

1 **Spinal cord injury level influences acute plasma caffeine responses.**

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15 **Abstract**

16 **Purpose.** To investigate the absorption curve and acute effects of caffeine at rest in
17 individuals with no spinal cord injury (SCI), paraplegia (PARA) and tetraplegia (TETRA).

18 **Methods.** Twenty-four healthy males (8 able-bodied (AB), 8 PARA and 8 TETRA)
19 consumed 3 mg·kg⁻¹ caffeine anhydrous (CAF) in a fasted state. Plasma caffeine [CAF],
20 glucose, lactate, free-fatty acid [FFA] and catecholamine concentrations were measured
21 during a 150 min rest period. **Results.** Peak [CAF] was greater in TETRA (21.5 μM)
22 compared to AB (12.2 μM) and PARA (15.1 μM), and mean peak [CAF] occurred at 70, 80
23 and 80 min, respectively. Moderate and large ES were revealed for TETRA compared to
24 PARA and AB (-0.55 and -1.14, respectively) for the total area under the [CAF] versus time

25 curve. Large inter-individual responses were apparent in SCI groups. The change in plasma
26 catecholamine concentrations following CAF did not reach significance ($p>0.05$) however
27 both adrenaline and noradrenaline concentrations were lowest in TETRA. Significant
28 increases in [FFA] were seen over time ($p<0.0005$) but there was no significant influence of
29 SCI level. Blood lactate concentration reduced over time ($p=0.022$) whereas blood glucose
30 concentration decreased modestly ($p=0.695$), and no difference between groups was seen
31 ($p>0.05$). **Conclusion.** Level of SCI influenced the caffeine absorption curve and there was
32 large inter-individual variation within and between groups. Individual curves should be
33 considered when using caffeine as an ergogenic aid in athletes with an SCI. The results
34 indicate TETRA should trial low doses in training and PARA may consider consuming
35 caffeine greater than 60 min prior to exercise performance. The study also supports caffeine's
36 direct effect on adipose tissue, which is not secondary to catecholamine release.

37 **Keywords:** adrenaline; noradrenaline; free fatty acid; ergogenic; wheelchair athletes

38 **Introduction**

39 Supplementation with caffeine (3-6 mg·kg⁻¹) can improve long and short-term endurance
40 performance (7,9) in able-bodied (AB) participants. However, there is a paucity of research
41 on the effects of caffeine on exercise performance in physically impaired populations e.g.
42 persons with a spinal cord injury (SCI). While current evidence is equivocal, a beneficial
43 effect of caffeine (4-6 mg·kg⁻¹ body mass (BM) in capsule form) on short-term wheelchair
44 propulsion exercise has been reported (5,13). These studies highlighted that there was great
45 inter-individual variability in wheelchair performance responses during a 1500 m time trial, 4
46 min maximal push and repeated sprints, especially in individuals with an SCI. The authors
47 highlighted the potential for slower caffeine absorption due to delayed gastrointestinal (GI)
48 transit times and prolonged gastric emptying (GE), especially in those with a cervical lesion
49 level (18). Understanding an individual's time to peak caffeine concentration has been shown
50 to have little impact on prolonged AB endurance cycling performance (34) but is likely to be
51 important prior to short-term upper-body exercise (UBE), and may require further
52 consideration in persons with an SCI.

53 Both metabolic and physiological functions are altered in individuals with an SCI, and the
54 level and completeness of injury has been shown to influence drug pharmacokinetics (15,23).
55 A review of the literature by Mestre et al. (23) indicated that the delayed absorption seen in
56 some individuals with an SCI increased the time to achieve the required therapeutic dose.
57 One drug reportedly affected by delayed GE and decreased GI motility is theophylline (32),
58 which can be used by individuals with an SCI to help treat bradycardia or to promote the
59 recovery of hemidiaphragmatic function. Diminished bioavailability could result in
60 underestimating the load and maintenance dose of theophylline in individuals with tetraplegia
61 (32). As a methylxanthine drug, theophylline has similar pharmacodynamic actions to
62 caffeine (28) and it has also been linked to improved endurance performance (14,22). There

63 is therefore reason to believe that caffeine absorption may also be delayed in persons with an
64 SCI. In disagreement however, Van Soeren et al. (37) suggested that the time to peak caffeine
65 concentration ($6 \text{ mg}\cdot\text{kg}^{-1}$) in individuals with tetraplegia ($\sim 47 \text{ }\mu\text{M}$ at 40 min ($n=6$)) did not
66 differ to those of AB individuals. The authors however could not assess the influence of SCI
67 lesion level on caffeine absorption because there was no direct control group and only two
68 individuals with paraplegia. They also did not report individual participant data, which may
69 help to explain inter-individual performance responses. Flueck et al. (4) measured plasma
70 caffeine concentrations (median) at 60 min only in AB individuals ($45.1 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$) and
71 individuals with paraplegia ($\sim 54 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$) and tetraplegia ($66.1 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$). It therefore
72 remains difficult to determine whether the time course of caffeine absorption differs based on
73 an individual's SCI level.

74 Numerous mechanisms of action have been proposed to help explain the beneficial effects of
75 an acute dose of caffeine on exercise performance. Current research suggests the main
76 mechanism at physiological caffeine doses is the blockade of central nervous system (CNS)
77 | adenosine receptors, which indirectly ~~effects~~affects neurotransmitter release (19) to increase
78 arousal, alertness and attention. Individuals with tetraplegia are therefore an interesting study
79 population given the reduced sympathetic activity caudal to the lesion level and associated
80 impaired catecholamine response (27). The study of this population has lent support to the
81 hypothesis that caffeine can have a direct effect on tissues following reports of adrenaline-
82 independent free fatty acid (FFA) mobilisation (37). No study has directly investigated the
83 acute effects of caffeine in a group of individuals with no SCI, paraplegia and tetraplegia.
84 Hence, the current study aimed to explore the time course of caffeine absorption and its
85 effects at rest in these three groups, with the aim of providing safe and accurate
86 | recommendations for its use as an ergogenic aid by individuals with an SCI. It was

87 hypothesised that caffeine absorption will be delayed in individuals with tetraplegia
88 compared to those with paraplegia and no SCI.

89 **Methods**

90 **Participants.** Twenty-four ~~healthy~~ recreationally active males (8 able-bodied controls (AB),
91 8 individuals with paraplegia (PARA) and 8 with tetraplegia (TETRA)) provided informed
92 consent to participate in the current study. Participants were classified using the American
93 Spinal Injury Association (ASIA) scale (20). A health screening questionnaire was completed
94 by all participants and individuals were excluded if any of their medication had known
95 interactions with caffeine. Average daily caffeine intake was assessed using a modified
96 version of the caffeine consumption questionnaire (21). All procedures were approved by the
97 University Ethical Advisory Committee and performed following the Declaration of Helsinki.
98 Participants' characteristics are shown in Table 1.

99 **Procedures.** In the days prior to visiting the laboratory, participants maintained their normal
100 dietary and activity patterns (light-moderate intensity exercise only) and their individual
101 medication regimes. Participants were provided with a list of caffeine containing foods and
102 drinks, and were asked to abstain from consumption in the 36 h preceding their laboratory
103 visit. Participants were also asked to refrain from alcohol consumption for 24 h prior to their
104 visit.

105 Participants arrived at the laboratory (~~between 08:00 and -10:-00~~) after fasting from 21:00 the
106 previous evening in a following an overnight fast fasted state (no food intake after 21:00).
107 Water consumption was encouraged to help ensure the participant arrived euhydrated. On
108 arrival participants were asked to void their bladder, if necessary, prior to lying in a semi-
109 supine position on a laboratory bed. Participants were asked to report any side-effects to the
110 investigators immediately at any point during the trial. A cannula (Venflon, Becton

111 Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein for subsequent venous
112 sampling. The cannula was kept patent using 5-10 ml sodium chloride (0.9%) after each
113 blood sample.

114 After a minimum of 15 min rest, a baseline venous blood sample was taken. Participants then
115 consumed cellulose capsules (Bulk Powders, Colchester, UK) containing 3 mg·kg⁻¹ BM
116 caffeine anhydrous (My Protein, Northwich, UK), which were filled manually by the
117 investigators to the nearest 0.1 mg. Participants remained rested for 150 min during which a
118 further 9 blood samples were taken. The blood sampling schedule can be seen in Figure 1.
119 After the final blood sample, participants were again asked once more to record whether they
120 experienced any side-effects during the experimental trial.

121 **Blood sampling and analysis.** Blood samples were immediately separated into tubes that
122 contained the relevant preservative(s). At every sampling time-point 5 ml blood was added to
123 an EDTA K2 vacutainer for subsequent plasma caffeine concentration ([CAF]) analysis. A 20
124 µl blood sample was removed and analysed in duplicate for blood lactate ([BLa]) and glucose
125 ([GLU]) concentrations using an automatic analyser (Biosen C-Line, EKF Diagnostic GmbH,
126 Barleben, Germany). For catecholamine and FFA analysis (baseline, 60, 90 and 150 min), a
127 further 10 ml of blood was dispensed into two lithium-heparin tubes containing 37.5 µl of
128 EGTA-Glutathione for the subsequent analysis of plasma adrenaline ([A]), noradrenaline
129 ([NA]) and FFA ([FFA]) concentrations. In addition, 25 µl of 3 mg·ml⁻¹ tetrahydrolipstatin
130 (THL) was added to the tube for [FFA] analysis. All tubes were centrifuged at 1000g for 10
131 min at 4°C as previously described (12). Plasma samples were aliquoted into Eppendorfs and
132 stored at -80°C until analysis.

133 Plasma [CAF] was analysed using high-performance liquid chromatography (HPLC) as
134 described by Holland et al. (16) with the following minor modifications; prior to injection

135 onto the HPLC column each sample was individually filtered (Mini-UniPrep syringeless
136 filters, Fisher Scientific, UK) and no guard column was used. The method produced a
137 coefficient of variation (CV) of 1.06% (range 0.24-1.45%).

138 Plasma [A] and [NA] were also determined using HPLC as previously described by Forster &
139 Macdonald (6). A plasma volume of 500 μ l was used for analysis. The method produced CVs
140 of 0.31 and 0.17% for [A] and [NA] respectively.

141 Plasma was analysed enzymatically for [FFA] using an in vitro enzymatic colorimetric
142 method (Wako Instrument kit) and a Pentra 400 analyser (Horiba Medical, California, USA).
143 The method produced an intra-assay CV of 1.68 and 1.28% for high and low FFA quality
144 controls (QC) (4 repeats of the QC samples at intervals during the analysis).

145 **Statistical analyses**

146 Data were analysed using the IBM Statistics Software Package for the Social Sciences (SPSS)
147 version 22 (IBM Corporation, New York, USA). The trapezium rule was used to calculate the
148 total area under the variable versus time curve for [CAF] (TAUC-CAF), [FFA] (TAUC-FFA),
149 [A] (TAUC-A) and [NA] (TAUC-NA). The incremental area under the plasma concentration
150 versus time curve for [FFA] (iAUC-FFA), [A] (iAUC-A) and [NA] (iAUC-NA) was also
151 calculated using the same method after adjusting for baseline concentrations.

152 Normal distribution was checked using Shapiro-Wilk tests and the data are presented as mean
153 \pm SD. Data for [FFA] were not normally distributed and were log transformed prior to
154 analysis. These data are presented as geometric mean (95% confidence intervals (CI)) and
155 analysis is based on the ratios of geometric means and 95% CI for ratios. Homogeneity of
156 variances was confirmed by Mauchly's test of sphericity, and where the sphericity
157 assumption was violated, the Greenhouse Geisser correction was applied to the degrees of
158 freedom.

159 Repeated measures analysis of variance (ANOVA) for group and time were used to examine
160 differences between [FFA], [A], [NA], [Bla] and [GLU].

161 An analysis of covariance (ANCOVA) was used to examine differences between [CAF], with
162 daily caffeine consumption (low $<50 \text{ mg}\cdot\text{d}^{-1}$, moderate $50\text{-}250 \text{ mg}\cdot\text{d}^{-1}$ and high $>250 \text{ mg}\cdot\text{d}^{-1}$)
163 as a covariate. One-way repeated measures ANOVAs were used to analyse TAUC and iAUC
164 data. Planned simple and difference contrasts were applied to explain any significant results.

165 Statistical significance was accepted at $p \leq 0.05$ and absolute standardised effect sizes (ES)
166 are included to supplement important findings. An ES of 0.2 was considered small, 0.5
167 moderate and 0.8 large according to Cohen (3). Due to incomplete data sets (e.g. insufficient
168 blood flow or a cannula change) the number of participants included in each analysis differs.
169 Data sets were A (7/6/7), NA (7/7/8), FFA (7/7/7), Bla (5/5/6) and GLU (8/6/8) for AB,
170 | PARA and TETRA groups, respectively.

171 Power analysis was performed using the [CAF] observed in 3 groups of participants with no
172 SCI, paraplegia and tetraplegia 60 min post-ingestion of $6 \text{ mg}\cdot\text{kg}^{-1}$ caffeine (46.4 (6.8), 55.3
173 (19.8) and $64.1 (6.9) \mu\text{M}$, respectively) (4). The *a priori* analysis, conducted in G*Power 3.1,
174 revealed that six participants would be required in each group to detect a similar change in
175 [CAF] with ES of 0.59, 0.66 and 2.74 (4), 90% power, and an α of 5%. Given the novel
176 nature of this investigation and the heterogeneity of the population, an additional two
177 participants per group were recruited to increase statistical power ($n = 8$).

178 **Results**

179 **Plasma caffeine**

180 At baseline, [CAF] was either undetectable or very low, which indicates that all participants
181 adhered to the withdrawal guidelines. Differences over time and across groups were revealed
182 (main effect time $p < 0.0005$; main effect group $p = 0.026$; time by SCI level interaction $p =$

183 0.019) (Figure 2). Planned simple contrasts revealed these group differences occurred
184 between AB and TETRA ($p = 0.017$), whereas no difference was observed between AB and
185 PARA ($p=0.913$). Peak [CAF] in TETRA was significantly greater than AB ($p = 0.008$) yet
186 non-significantly ($p = 0.058$), but meaningfully ($ES = 0.9$) greater than PARA (21.5 ± 7.0 ,
187 12.2 ± 2.3 and $15.1 \pm 8.1 \mu\text{M}$, respectively). Time to peak [CAF] varied greatly between
188 individuals but group means were 80 min for AB and PARA, and 70 min for TETRA. There
189 was no influence of habitual caffeine use on [CAF] ($p = 0.943$).

190 No significant difference in TAUC-CAF was observed between groups ($p = 0.135$; AB $3.74 \pm$
191 $0.96 \mu\text{M}$, PARA $4.62 \pm 3.12 \mu\text{M}$, and TETRA $6.08 \pm 2.15 \mu\text{M}$). However, small (AB vs.
192 PARA, $ES = 0.38$), moderate (PARA vs. TETRA, $ES = 0.55$) and large (AB vs. TETRA, ES
193 $= 1.14$) ES were apparent.

194 Seven participants (3 AB/2 PARA/2 TETRA) reported adverse effects prior to/during the first
195 30 min of testing (headache/light-headed (2)) and during testing; (struggling with quick
196 decision making (1), tingling arm (1), twitching eye (1)) and five participants reported feeling
197 more alert.

198 **Plasma catecholamines (adrenaline and noradrenaline)**

199 All catecholamine analysis excluded the participant with a T4 lesion level due to a missed
200 sample and hence statistical analysis for PARA was calculated based on injuries at or below
201 T6/7. The change in [A] over the course of the resting protocol did not reach statistical
202 significance, but did differ between groups (main effect of time $p = 0.088$; main effect of
203 group $p = 0.027$; time by SCI level $p = 0.618$) (Figure 3). Planned difference contrasts
204 revealed these group differences occurred between PARA and TETRA ($p = 0.019$) only.
205 There was no significant difference in TAUC-A ($p = 0.075$) between groups (AB 0.43 ± 0.17
206 $\text{nmol}\cdot\text{L}^{-1}$, PARA $0.57 \pm 0.22 \text{ nmol}\cdot\text{L}^{-1}$, and TETRA $0.22 \pm 0.10 \text{ nmol}\cdot\text{L}^{-1}$) though ES were

207 large for both AB (ES = 2.02) and PARA (ES = 1.04) compared to TETRA. There was no
208 difference in iAUC-A ($p = 0.733$).

209 The [NA] did not change significantly during the 150 min protocol ($p = 0.423$) but did differ
210 between groups ($p = 0.003$), and no interaction was evident ($p = 0.772$). Planned difference
211 contrasts revealed these group differences occurred between AB and TETRA ($p = 0.001$), and
212 PARA and TETRA ($p = 0.006$), but no significant difference was observed between AB and
213 PARA ($p = 0.505$). There was a significant difference in TAUC-NA ($p = 0.003$) between
214 groups (AB $4.04 \pm 0.92 \text{ nmol}\cdot\text{L}^{-1}$, PARA $3.68 \pm 1.01 \text{ nmol}\cdot\text{L}^{-1}$, and TETRA 2.01 ± 1.21
215 $\text{nmol}\cdot\text{L}^{-1}$). Small (AB vs. PARA, ES = 0.38) and large (AB vs. TETRA, ES = 1.89, and
216 PARA vs. TETRA, ES = 1.50) ES were revealed. However, no significant difference in
217 iAUC-NA was observed ($p = 0.827$).

218 Plasma FFA, lactate and glucose

219 Differences in [FFA] were observed over time and between groups; with the latter not
220 reaching significance, but displaying a large effect ~~however the latter failed to reach~~
221 ~~significance~~ (ES = 0.80) (main effect time $p < 0.0005$; main effect group $p = 0.054$; time by
222 group interaction $p = 0.035$). ~~Means (95% CI) [FFA] were as 51% (-42 to 24%), 64% (-47 to~~
223 ~~-30%) and 84% (-54 to -37%) higher than baseline at 60, 90 and 150 min, respectively.~~
224 Geometric mMeans (95% CI) [FFA] were as 26% (-46 to 2%) lower in PARA than AB, but
225 and 9% higher than AB in PARA (95% CI -2.46 to 862%) in and TETRA than AB (95% CI-
226 34.21 to 2750%), respectively; furthermore, Mean [FFA] results were 47% (6 to 103%)
227 higher in TETRA than compared to PARA (95% CI -516 to -6103%) (Figure 3). The
228 interaction indicated that while PARA experienced only a marginal increase in [FFA] from
229 baseline to 150 min ($\Delta \sim 0.13 \text{ mmol}\cdot\text{L}^{-1}$), AB and TETRA increased to a greater extent over
230 the course of the protocol ($\Delta \sim 0.36$ and $0.31 \text{ mmol}\cdot\text{L}^{-1}$, respectively).

231 ~~Although the main effect for No significant differences~~ in TAUC-FFA was not
232 ~~significant~~observed ($p = 0.072$), ~~the effect sizes ranged from small yet moderate~~ (AB vs.
233 PARA, $ES = 0.47$; ~~AB vs. TETRA, $ES = 0.85$~~) ~~to~~and large (AB vs. TETRA, $ES = 0.85$;
234 PARA vs. TETRA, $ES = 1.16$) ~~ES were revealed~~. No significant difference in iAUC-FFA
235 was observed ($p = 0.357$).

236 Differences in [Bla] were observed over time but not between groups (main effect time $p =$
237 0.022 ; main effect group $p = 0.463$; time by group interaction $p = 0.065$). ~~Planned difference~~
238 ~~contrasts revealed a significant decrease in [Bla] between baseline and 60 min ($p = 0.049$);~~
239 ~~and between 90 and 150 min ($p < 0.0005$).~~ No significant difference in [GLU] was seen over
240 the course of the 150 min protocol ($p = 0.695$) or between groups ($p = 0.983$).

241 **Discussion**

242 The current study is the first to report large inter-individual differences in caffeine absorption
243 within and between groups when separated for level of SCI (AB, PARA and TETRA).
244 Consequently, dosage and timing recommendations provided to individuals with an SCI may
245 need to be adapted from the AB literature. In addition, the pattern of caffeine absorption
246 differs in TETRA compared to AB and PARA. There were small differences in [A], [NA]
247 and [FFA] between the AB and SCI groups, which were non-significant when baseline values
248 were accounted for using the incremental area under the curve. No differences in [Bla] and
249 [GLU] were seen between groups.

250 **Plasma caffeine**

251 Participant's [CAF] increased in all three groups following the ingestion of $3 \text{ mg}\cdot\text{kg}^{-1}$ caffeine.
252 The [CAF] in AB at 60 min ($10.8 \pm 3.1 \text{ }\mu\text{M}$) is in line with that reported 60 min post-
253 ingestion of 2, 3 and $4 \text{ mg}\cdot\text{kg}^{-1}$ caffeine (5.7, ~15 and $14.6 \text{ }\mu\text{M}$, respectively) (11,33). This

254 study is the first to investigate the caffeine absorption curve in a group of participants with
255 paraplegia. In agreement with the hypothesis, the PARA results do not differ from the AB
256 responses at 60 min ($11.1 \pm 7.9 \mu\text{M}$) and both groups reached mean peak [CAF] at 80 min.
257 The TETRA responses were significantly greater than AB and the mean peak [CAF] was
258 reached 10 min earlier (70 min). Flueck et al. (4) also reported a higher [CAF] 60 min post-
259 ingestion of $\sim 6 \text{ mg}\cdot\text{kg}^{-1}$ caffeine in individuals with tetraplegia compared to those with
260 paraplegia (66.1 and $45.1 \mu\text{M}$, respectively). Interestingly, Van Soeren et al. (37) also
261 reported a high peak [CAF] of $46.7 \pm 5.0 \mu\text{M}$ in individuals with tetraplegia ($n=6$) yet this
262 was reached after only 40 min post-ingestion of $6 \text{ mg}\cdot\text{kg}^{-1}$ caffeine. The current study
263 therefore adds further support to reports of higher [CAF] in TETRA compared to individuals
264 with lower lesion levels and no spinal injury. Furthermore, the data also highlight the large
265 variability that exists within each group. Based on the current study there does not appear to
266 be an influence of habitual caffeine use on the participants' [CAF] in response to a single
267 dose, as seen previously (1). Seven participants reported adverse effects which were likely a
268 result of withdrawal (headache), fasting (light headed) and CAF (tingling arm, twitching eye
269 and struggling to make quick decisions). All symptoms were mild and only lasted for a short
270 duration. The $3 \text{ mg}\cdot\text{kg}^{-1}$ caffeine dose is therefore deemed safe in this population.

271 An interaction effect occurred due to the sharp increase in [CAF] in TETRA while both AB
272 and PARA groups [CAF] increased gradually followed by a plateau. The rapid increase in
273 [CAF] in TETRA means that the hypothesis of slowed absorption in this population can be
274 rejected. The sharp rise may be due to a number of factors. Firstly, individuals with
275 tetraplegia have a smaller blood volume compared to AB individuals (17) due to atrophy of
276 the musculature and vessels of the lower limbs. This reduced blood volume may result in a
277 falsely large [CAF] in TETRA following the administration of a standardised dose per
278 kilogram body mass. Secondly, following a cervical or thoracic SCI sympathetic outflow to

279 the liver is also disrupted, which in turn can lead to hepatic pathology (30). The liver is
280 innervated by both sympathetic and parasympathetic nerves, and the sympathetic splanchnic
281 nerves originate from neurons which are located in the spinal column (T7-T12) (38). Acute
282 changes to the liver occur due to the complete (cervical level) and partial (thoracic level)
283 disruption to the descending control of sympathetic neurons innervating the organ (30). It has
284 been proposed that abnormal liver function may affect the metabolism and bioavailability of
285 drugs (23,30). The half-life of many drugs can be prolonged in individuals with an SCI who
286 display suboptimal liver function and slow renal clearance (23,30). Serum caffeine half-life
287 has also been shown to be severely prolonged in individuals with compromised liver function
288 e.g. those with alcoholic hepatic liver disease (36). The half-life of caffeine in healthy
289 individuals is ~4-6 h (1). This may help explain the sharp rise to peak [CAF] in TETRA
290 (slowed metabolism) which remains higher than AB and PARA (slowed renal clearance).
291 This TETRA response indicates that individuals with a cervical SCI may consider using a
292 lower dose of caffeine to produce similar [CAF] as AB and PARA while avoiding any
293 potential side-effects that are reported anecdotally and in previous research (13). It also
294 suggests that individuals with a high lesion level may need to consider reducing the
295 frequency of caffeine intake to prevent the potential negative effects of high doses of caffeine
296 e.g. nervousness, jitters, restlessness, sleeplessness and irritability.

297 The TAUC-CAF did not statistically differ between groups yet a large ES of 1.14 was evident
298 between AB and TETRA. Large inter-individual responses were evident in both SCI groups
299 (Figure 2) due to the heterogeneous nature of this population. The equivocal findings
300 regarding the beneficial effects of caffeine during short-term exercise performance (4) may
301 be partly explained by these inter-individual differences, highlighted by the current PARA
302 and TETRA responses. Examination of individual data within PARA reveals some interesting
303 findings. Participant 9 (L1 lesion; ASIA B) produced a similar curve to the AB participants,

304 with a peak (albeit larger) at 45 min followed by a steady decline. However, caffeine did not
305 appear in the bloodstream of participant 10 (T7 lesion; ASIA B) until 70 min and continued
306 to rise for the remaining 80 min. Hence the implementation of a standard caffeine protocol
307 whereby caffeine is administered 60 min prior to short-term exercise performance would
308 result in participant 10 exhibiting a [CAF] associated with a placebo dose at the
309 commencement of exercise. For short-term exercise performance it is therefore recommended
310 that an athlete with an SCI determines their individual absorption curve to produce
311 individualised dose and timing recommendations. If this is impractical it is recommended that
312 caffeine is provided to individuals with paraplegia earlier to ensure it enters the bloodstream
313 prior to exercise performance. Research into the use of caffeine gum or mouth rinse is
314 emerging yet the evidence of a consistent positive effect is currently limited (26,29).
315 Consuming caffeine in this format allows direct absorption into the bloodstream through the
316 buccal mucosa and may eliminate any potential issues regarding caffeine absorption in
317 individuals with an SCI.

318 Body mass and habitual caffeine intakes were similar between all three groups, but PARA
319 were significantly older than AB (Table 1). However, previous research has suggested that
320 age is not associated ($p > 0.612$) with C_{max} or time to C_{max} following caffeine ingestion
321 (Skinner et al., 2014) nor does it affect gastric emptying (Kao et al., 1999). Furthermore, no
322 significant correlations were observed between age and C_{max} ($r = 0.07$) or time to C_{max} ($r = -$
323 0.11) within the current cohort.

324 **Plasma catecholamines (adrenaline and noradrenaline)**

325 Resting plasma catecholamine concentrations did not significantly increase over the course of
326 the 150 min protocol in any group (Figure 3). In contrast, Flueck et al. (4) and Van Soeren et
327 al. (37) reported increases in adrenaline in both AB individuals and individuals with

328 paraplegia, which may in part be due to the larger $6 \text{ mg}\cdot\text{kg}^{-1}$ dose administered in these
329 studies. In line with previous findings, baseline catecholamine concentrations were lower in
330 TETRA compared to AB and PARA due to the impaired sympathetic activation of the
331 nervous system (27,31).

332 **Plasma FFA, lactate and glucose**

333 Mean resting [FFA] increased over time from $0.36 \pm 0.19 \text{ mmol}\cdot\text{L}^{-1}$ at baseline to 0.61 ± 0.25
334 at 150 min, in agreement with previous research in an AB and SCI population (10,37). In the
335 absence of a catecholamine response, the current results lend further support for a direct
336 effect of caffeine on human tissue, specifically adipocytes. The majority of research suggests
337 that FFA availability does not result in greater FFA oxidation and therefore does not alter
338 substrate use at rest or during exercise (12,24). It is also unlikely to aid performance during
339 short-term upper-body exercise where participants/athletes predominantly work anaerobically,
340 and therefore utilise carbohydrate as the main substrate.

341 Baseline [FFA] was higher in TETRA than AB or PARA (Figure 3). The lack of muscle
342 innervation of paralysed lower limbs in individuals with an SCI leads to rapid muscle atrophy
343 and a reduction in resting metabolic rate (25). Alongside poor nutritional choices and a
344 disruption in the secretion of anabolic hormones, these changes can result in an increase in fat
345 mass (35). An expanding fat mass which releases more FFA and a potential reduction in FFA
346 clearance leads to increased plasma [FFA] (2). The [FFA] responses only significantly
347 differed between the two SCI groups (PARA vs. TETRA). One possible explanation for this
348 could be the difference in the group's time since injury (PARA $4.3 \pm 4.3 \text{ yr}$ and TETRA 12.2
349 $\pm 6.3 \text{ yr}$) which has been positively associated with loss of lean tissue and increased fat mass
350 (35). Unfortunately no body composition or respiratory exchange ratio (RER) data were

351 collected to enable a greater understanding of the [FFA] responses and whether substrate use
352 was influenced at rest. However, previous research would suggest this does not occur (12,37).
353 Many studies report an increase in [Bla] during exercise following the ingestion of caffeine,
354 sometimes in the absence of increased workload/speed/power. At rest however, the current
355 data show [Bla] decreased slightly over the course of the 150 min protocol, which is in line
356 with previous data (37). On the other hand, [GLU] decreased modestly (non-significantly)
357 during the current protocol, as previously reported (24) and is unlikely a result of caffeine
358 ingestion.

359 **Conclusion**

360 The current study demonstrates that there is large inter-individual variability in caffeine
361 absorption in individuals with an SCI and that this should be assessed prior to making
362 specific recommendations for its use. Individuals with tetraplegia may consider using a lower
363 dose and individuals with paraplegia may consider consuming supplementary caffeine earlier
364 than the 60 min recommended to AB individuals.

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368 fatty acid concentration analysis, and The Peter Harrison Centre for Disability Sport for their
369 support.

370 **Conflicts of interest**

371 The authors declare no conflict of interest. The results of the present study do not constitute
372 endorsement by ACSM. The authors declare that the results of the study are presented clearly,
373 honestly, and without fabrication, falsification, or inappropriate data manipulation.

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472 **Tables**

473 **Table 1.** Participants' characteristics.

474

475 **Figures**

476 **Figure 1.** Schematic of the experimental protocol.

477 **Figure 2.** Mean \pm SD plasma caffeine concentration following the consumption of 3 mg·kg⁻¹
478 caffeine anhydrous (**Aa**). Individual data from able-bodied participants (**Bb**), and participants
479 with paraplegia (**Cc**) and tetraplegia (**Dd**) (dotted/bold lines represent individuals with an
480 ASIA A/B classification).

481 **Figure 3.** Plasma **adrenaline (a), noradrenaline (b),** free fatty acid (**Ac**), lactate (**Bd**), glucose
482 (**Ce**), ~~noradrenaline (D) and adrenaline (E)~~ concentrations (mean \pm SD) following the
483 consumption of 3 mg·kg⁻¹ caffeine anhydrous in able-bodied (AB) individuals and individuals
484 with paraplegia (PARA) and tetraplegia (TETRA). * significant main effect for group; †
485 significant time by group interaction effect.

486 ~~-^aSignificantly different to baseline values, ^bsignificantly different to 60 min, ^csignificantly~~
487 ~~different to 90 min.~~