MG (see

283 below). Our data show that soleus exhibited consistent contractile behaviour when walking for up to

284 60 minutes, as well as when walking at inefficient speeds. This is consistent with recent data

285 showing that soleus operates over a similar region on its force-length curve in both walking and

286 running, thus conserving a near optimal (~95%) length-dependent force generating capacity around

287 the time the highest forces are required (Rubenson et al., 2012). Furthermore, in the pushoff phase,

288 we observed that soleus always shortened at lower velocities than MG, implying lower metabolic

289 energy consumption (Hill, 1938). We therefore suggest that when walking for prolonged periods or

290 at faster speeds, the decrease in MG force capacity is compensated by an increase in soleus force.

291 This phenomenon is presumably more evident at faster walking speeds due to the decrease in

292 fascicle operating length and increase in shortening velocity of MG at faster speeds. It should be

293 noted that in the case of prolonged walking at a constant speed, a decrease in MG force could be

294 compensated by a change in the distribution of relative torque between joints. Relative joint powers

295 have been shown to remain constant with changes in walking speed (Farris and Sawicki, 2011), but

296 it is not known whether this mechanism is involved in prolonged walking. However, our previous

297 finding of neural compensation within the triceps surae in prolonged walking (Cronin et al., 2011)

298 suggests this is unlikely.

299

300 It is noteworthy that changes in the contractile behaviour of MG (and presumably also MG

301 efficiency) did not appear to affect COT values. As COT is a measure of the cumulative metabolic

302 rates of the muscles that power locomotion, this apparent discrepancy may be due to the relatively

303 low importance of MG in the overall efficiency of walking. Alternatively, compensatory

304 mechanisms within the triceps surae group (e.g. increased soleus activation) may occur at

305 sufficiently small energetic cost that there is no observable net effect on COT. Future studies should

306 examine the contribution of individual muscles to total COT during locomotion.

307

308 Conclusions

309

310 The results of this study may be indicative of a more important functional role for soleus than MG

311 in human walking, particularly when walking for prolonged periods or at faster speeds. In addition

312 to the lower shortening velocities of soleus observed here, this monoarticular muscle has been

313 suggested to make a larger contribution to ankle torque, make greater use of elastic energy storage

314 and reuse, and be metabolically more efficient than the biarticular MG in walking (Krishnaswamy

315 et al., 2011; Neptune et al., 2008). Soleus also has a larger cross-sectional area (Ward et al., 2009)

316 and shorter fascicles than either gastrocnemius head (Maganaris et al., 1998), reducing the required

317 activation volume for a given force. Furthermore, soleus contains ~88% slow-twitch fibres

318 compared to ~50% in MG (Edgerton et al., 1975; Gollnick et al., 1974), and thus relies more on

319 slower fibres with a lower maximal shortening velocity (Vmax). Therefore, soleus is probably able

320 to function at higher activation levels more economically than MG, particularly over prolonged

321 periods. Interestingly, in the pushoff phase, soleus shortened at velocities between 0.12-0.16 Vmax

322 (based on a resting fascicle length of 35.4 mm [Maganaris et al., 1998] and a Vmax for soleus of 6

323 lengths s^{-1} [Geyer and Herr, 2010]), which is close to the predicted optimum efficiency (Hill, 1964)

324 and was independent of walking speed and duration. Based on these findings, it is more

325 energetically sensible that decreases in MG force would be compensated by increased recruitment

326 of soleus, rather than increased recruitment of MG. We thus hypothesise that during walking, soleus

327 acts as the primary force generator in the triceps surae group by providing efficient propulsion,

328 while MG is more metabolically demanding and less important in this context (Krishnaswamy et

329 al., 2011). The functional importance of MG likely declines with increasing walking speed,

330 whereby MG operates at a progressively shorter length and shortens faster, and thus the

331 contribution of MG force to total ankle torque decreases (Farris and Sawicki, 2012). However, it

332 should be noted that the functional roles of the individual muscles may differ between walking and

333 running (Carrier et al., 2011; Farris and Sawicki, 2012).

334

335   Funding

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341 <u>Figure legends</u>

342

343 Fig 1. A: Group mean COT data obtained from the first session at seven walking speeds (N = 11). *

344 denotes a significant difference from 4.5 km/h. B: Group mean COT data averaged per minute from

345 the Optimal and Fast sessions. COT was significantly higher at each 10 minute interval in the Fast

346 condition. C: The corresponding values for respiratory quotient, which was always below 1.0,

347 indicating aerobic conditions. RQ increased significantly between 0-10 minutes at both speeds, but

348 showed no other significant changes. In all plots, error bars denote 1 SD. Note that the 0 time

349 interval began approximately 3 minutes after the treadmill started to allow steady state to be

350 reached.

351

352 Fig 2. Mean soleus and MG fascicle lengths from the first (0 minutes) and last (60 minutes)

353 measurement intervals (N = 11). Each trace represents the average of 4-5 steps per subject and

354 interval, which were then normalised to 100 data points and group averaged. In the left column, the

355 averaged data are shown, and in the right column each trace has been corrected for differences in

356 length at ground contact to facilitate comparisons. Note that stride duration differed between

357 speeds, which is not evident in this figure due to normalisation.

358

359 Fig 3. Mean fascicle shortening velocities calculated during the pushoff phase (N = 11). Error bars

360 denote 1 SD. * denotes a significant difference between speeds in MG.

361

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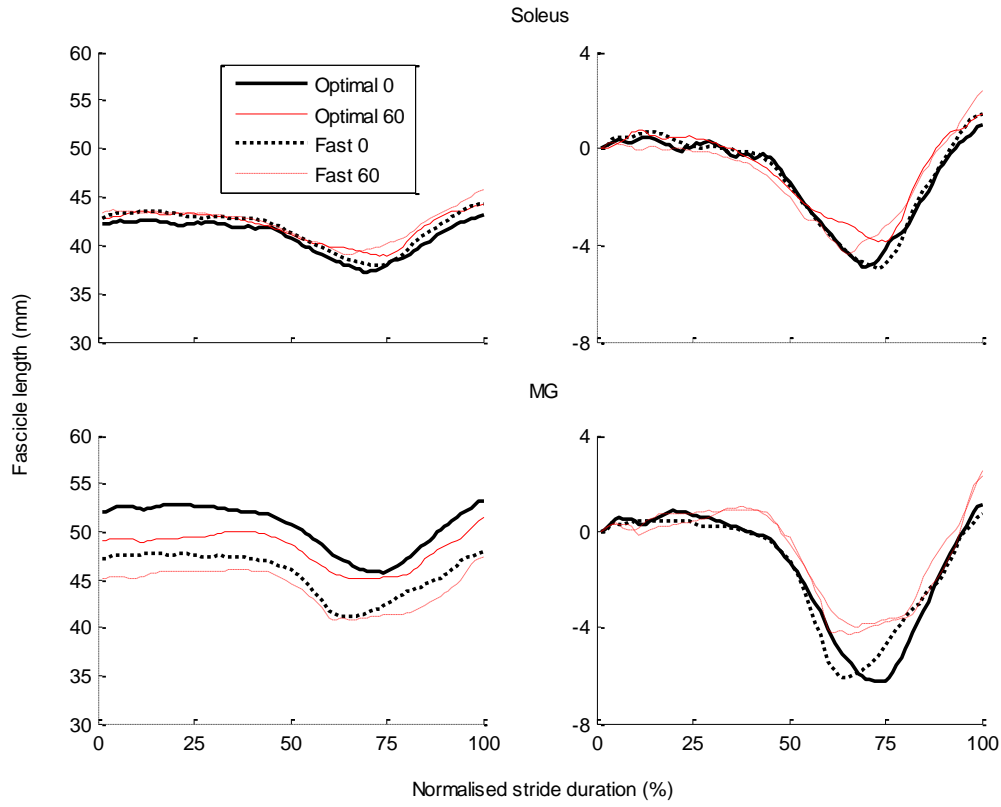431 *Metabolism* **54,** 793-799.

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Figure 1
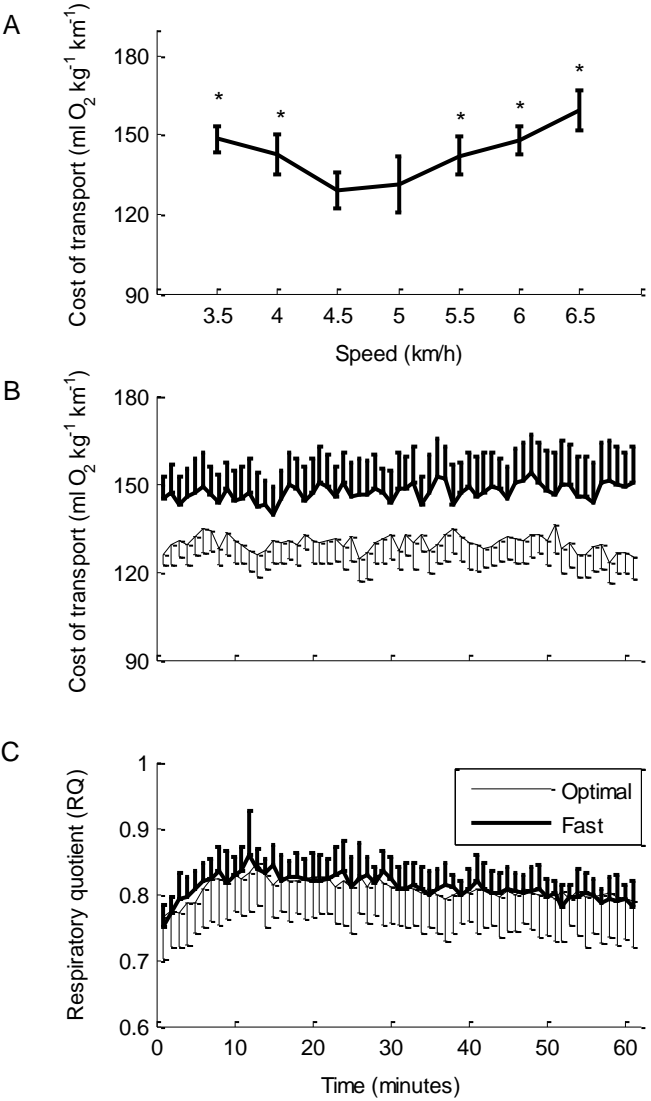
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