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A comparison of muscle stiffness and musculoarticular stiffness of the knee joint in young athletic males and females

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Abstract: The objective of this study was to investigate the gender-specific differences in peak torque (PT), muscle stiffness (MS) and musculoarticular stiffness (MAS) of the knee joints in a young active population. Twenty-two male and twenty-two female recreational athletes participated. Peak torque of the knee joint extensor musculature was assessed on an isokinetic dynamometer, MS of the vastus lateralis (VL) muscle was measured in both relaxed and contracted conditions, and knee joint MAS was quantified using the free oscillation technique. Significant gender differences were observed for all dependent variables. Females demonstrated less normalized peak torque (mean difference (MD) = 0.4 Nm/kg, $p = 0.005$, $\eta^2 = 0.17$), relaxed MS (MD = 94.2 N/m, $p < .001$, $\eta^2 = 0.53$), contracted MS (MD = 162.7 N/m, $p < .001$, $\eta^2 = 0.53$) and MAS (MD = 422.1 N/m, $p < .001$, $\eta^2 = 0.23$) than males. MAS increased linearly with the external load in both genders with males demonstrating a significantly higher slope ($p = 0.019$) than females. The observed differences outlined above may contribute to the higher knee joint injury incidence and prevalence in females when compared to males.

Title: A comparison of muscle stiffness and musculoarticular stiffness of the knee joint in young athletic males and females

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Abstract:

The objective of this study was to investigate the gender-specific differences in peak torque (PT), muscle stiffness (MS) and musculoarticular stiffness (MAS) of the knee joints in a young active population. Twenty-two male and twenty-two female recreational athletes participated. Peak torque of the knee joint extensor musculature was assessed on an isokinetic dynamometer, MS of the vastus lateralis (VL) muscle was measured in both relaxed and contracted conditions, and knee joint MAS was quantified using the free oscillation technique. Significant gender differences were observed for all dependent variables. Females demonstrated less normalized peak torque (mean difference (MD) = 0.4 Nm/kg, $p = 0.005$, $\eta^2 = 0.17$), relaxed MS (MD = 94.2 N/m, $p < .001$, $\eta^2 = 0.53$), contracted MS (MD = 162.7 N/m, $p < .001$, $\eta^2 = 0.53$) and MAS (MD = 422.1 N/m, $p < .001$, $\eta^2 = 0.23$) than males. MAS increased linearly with the external load in both genders with males demonstrating a significantly higher slope ($p = 0.019$) than females. ~~It is hypothesized that~~ the observed differences outlined above may contribute to the higher knee joint injury incidence and prevalence in females when compared to males.

1 Introduction

2 Epidemiological research has reported that female athletes have an increased risk of
3 lower limb musculoskeletal sports related injuries when compared to their male
4 counterparts (Jones et al., 1993, Messina et al., 1999). This observation is particularly
5 relevant in relation to anterior cruciate ligament (ACL) injuries and patellofemoral
6 pain (PFP). Female soccer players have been reported to have a 2-3 times higher risk
7 of ACL injuries when compared to males (Walden et al., 2011); ~~∓~~ this is also seen in
8 ~~female athletes~~ in other high velocity, intermittent sports such as basketball and
9 volleyball (Hewett, 2000). PFP is a prevalent lower limb musculoskeletal disorder,
10 observed in young, physically active female athletes (Heintjes et al., 2003, Natri et al.,
11 1998), and is associated with reduced participation in field and court based sports.
12 Furthermore, it may precipitate the onset of patellofemoral osteoarthritis (Utting et al.,
13 2005), as well as being potentially linked to non-contact ACL injury risk (Myer et al.,
14 2014).

15

16 Factors that are thought to contribute to gender differences in the incidence and
17 prevalence of knee joint injuries include; differences in the mechanical properties of
18 the knee joint ligaments, knee joint kinematics during landing, cutting and pivoting,
19 as well as skeletal alignment (Bonci, 1999, Harner et al., 1994, Rosene and Fogarty,
20 1999). During sport related activities, joint loads increase and knee joint stability is
21 dependent upon activation of the dynamic muscular constraint system, aimed at
22 protecting joints against injury. [Kim et al. \(Kim et al., 2011\) summarized from](#)

23 previous studies that co-contraction of agonist and antagonist muscles is important for
24 joint stabilization during dynamic movement; the amount of co-contraction could
25 significantly influence the resultant torque at the knee joint. Billot et al. indicated that
26 agonist-antagonist muscles have a common descending drive control (Billot et al.,
27 2014). Imbalance of quadriceps and hamstring strength (hamstring/quadriceps ratio <
28 0.6) has been reported as a contributing factor to non-contact knee injuries (Kim et al.,
29 2011). Furthermore, neuromuscular imbalance of decreased hamstring activation
30 relative to quadriceps activation is also well documented as a risk factor for ACL
31 injury (Alentorn-Geli et al., 2009). The role of hamstring muscles during landing or
32 cutting is to provide a counterbalancing force to resist the relatively higher quadriceps
33 force; hHigher quadriceps muscle activity and altered co-activation patterns in
34 females have been inferred to change the knee joint loads and thereby increase their
35 risk for knee injury (Krishnan et al., 2009). In this context, strength is only one
36 component of injury mechanism; neuromuscular function is actually the primary
37 contributor to the higher risk of non-contact lower limbs injuries in females when
38 compared to males. In contrast, stiffness is a more comprehensive variable which
39 represents the shock absorption characteristics of an individual muscle-tendon unit,
40 joint, or system (Watsford et al., 2010). ~~Indeed, muscle stiffness is a primary control~~
41 variable related to kKnee joint stability is mainly determined by muscle stiffness
42 (Needle et al., 2014). Additionally, stiffness is a primary determinant of the shock
43 absorption characteristics of an individual muscle tendon unit, joint, or system
44 (Watsford et al., 2010). A recent consensus paper published by Shultz and colleagues

45 (Shultz et al., 2012) advocated that further insight into the dynamic-restraint systems
46 of the knee joint beyond absolute strength is ~~are~~ required to understand more
47 comprehensively the potential mechanisms associated with the observed gender
48 disparity in knee joint injuries amongst athletes, with the authors recommending that
49 further research regarding knee joint stiffness is warranted.

50

51 Musculoarticular stiffness (MAS), assessed with the free-oscillation technique, is a
52 comprehensive measurement incorporating the stiffness of the muscle-tendon unit,
53 surrounding articular surfaces, ligaments, and skin. The same technique can be
54 applied to a single muscle using a specific device, thus obtaining a more localized

55 measurement of muscle stiffness (MS) than MAS evaluation in joint. It has been

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56 advocated that some level of stiffness is beneficial to enhance athletic performance,

57 however too much or too little stiffness may increase the risk of injury (Butler et al,

58 2003). Further, whilst an elevated level of stiffness appears to be beneficial for rapid

59 stretch-shortening cycle (SSC) movements, during relatively slow SSC movements a

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60 more compliant structure can better utilize, the eccentric pre-stretch and cushion the

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61 impact (Pruyn, et al. 2014). That's why MS and MAS have the potential to play

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62 crucial roles in neuromuscular control of joint stability, injury prevention and athletic

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63 performance (Ditroilo et al., 2012, Ditroilo et al., 2011b). The level of stiffness

64 contributes to the ability to attenuate excessive external forces, which is why MS and

65 MAS have the potential to play crucial roles in neuromuscular control of joint

66 | ~~stability, injury prevention and athletic performance (Ditroilo et al., 2012, Ditroilo et~~
67 | ~~al., 2011b).—~~

68 |
69 | To the present authors knowledge, no studies to date have concomitantly measured
70 | and compared knee joint MAS and quadriceps MS in male and female recreational
71 | athletes. ~~In the present study, v~~Vastus lateralis (VL) was utilized as representative of
72 | the quadriceps muscle ~~in accordance with previous research by Cafarelli~~(Cafarelli,
73 | 1977). Thus, the aim of the present study was to concurrently investigate MAS of
74 | knee joints and MS of VL in young male and female athletes. It was hypothesized that
75 | females would be characterized by lower knee joint MAS and MS of the VL when
76 | compared to males, which could help to explain an important mechanism linked to
77 | gender disparities in knee joint musculoskeletal injuries.

79 | **Methods**

80 | **Participants**

81 | Twenty-two male (age = 26.7 ± 2.6 years, ~~height-stature~~ = $1.77.2 \pm 0.06.67$ em, body
82 | ~~e~~mass = 72.6 ± 9.1 kg, BMI = 23.1 ± 2.4 kg/m²) and twenty-two female recreational
83 | athletes (age = 23.8 ± 4.1 years, ~~stature height~~ = $1.654.8 \pm 07.087$ em, body mass =
84 | 63.0 ± 12.0 kg, BMI = 23.1 ± 3.5 kg/m²) volunteered to participate. The study
85 | protocol was approved by the University Human Research Ethics Committee, and all
86 | participants signed consent forms. The specific inclusion criteria were: (1)
87 | recreational athletes who participated in organized sports ; (2) aged: 18-35 years; (3)

88 BMI \leq 25 (if a participant's BMI was $>$ 25, body fat \leq 25% (males) or 35% (females)
89 (assessed via skinfold thickness) were deemed acceptable (Ho-Pham et al., 2011));
90 (4) no recent significant soft-tissue injury to the lower limbs in the last 6 months; (5)
91 no reported medical condition that could influence performance. Furthermore,
92 participants were also screened using a medical history questionnaire (Ditroilo et al.,
93 2011a) and the Physical Activity Readiness Questionnaire form.

94

95 **Study design**

96 Each participant was required to visit the laboratory on one occasion and undergo the
97 following evaluations: (1) peak torque (PT) testing of their right knee joint extensor
98 musculature; (2) relaxed MS testing of their right VL; (3) contracted MS testing of
99 their right VL; (4) contracted MAS testing of their right knee joint.

100

101 **Peak Torque (PT)**

102 Each participant underwent PT testing of their right knee joint extensor musculature
103 on a dynamometer (Bodymax Fitness, Clydebank, UK). The participant was seated on
104 the dynamometer with their; hip flexed at 105° and their right knee flexed at 80°
105 (where full extension represents 0°) (Ditroilo et al., 2012), with the lateral femoral
106 condyle aligned with the axis of the dynamometer. The force transmission point was a
107 bar that was positioned anteriorly to the participant's lateral malleolus. The machine
108 was equipped with a load cell (Leane International, Parma, Italy, measurement range:
109 0-500 kg, output: 2.00 mV/V) applied in series with the plane of force application.

110 The load cell was secured to the leg-extension machine with a chain. This prevented
111 movements of the bar and therefore allowed an isometric contraction when the
112 participant attempted to extend their leg. Participants were stabilized with straps at the
113 pelvis to avoid movements towards hip extension during the test. Furthermore, to
114 minimize any contribution from the upper body, participants were required to cross
115 their hands across their body throughout. After familiarization with the procedures,
116 participants were instructed to produce a maximum voluntary isometric contraction
117 (MVIC) of their knee joint extensor musculature, as quickly as possible for
118 approximately 3 seconds. Each participant was required to perform three MVICs,
119 with the highest value recorded being used to determine the load with which MAS
120 was assessed. During performance of each MVIC, strong verbal encouragement and
121 visual target stimulation were provided to motivate maximal contraction. The force
122 signal was sampled at 1000 Hz and stored on a PC using a 16 bit A/D converter data
123 acquisition system (Biopac Systems, Inc. Goleta, CA, USA). Prior to data analysis,
124 the signal was filtered using a 5-ms moving average. The force signal was then
125 multiplied by the individual lever arm length to convert it into torque (Nm). The
126 highest torque value was identified as PT, which was normalized to body mass of
127 each individual (Pincivero et al., 2003) for further analysis.

128

129 **Muscle stiffness (MS)**

130 MS of the VL muscle was measured using a device incorporating a probe and an
131 accelerometer (Myometer, Myoton-3, Müomeetria AS, Tallinn, Estonia) sampled at

132 3200 Hz. During MS recordings, the subjects were seated in the same position used
133 for MVIC measurements. The probe was manually positioned perpendicular to the
134 muscle belly with the recording site being 2/3 the distance along a line measured from
135 the anterior superior iliac spine to the midpoint on the lateral side of the patella. The
136 probe was gently lowered onto the muscle belly of the VL with a resultant automatic
137 mechanical impact being delivered to the muscle (duration of 15 ms, a force of
138 0.3-0.4 N and a local deformation in the order of a few millimeters) (Ditroilo et al.,
139 2012). The damped natural oscillations were recorded by the accelerometer within the
140 probe giving an instantaneous digital output of the MS. Five consecutive
141 measurements were taken during relaxed (no external load) and contacted (external
142 load = 30% MVIC) (Fig. 1.) conditions. The average of the five measurements was
143 used for later analysis.

144

145 **Musculo-articular stiffness (MAS)**

146 MAS of each participant's right knee joint was measured using a technique previously
147 published by Ditroilo et al., 2012 (Fig. 2.). Participants sat in the same position used
148 previously for MVIC assessments. To quantify submaximal MAS stiffness, the
149 participants were required to support a load corresponding to 30% of MVIC on the
150 anterior distal portion of their lower leg. An external perturbation of 100-150N was
151 applied to the bar by the investigator and the ensuing oscillations were recorded by a
152 uniaxial accelerometer (Crossbow, Milpitsa, CA, USA) attached to the distal end of
153 the lever arm of the leg-extension dynamometer. Accelerometer data were sampled at

154 1000 Hz and recorded on a personal computer using a 16-bit A/D converter. A
155 Butterworth low-pass filter (third order) with a cutoff frequency of 4 Hz was used to
156 filter the signal. Each participant completed five MAS trials separated by a 1-min rest
157 period, with the average of the three trials being used for analysis. Considering the
158 positive relationship between the active joint stiffness and the applied load, stiffness
159 gradient, defined as the ratio of the two parameters, was subsequently calculated
160 afterwards and utilized as an independent variable in the statistical analysis
161 (Gardner-Morse et al., 1995).

162

163 **Statistical Analysis**

164 Independent samples *t*-tests (two tailed) were undertaken to investigate differences
165 between males and females on the following four dependent variables: (1) ~~PT_{peak}~~
166 ~~torque~~; (2) relaxed MS; (3) contracted MS; (4) MAS. Statistical analyses were
167 conducted in IBM SPSS Statistics 20 (IBM Ireland Ltd, Dublin, Ireland). To account
168 for the number of analyses undertaken, statistical significance was set a priori at $p \leq$
169 0.0125 (Bonferroni adjustment). Furthermore, a one-way between-groups analysis of
170 covariance (ANCOVA) was conducted to investigate differences in stiffness gradient
171 across genders with the external load as the covariate; the level of significance was set
172 at $p < 0.05$.

173

174 **Results**

175 A significant difference was observed between males and females in; normalized
176 ~~PT~~peak torque (~~PT~~peak torque/ body mass) (males 2.8 ± 0.4 Nm/kg, females 2.4 ± 0.4
177 Nm/kg (Fig. 3.); $t(42) = 2.96$, $p = 0.005$), relaxed MS (males 364.4 ± 52.0 N/m,
178 females 270.3 ± 33.3 N/m (Fig. 4.); $t(42) = 6.90$, $p < .001$), contracted MS (males
179 495.1 ± 71.0 N/m, females 332.3 ± 85.4 N/m (Fig. 5.); $t(42) = 6.9$, $p < .001$) and
180 MAS (males 1450.1 ± 508.0 N/m, females 1028.0 ± 227.3 N/m (Fig. 6.); $t(42) =$
181 3.55 , $p < .001$).

182

183 The magnitude of the difference in means was also large for; normalized ~~peak torque~~
184 ~~PT~~ (mean difference (MD) = 2.3 Nm/kg, 95% CI: 0.1 to 0.6 , $\eta^2 = 0.17$), relaxed MS
185 (MD = 94.2 N/m, 95% CI: 66.6 to 121.7 $\eta^2 = 0.53$), contracted MS (MD= 162.7 N/m,
186 95% CI: 114.9 to 210.5 , $\eta^2 = 0.53$) and MAS (MD = 422.1 N/m, 95% CI: 179.5 to
187 664.8 $\eta^2 = 0.23$)

188

189 ~~The one-way ANCOVA Pp~~ preliminary checks were conducted to ensure that there was
190 no violation of the assumptions of normality, linearity, homogeneity of variances and
191 regression slopes, and reliable measurement of the covariate before one-way
192 ANCOVA was -processed. After adjusting for external load, there was significant
193 difference for MAS between the two groups, $F(1, 42) = 6.02$, $p = 0.019$, with males
194 having a steeper stiffness gradient slope than females (Males, $Y = 36.92X - 786.51$, $r^2 =$
195 0.80 ; Females, $Y = 18.32X + 224.49$, $r^2 = 0.33$). (Fig. 7).

196

197 **Discussion**

198 ~~This investigation aimed to identify whether differences in the stiffness characteristics~~
199 ~~of the knee joint exist between young recreationally athletic males and females. To~~
200 ~~the best of the authors' knowledge, this is the first study to concurrently measure MS~~
201 ~~of the VL and MAS of the knee joint (extensor) in young recreational athletes.~~ The
202 primary findings were that females have lower relaxed and contracted MS of the VL
203 and were characterized by lower knee joint MAS, which are important mechanisms
204 underlying gender disparity. ~~It is possible that these observed stiffness discrepancies~~
205 ~~across genders may contribute to higher rates of knee injury incidence and prevalence~~
206 ~~observed in female athletes.~~

207

208 ~~MS is a localized evaluation of the muscle's ability to resist external load. It is~~
209 influenced by geometry (physiological cross-sectional area, PCSA) (Foure et al.,
210 2012) and hence muscle mass (muscle mass= PCSA*fiber length* ρ) (Narici et al.,
211 1992), as well as intrinsic properties (actin-myosin cross-bridge, and protein titin)
212 (Proske and Morgan, 1999, ~~Wu et al., 2000~~). Therefore, gender differences in relaxed
213 MS could be attributable to the fact that males have a larger PCSA, greatermore
214 muscle mass and therebythus a greater amountmore of muscle fiber cross-bridges
215 (Blackburn et al., 2004) and titin than females. Gajdosik et al. (Gajdosik et al., 1990)
216 for instance suggested that higher hamstring stiffness values in males were ascribed to
217 greater muscle mass, compared to their female, whilst Blackburncounterparts.
218 Blackburn et al., ~~2004~~, also postulated that greater thigh segment mass in males could

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219 be responsible for observed gender differences in passive knee flexor stiffness.
220 Furthermore, increased muscle mass in males implies more passive connective tissue,
221 and hence a greater number of collagen fibers for lengthening resistance when
222 compared to those in females, leading to increased passive stiffness (Blackburn et al.,
223 2004). In addition, in contracted muscles, the amount of cross-bridges formed should
224 also be considered, as contracted MS has been found to be proportional to contractile
225 forces in muscle (Needle et al., 2014). Previous studies ~~has~~ ~~ve~~ shown that males are
226 stronger than females (~~Hannah et al., 2012~~, Wojtys et al., 2002a), a finding also
227 confirmed by the present study, whereby males produced significantly higher
228 normalized ~~PT~~ ~~peak torques~~ values compared to females (2.8 ± 0.4 Nm/kg vs 2.4 ± 0.4
229 Nm/kg).

230

231 Males were also found to have greater MAS compared to females, which is consistent
232 with conclusions of a previous study (Blackburn et al., 2009). Sinkjaer et al. (Sinkjaer
233 et al., 1988) divided MAS into two parts: the intrinsic component (deformation and
234 breakdown of actin-myosin filament cross-bridges) and the reflexive component
235 (occurs after the establishment of intrinsic portions during rapid muscle stretches).
236 The intrinsic component increases linearly with background torque (pre-activation)
237 (Mrachacz-Kersting and Sinkjaer, 2003) which is the external stretch on quadriceps;
238 whilst the reflexive component is integrated by the central nervous system ~~(CNS)~~, and
239 accounts for approximately 50% of the total stiffness (Hinsey, 2011). Muscle
240 contraction plays an essential role in joint stiffness (Needle et al., 2014), leading to a

241 2-4 times increase in knee joint stability (Markolf et al., 1976). Furthermore, studies
242 have reported that active joint stiffness is proportional to the force generated by
243 muscles (Morgan, 1977, ~~Morgan et al., 1978~~). Thus, factors related to muscle force
244 production, such as geometric mechanisms (Granata et al., 2002b), cross-bridge
245 mechanics and material qualities (Hinsey, 2011) are promising explanations for the
246 gender differences in joint stiffness found in the current investigation.

247

248 In addition to the aforementioned mechanisms, knee joint stiffness properties can also
249 be influenced by hormones, specifically free testosterone (FT) (Bell et al., 2012,
250 Granata et al., 2002b). An early study showed that when compared to females, male
251 adults possess approximately 7-8 times more FT (Southren et al., 1965). It has been
252 observed that an inverse relationship exists between FT and time to 50% peak torque;
253 with shorter time to 50% ~~peak torque~~PT being more advantageous to overall joint
254 stability (Bell et al., 2012, Blackburn et al., 2009). Bell et al., 2012, have reported that
255 a negative relationship exists between estrogen and MAS, offering some explanation
256 for the lower MAS observed in females. We hypothesize that this is the case for the
257 present study although no experimental measurements were carried out.

258

259 Stiffness gradient is an essential tool to describe active stiffness characteristics. The
260 results of the current study demonstrated a significantly higher stiffness gradient in
261 males in comparison to females, indicating that when an applied moment increases,
262 joint stiffness subsequently increases, and males manifest a higher degree of increased

263 stiffness. Therefore, it is reasonable to assume that males are characterized by greater
264 ability to resist external loads which has implications for injury risk in females. The
265 observed difference in stiffness gradient between males and females is also supported
266 by the findings of Granata et al., 2002b [which reported that stiffness increased with](#)
267 [the external load, and there was a significant difference in slope of linear regressions](#)
268 [between stiffness and applied load with females demonstrating a reduced regression](#)
269 [slope](#) .

270

271 Joint stiffness parameters are integrated by the CNS internally and exhibit mechanical
272 characteristics externally. As a consequence, it is an important variable capable of
273 comprehensively representing joint stability and muscle performance. A higher degree
274 of stiffness may provide more resistance to external load during functional
275 performance and hence protect joints from musculoskeletal injury (Granata et al.,
276 2002a). A decrease in joint stiffness or MS reduces structures' capacity to resist
277 external applied loads, and hence the gender differences in stiffness observed in the
278 present study could help explain the higher risk of lower-limb injuries in females. It
279 could also point out one possible solution for preventing injuries in females and
280 males. Training; such as weight (Kubo et al., 2007), ~~isometric (Burgess et al., 2007),~~
281 ~~eccentric (Pousson et al., 1990)~~, and plyometric training (Spurrs et al., 2003) have all
282 been suggested to be beneficial for stiffness augmentation. In the future, it is
283 important to investigate what kind of training is best for stiffness enhancement.

284

285 Limitations of this study include; not measuring the participants' testosterone and
286 estrogen levels, and also not controlling females' menstrual cycle due to time and
287 financial limits. The effect of menstrual cycle hormone fluctuations on stiffness
288 properties and the injury occurrence is still controversial. The study of Eiling et al.
289 (Eiling et al., 2007) indicated significant effect of estrogen levels on
290 musculotendinous stiffness at the time of ovulation when compared to the menstrual
291 and follicular phase; and more acute ACL tears were reported in females during
292 mid-cycle by Wojtys et al. (Wojtys et al., 2002b). However, Bryant et al. (Bryant et
293 al., 2011) attested no significant leg stiffness difference between non-MOCP
294 (monophasic oral contraceptive pill) and MOCP users.

295

296 **Conclusions**

297 Gender differences exist in the knee joint stiffness properties of young active
298 populations. Females exhibit a lower level of MS and MAS when compared to males.
299 The mechanism explaining this difference is still unknown, but neuromuscular control
300 and muscle volume differences may affect MS and MAS. This study's results may
301 provide some interpretation as to why females incur more knee injuries than their
302 male counterparts. Investigation of optimal training programmes for the augmentation
303 of MS and MAS should be of interest in future.

304

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308

309 **References**

310 1. Alentorn-Geli E, Myer GD, Silvers HJ, Samitier G, Romero D, Lázaro-Haro C,
311 Cugat R. Prevention of non-contact anterior cruciate ligament injuries in soccer
312 players. Part 1: Mechanisms of injury and underlying risk factors. Knee surgery,
313 sports traumatology, arthroscopy. 2009;17(7):705-29.

314 ~~1~~2. Bell DR, Blackburn JT, Norcross MF, Ondrak KS, Hudson JD, Hackney AC,
315 Padua DA. Estrogen and muscle stiffness have a negative relationship in females.
316 Knee surgery, sports traumatology, arthroscopy. 2012;20(2):361-7.

317 3. Billot M, Duclay J, Simoneau-Buessinger EM, Ballay Y, Martin A. Is
318 co-contraction responsible for the decline in maximal knee joint torque in older
319 males? AGE. 2014;36(2):899-910.

320 ~~2~~4. Blackburn JT, Bell DR, Norcross MF, Hudson JD, Engstrom LA. Comparison of
321 hamstring neuromechanical properties between healthy males and females and
322 the influence of musculotendinous stiffness. Journal of electromyography and
323 kinesiology. 2009;19(5):e362-9.

324 ~~3~~5. Blackburn JT, Riemann BL, Padua DA, Guskiewicz KM. Sex comparison of
325 extensibility, passive, and active stiffness of the knee flexors. Clinical
326 biomechanics. 2004;19(1):36-43.

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327 | ~~4.6.~~ Bonci CM. Assessment and evaluation of predisposing factors to anterior cruciate
328 | ligament injury. Journal of athletic training. 1999;34(2):155-64.

329 | 7. Bryant AL, Crossley KM, Bartold S, Hohmann E, Clark RA. Estrogen-induced
330 | effects on the neuro-mechanics of hopping in humans. European journal of
331 | applied physiology. 2011;111(2):245-52.

332 | ~~5.8.~~ Butler RJ, Crowell HP, Davis IM. Lower extremity stiffness: implications for
333 | performance and injury. Clinical biomechanics. 2003; 8(6):511-7.

334 | ~~6.~~ Burgess KE, Connick MJ, Graham-Smith P, Pearson SJ. Plyometric vs. isometric
335 | training influences on tendon properties and muscle output. Journal of strength and
336 | conditioning research. 2007;21(3):986-9.

337 | ~~7.9.~~ Cafarelli E. Peripheral and central inputs to the effort sense during cycling
338 | exercise. European journal of applied physiology and occupational physiology.
339 | 1977;37(3):181-9.

340 | ~~8.10.~~ Ditroilo M, Cully L, Boreham CA, De Vito G. Assessment of
341 | musculo-articular and muscle stiffness in young and older men. Muscle & nerve.
342 | 2012;46(4):559-65.

343 | ~~9.11.~~ Ditroilo M, Watsford M, Fernandez-Pena E, D'Amen G, Lucertini F, De Vito
344 | G. Effects of fatigue on muscle stiffness and intermittent sprinting during cycling.
345 | Medicine and science in sports and exercise. 2011a;43(5):837-45.

346 | ~~10.12.~~ Ditroilo M, Watsford M, Murphy A, De Vito G. Assessing
347 | Musculo-Articular Stiffness Using Free Oscillations Theory, Measurement and
348 | Analysis. Sports medicine. 2011b;41(12):1019-32.

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349 | ~~11,13.~~ Eiling E, Bryant AL, Petersen W, Murphy A, Hohmann E. Effects of
350 | menstrual-cycle hormone fluctuations on musculotendinous stiffness and knee
351 | joint laxity. *Knee surgery, sports traumatology, arthroscopy*. 2007;15(2):126-32.

352 | ~~12,14.~~ Foure A, Cornu C, McNair PJ, Nordez A. Gender differences in both active
353 | and passive parts of the plantar flexors series elastic component stiffness and
354 | geometrical parameters of the muscle-tendon complex. *Journal of orthopaedic*
355 | *research*. 2012;30(5):707-12.

356 | ~~13,15.~~ Gajdosik RL, Giuliani CA, Bohannon RW. Passive compliance and length of
357 | the hamstring muscles of healthy men and women. *Clinical biomechanics*.
358 | 1990;5(1):23-9.

359 | ~~14,16.~~ Gardner-Morse M, Stokes IA, Laible JP. Role of muscles in lumbar spine
360 | stability in maximum extension efforts. *Journal of orthopaedic research*.
361 | 1995;13(5):802-8.

362 | ~~15,17.~~ Granata KP, Padua DA, Wilson SE. Gender differences in active
363 | musculoskeletal stiffness. Part II. Quantification of leg stiffness during functional
364 | hopping tasks. *Journal of electromyography and kinesiology*.
365 | 2002a;12(2):127-35.

366 | ~~16,18.~~ Granata KP, Wilson SE, Padua DA. Gender differences in active
367 | musculoskeletal stiffness. Part I. Quantification in controlled measurements of
368 | knee joint dynamics. *Journal of electromyography and kinesiology*.
369 | 2002b;12(2):119-26.

- 370 ~~17. Hannah R, Minshull C, Buckthorpe MW, Folland JP. Explosive neuromuscular~~
371 ~~performance of males versus females. Experimental physiology.~~
372 ~~2012;97(5):618-29.~~
- 373 ~~18.19. Harner CD, Paulos LE, Greenwald AE, Rosenberg TD, Cooley VC. Detailed~~
374 ~~Analysis of Patients with Bilateral Anterior Cruciate Ligament Injuries. American~~
375 ~~journal of sports medicine. 1994;22(1):37-43.~~
- 376 ~~19.20. Heintjes E, Berger MY, Bierma-Zeinstra SM, Bernsen RM, Verhaar JA,~~
377 ~~Koes BW. Exercise therapy for patellofemoral pain syndrome. The Cochrane~~
378 ~~database of systematic reviews. 2003(4):CD003472.~~
- 379 ~~20.21. Hewett TE. Neuromuscular and hormonal factors associated with knee~~
380 ~~injuries in female athletes - Strategies for intervention. Sports medicine.~~
381 ~~2000;29(5):313-27.~~
- 382 ~~21.22. Hinsey ML. Comparison of reactive knee stiffening strategies between sexes.~~
383 ~~2011. Masters Thesis, University of Delaware.~~
- 384 23. Ho-Pham LT, Campbell LV, Nguyen TV. More on body fat cutoff points.
385 Mayo Clinic proceedings. 2011;86(6):584; author reply -5.
- 386 24. Jones BH, Bovee MW, Harris JM, Cowan DN. Intrinsic Risk-Factors for
387 Exercise-Related Injuries among Male and Female Army Trainees. American
388 journal of sports medicine. 1993;21(5):705-10.
- 389 25. Kim D, Hong JG. Hamstring to quadriceps strength ratio and noncontact leg
390 injuries: A prospective study during one season. Isokinetics and exercise
391 science. 2011; 19(1):1-6.

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392 ~~24-26.~~ Krishnan C, Williams GN. Sex difference in quadriceps and hamstrings
393 EMG-Moment relationships. Medical & science in sports & exercise. 2009;
394 41(8):1652-60.

395 ~~25-27.~~ Kubo K, Morimoto M, Komuro T, Yata H, Tsunoda N, Kanehisa H,
396 Fukunaga T. Effects of plyometric and weight training on muscle-tendon
397 complex and jump performance. Medicine and science in sports and exercise.
398 2007;39(10):1801-10.

399 ~~26-28.~~ Markolf KL, Mensch JS, Amstutz HC. Stiffness and laxity of the
400 knee--the contributions of the supporting structures. A quantitative in vitro
401 study. The Journal of bone and joint surgery American volume.
402 1976;58(5):583-94.

403 ~~27-29.~~ Messina F, Farney WC, DeLee JC. The incidence of injury in Texas high
404 school basketball. A prospective study among male and female athletes.
405 American journal of sports medicine. 1999;27(3):294-9.

406 ~~28-30.~~ Morgan DL. Separation of active and passive components of short-range
407 stiffness of muscle. The American journal of physiology. 1977;232(1):C45-9.

408 ~~29. Morgan DL, Proske U, Warren D. Measurements of muscle stiffness and the~~
409 ~~mechanism of elastic storage of energy in hopping kangaroos. The Journal of~~
410 ~~physiology. 1978;282:253-61.~~

411 ~~30-31.~~ Mrachacz-Kersting N, Sinkjaer T. Reflex and non-reflex torque
412 responses to stretch of the human knee extensors. Experimental brain research.
413 2003;151(1):72-81.

414 | ~~31-32.~~ Myer GD, Ford KR, Di Stasi SL, Barber Foss KD, Micheli LJ, Hewett
415 | TE. High knee abduction moments are common risk factors for patellofemoral
416 | pain (PFP) and anterior cruciate ligament (ACL) injury in girls: Is PFP itself a
417 | predictor for subsequent ACL injury? *British journal of sports medicine*. 2014.
418 | (epub ahead of print)

419 | ~~32-33.~~ Narici MV, Landoni L, Minetti AE. Assessment of human knee extensor
420 | muscles stress from in vivo physiological cross-sectional area and strength
421 | measurements. *European journal of applied physiology and occupational*
422 | *physiology*. 1992;65(5):438-44.

423 | ~~33-34.~~ Natri A, Kannus P, Jarvinen M. Which factors predict the long-term
424 | outcome in chronic patellofemoral pain syndrome? A 7-yr prospective
425 | follow-up study. *Medicine and science in sports and exercise*. 1998;30
426 | (11):1527-77.

427 | ~~34-35.~~ Needle AR, Baumeister J, Kaminski TW, Higginson JS, Farquhar WB,
428 | Swanik CB. Neuromechanical coupling in the regulation of muscle tone and
429 | joint stiffness. *Scandinavian journal of medicine & science in sports*. 2014.
430 | (epub ahead of print)

431 | ~~35-36.~~ Pincivero DM, Gandaio CM, Ito Y. Gender-specific knee extensor
432 | torque, flexor torque, and muscle fatigue responses during maximal effort
433 | contractions. *European journal of applied physiology*. 2003;89(2):134-41.

434 ~~36. Pousson M, Van Hoecke J, Goubel F. Changes in elastic characteristics of~~
435 ~~human muscle induced by eccentric exercise. Journal of biomechanics.~~
436 ~~1990;23(4):343-8.~~

437 37. Proske U, Morgan DL. Do cross-bridges contribute to the tension during
438 stretch of passive muscle? Journal of muscle research and cell motility.
439 1999;20(5-6):433-42.

440 ~~37-38. Pruyn EC, Watsford M, Murphy A. The relationship between~~
441 ~~lower-body stiffness and dynamic performance. Applied physiology, nutrition~~
442 ~~and metabolism. 2014; 39(109): 1144-50.~~

443 ~~38-39. Rosene JM, Fogarty TD. Anterior tibial translation in collegiate athletes~~
444 ~~with normal anterior cruciate ligament integrity. Journal of athletic training.~~
445 ~~1999;34(2):93-8.~~

446 ~~39-40. Shultz SJ, Schmitz RJ, Benjaminse A, Chaudhari AM, Collins M, Padua~~
447 ~~DA. ACL Research Retreat VI: An Update on ACL Injury Risk and~~
448 ~~Prevention. Journal of athletic training. 2012;47(5):591-603.~~

449 ~~40-41. Sinkjaer T, Toft E, Andreassen S, Hornemann BC. Muscle stiffness in~~
450 ~~human ankle dorsiflexors: intrinsic and reflex components. Journal of~~
451 ~~neurophysiology. 1988;60(3):1110-21.~~

452 ~~41-42. Southren AL, Tochimoto S, Carmody NC, Isurugi K. Plasma production~~
453 ~~rates of testosterone in normal adult men and women and in patients with the~~
454 ~~syndrome of feminizing testes. Journal of clinical endocrinology &~~
455 ~~metabolism 1965;25(11):1441-50.~~

456 | ~~42-43.~~ Spurr RW, Murphy AJ, Watsford ML. The effect of plyometric training
457 | on distance running performance. *European journal of applied physiology.*
458 | 2003;89(1):1-7.

459 | ~~43-44.~~ Utting MR, Davies G, Newman JH. Is anterior knee pain a predisposing
460 | factor to patellofemoral osteoarthritis? *The Knee.* 2005;12(5):362-5.

461 | ~~44-45.~~ Walden M, Hagglund M, Werner J, Ekstrand J. The epidemiology of
462 | anterior cruciate ligament injury in football (soccer): a review of the literature
463 | from a gender-related perspective. *Knee surgery, sports traumatology,*
464 | *arthroscopy.* 2011;19(1):3-10.

465 | ~~45-46.~~ Watsford ML, Murphy AJ, McLachlan KA, Bryant AL, Cameron ML,
466 | Crossley KM, Makdissi M. A prospective study of the relationship between
467 | lower body stiffness and hamstring injury in professional Australian rules
468 | footballers. *American journal of sports medicine.* 2010;38(10):2058-64.

469 | ~~46-47.~~ Wojtys EM, Ashton-Miller JA, Huston LJ. A gender-related difference
470 | in the contribution of the knee musculature to sagittal-plane shear stiffness in
471 | subjects with similar knee laxity. *The Journal of bone and joint surgery*
472 | *American volume.* 2002a;84-A(1):10-6.

473 | ~~47-48.~~ Wojtys EM, Huston LJ, Boynton MD, Spindler KP, Lindenfeld TN. The
474 | effect of the menstrual cycle on anterior cruciate ligament injuries in women
475 | as determined by hormone levels. *American journal of sports medicine.*
476 | 2002b;30(2):182-8.

477

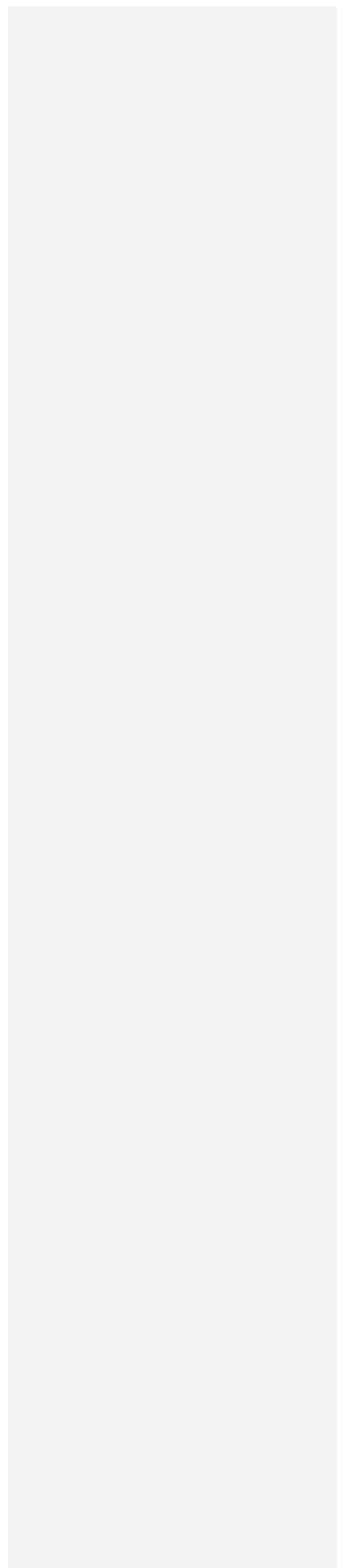
48. Wu Y, Cazorla O, Labeit D, Labeit S, Granzier H. Changes in titin and

478

collagen underlie diastolic stiffness diversity of cardiac muscle. *Journal of*

479

molecular and cellular cardiology. 2000;32(12):2151-62.



Captions to illustrations

Fig. 1. Myometer was utilized to evaluate cContracted MS-measurement technique. MS = muscle stiffness

Fig. 2. MAS measurement with free oscillation technique. MAS = musculoarticular stiffness

Fig. 3. Comparison of nNormalized peak torque (peak torque/body mass) between males and females (Mean ± SD (Standard Deviation)).

* indicates statistically significant difference compared to males.

Fig. 4. Comparison of rRelaxed MS between males and females (Mean ± SD). MS = muscle stiffness

* indicates statistically significant difference compared to males.

Fig. 5. Comparison of cContracted MS between males and females (Mean ± SD). MS = muscle stiffness

* indicates statistically significant difference compared to males.

Fig. 6. Comparison of MAS between males and females (Mean ± SD). MAS = musculoarticular stiffness

* indicates statistically significant difference compared to males.

Fig. 7. Relationship between MAS of the knee joint and applied load. MAS = musculoarticular stiffness

MAS increased with applied load in both genders. Linear regressions between stiffness and applied load for the male and female populations are significantly different in slope (Males, $Y=36.92X-786.51, r^2 = 0.80$; Females, $Y= 18.32X+224.49, r^2 = 0.33$).

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