

1 **Critical power is positively related to skeletal muscle capillarity and type I muscle fibers**
2 **in endurance trained individuals.**

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9 **Running head:** Critical power and skeletal muscle morphology

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24 **ABSTRACT**

25 The asymptote (critical power; CP) and curvature constant (W') of the hyperbolic power-
26 duration relationship can predict performance within the severe-intensity exercise domain.
27 However, the extent which these parameters relate to skeletal muscle morphology is less
28 clear, particularly in endurance trained individuals who, relative to their lesser trained
29 counterparts, possess skeletal muscles that can support high levels of oxygen transport and
30 oxidative capacity i.e. elevated type I fiber proportion and cross-sectional area (CSA) and
31 capillarity. Fourteen endurance trained males performed a maximal incremental test to
32 determine peak oxygen uptake ($\dot{V}O_{2\text{peak}}$; $63.2 \pm 4.1 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$), maximal aerobic power
33 ($406 \pm 63 \text{ W}$), and 3-5 constant load tests to task failure for the determination of CP ($303 \pm$
34 52 W) and W' ($17.0 \pm 3.0 \text{ kJ}$). Skeletal muscle biopsies were obtained from the vastus
35 lateralis and analyzed for % fiber type proportion, CSA and indices of capillarity. CP was
36 positively correlated with the % proportion ($r = 0.79$; $P = 0.001$) and CSA ($r = 0.73$; $P =$
37 0.003) of type I fibers, capillary to fiber ratio ($r = 0.88$; $P < 0.001$) and capillary contacts
38 around type I fibers ($r = 0.94$; $P < 0.001$) and type II fibers ($r = 0.68$; $P = 0.008$). W' was not
39 correlated with any morphological variables. These data reveal a strong positive association
40 between CP and skeletal muscle capillarity. Our findings support the assertion that CP is an
41 important parameter of aerobic function and offer novel insights into the physiological bases
42 of CP.

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44 **NEW & NOTEWORTHY**

45 This investigation demonstrated very strong positive correlations between critical power (CP)
46 and skeletal muscle capillarity, particularly around type I fibers, and type I fiber composition.
47 These correlations were demonstrated in endurance trained individuals expected to possess
48 well-adapted skeletal muscles, such as high levels of oxygen transport structures and high
49 oxidative capacities; supporting the view that CP is an important parameter of aerobic
50 function. In contrast, W' was not associated with fiber type composition or capillarity.

51 **KEYWORDS:** power-duration relationship, very heavy-intensity exercise, severe-intensity
52 exercise, capillarization, muscle fiber composition

53 INTRODUCTION

54 The hyperbolic relationship between time to exhaustion and power output during high-
55 intensity exercise is defined by a power asymptote, critical power (CP), and curvature
56 constant, W' (26, 27, 33). Together, these parameters determine exercise performance
57 capabilities within the severe-intensity exercise domain (20, 46) or alternatively termed the
58 very-heavy intensity domain (49). These parameters are, therefore, of significance to athletes,
59 coaches and exercise physiologists interested in fatigue development and its underpinning
60 mechanisms (6, 20, 31, 45, 47). It is well established that CP demarcates the heavy and
61 severe exercise intensity domains (21, 33, 49). During heavy-intensity exercise, pulmonary
62 $\dot{V}O_2$ and intramuscular substrates (e.g. phosphocreatine (PCr)) and metabolites (e.g. Pi and
63 H^+) achieve sub-maximal steady-state values. In contrast, during severe-intensity exercise,
64 i.e. above CP, pulmonary $\dot{V}O_2$, and intramuscular substrates and metabolites continue to
65 increase/decrease until their respective maxima/minima are obtained and task failure occurs
66 (21, 33, 43). CP is, therefore, considered to reflect the greatest sustainable rate of oxidative
67 metabolism in the absence of a progressive loss of muscle metabolic homeostasis, and is an
68 important determinant of endurance exercise performance (20).

69 Whilst the relationship between CP and the broad parameters of aerobic function, such as
70 oxygen delivery and $\dot{V}O_2$ kinetics, have been well established (9, 29, 45, 49), the association
71 between CP and aspects of skeletal muscle morphology is not fully understood. Vanhatalo *et*
72 *al.* (43) previously reported a positive relationship between CP and the proportion of type I
73 muscle fibers in recreationally active individuals. Since type I skeletal muscle fibers possess a
74 superior phenotype for oxidative metabolism and enhanced fatigue resistance compared to
75 type II fibers (for review see 41), this observation is compatible with the interpretation that
76 CP is principally a parameter of oxidative metabolism. Another aspect of skeletal muscle
77 morphology that will influence oxidative metabolism and fatigue resistance is capillarity (22).

78 Muscle capillarity is an important determinant of oxygen extraction which itself is a function
79 of the muscle oxygen diffusion capacity and muscle blood flow (37, 48). The former is
80 primarily determined by the number of red blood cells that are in contact with the contracting
81 skeletal muscle fibers (10) which is facilitated by a high capillary network and the likelihood
82 that most capillaries support red blood cell flux (32). Taken together, therefore, it seems
83 logical, that CP would be related to skeletal muscle capillary supply. Iaia *et al.* (16) have
84 previously demonstrated a positive relationship between capillary supply and time to task
85 failure over a performance range of ~1-20 minutes. Although this study did not partition the
86 exercise intensity domains as defined by CP, these exercise durations would be expected to
87 fall close to or within the severe intensity domain. Therefore, the correlation between time to
88 task failure, over a range that spans the severe-intensity exercise domain, and skeletal muscle
89 capillarity could be linked to the CP, but the relationship between skeletal muscle capillarity
90 and CP has yet to be assessed.

91 Compared to CP, the physiological understanding of W' is less clear. Classically, W' was
92 considered to represent a fixed anaerobic energy store (28). However, more recent
93 observations have challenged this interpretation since W' appears to be sensitive to changes in
94 oxygen delivery (45). Instead, W' appears to be linked to the development of the $\dot{V}O_2$ slow
95 component, and the attainment of critical levels of intramuscular pH, PCr and Pi (21, 47),
96 both of which are dependent on muscle fiber composition (25, 35). Moreover, as exercise
97 intensity exceeds CP and the utilization of W' ensues, muscle blood flow is preferentially
98 distributed to type II muscle fibers (7) suggesting a potential dependence of W' utilization on
99 type II skeletal muscle fiber recruitment and perfusion. However, Vanhatalo *et al.* (43) did
100 not report any relationship between the magnitude of W' and the proportion of type II fibers
101 in recreationally active individuals.

102 It has long been established that endurance trained individuals possess well-adapted skeletal
103 muscles with a significantly greater capillary supply (14, 17, 39) and oxidative capacity (8,
104 39) compared to untrained individuals. This permits athletes to achieve high levels of oxygen
105 extraction and, consequently, very high values of leg $\dot{V}O_2$ ($>600 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$) have been
106 observed (36). Therefore, the expected high level of oxidative capacity and oxygen transport
107 structures in endurance trained athletes suggest this population would achieve greater values
108 of CP. Indeed, CP typically occurs at 80-90% of $\dot{V}O_{2\text{max}}$ in athletes compared to 70-80% of
109 $\dot{V}O_{2\text{max}}$ in healthy young individuals (31). However, despite findings of a higher CP and
110 skeletal muscle capillarity and type I fiber percentage in athletes, a direct relationship
111 between capillary supply and muscle fiber composition and CP in endurance trained
112 individuals has yet to be established.

113 Therefore, the aim of the current study was to assess the relationship between parameters of
114 the power-duration relationship (CP and W') and indices of capillarity and muscle fiber
115 morphology in endurance trained individuals. It was hypothesized that CP would be
116 positively related to indices of skeletal muscle capillarity and the proportion and cross-
117 sectional area of type I skeletal muscle fibers.

118 **METHODS**

119 *Participants*

120 Fourteen healthy males (Table 1) volunteered to take part in the study. Participants were
121 competitive cyclists or triathletes and had to achieve the inclusion criteria of $\dot{V}O_{2\text{peak}}$
122 approximately $60 \text{ ml}\cdot\text{min}^{-1}\text{kg}^{-1}$. All completed health and muscle biopsy screening
123 questionnaires prior to participation to mitigate for contraindications to maximal exercise and
124 muscle biopsy procedures. Participants did not have a history of neuromuscular,
125 hematological or musculoskeletal abnormalities and were not using pharmacological
126 treatments during the study period. All experimental procedures were approved by the
127 Loughborough University Ethics Approvals (Human Participants) Sub-Committee and
128 conformed in all respects with the Declaration of Helsinki. Participants were fully informed
129 of the risks and discomforts associated with all experimental trials before providing written,
130 informed consent.

131 *Experimental protocol*

132 Participants attended the laboratory on five to seven occasions over a period of approximately
133 10 days. $\dot{V}O_{2\text{peak}}$ was initially tested to ensure participants attained the appropriate inclusion
134 criteria. Following a minimum of 48 hours a muscle biopsy was then obtained. After a further
135 48 hours participants undertook a series of 3-5 constant load tests to the limit of tolerance,
136 each separated by a minimum of 24 hours, to determine CP and W' .

137 All performance tests were conducted upon an electronically braked cycle ergometer (Lode
138 Excalibur Sport, Lode B.V. Gronigen, The Netherlands). Ergometer saddle and handle bar
139 dimensions were recorded for each participant during preliminary testing and remained
140 standardized for the remainder of the testing period. Participants were instructed to maintain a

141 normal diet during the testing period and refrain from ingesting alcohol and caffeine during
142 the 48 h preceding testing. All tests were conducted in constant laboratory ambient conditions
143 (19-21°C, 40-50% humidity).

144 *Performance measures*

145 $\dot{V}O_{2\text{peak}}$ and maximal aerobic power

146 Participants performed an incremental test to exhaustion to establish $\dot{V}O_{2\text{peak}}$ and maximal
147 aerobic power. Participants began cycling, at a freely chosen, constant pedal cadence for 1
148 min at 50 W, after which power increased 25 W every 60 s until volitional exhaustion or
149 when cadence fell 10% below the chosen cadence for more than 5 s, despite strong verbal
150 encouragement. Pulmonary gas exchange was measured continuously throughout exercise
151 (Cortex MetaLyzer 3B, Leipzig, Germany). $\dot{V}O_{2\text{peak}}$ and maximal aerobic power were defined
152 as the highest $\dot{V}O_2$ and power output achieved for a 30 and 60 s period during the test,
153 respectively.

154 CP and W'

155 Participants performed a minimum of 3 constant-load tests that were continued until the limit
156 of tolerance at between 75-100% of maximal aerobic power, the sequence of which was
157 randomized. These were designed to elicit exhaustion within 2- to 15-min (33). Each test was
158 preceded with an initial warm-up at 50 W for 5 min. Time to exhaustion (t) was recorded to
159 the nearest second and was taken as either volitional exhaustion or when pedal cadence fell
160 10% below the freely chosen cadence for more than 5 s, despite strong verbal encouragement.
161 No feedback regarding the power output or times achieved were provided, however
162 participants were permitted to view pedal cadence throughout. To enhance the accuracy of

163 parameter estimates, when the standard error (SE) of CP was >5% and W' >10% an
164 additional test was performed (15)

165 The parameters of the power-duration relationship, CP and W' , were calculated using the
166 inverse linear model (equation 1), the linear work-time model (equation 2) and the hyperbolic
167 model (equation 3). The equation associated with the lowest combined standard error was
168 selected and used for all further analysis.

$$169 \quad P = W' \cdot (1/t) + CP \quad (1)$$

$$170 \quad W = CP \cdot t + W' \quad (2)$$

$$171 \quad t = W' / (P - CP) \quad (3)$$

172 *Muscle sampling and analysis*

173 Muscle biopsies were obtained from the lateral portion of the vastus lateralis muscle under
174 local anaesthesia (1% lidocaine) using the percutaneous needle biopsy technique with
175 suction. Muscle samples were immediately embedded in mounting medium (Tissue-Tek OCT
176 Compound, Sakura Finetek Europe, The Netherlands) and frozen in liquid-cooled isopentane.
177 All samples were then stored at -80°C until analysis.

178 *Immuno-histochemistry*

179 Transverse serial sections (8 μ m) were obtained using a cryotome and placed onto poly L-
180 lysine coated glass slides. Sections were fixed for 10 min in 3.7% formaldehyde at room
181 temperature and blocked with phosphate buffered saline (PBS) containing 2% bovine serum
182 albumin (BSA) and 5% goat serum for 1 h at room temperature. Serial muscle sections were
183 then incubated with either primary antibodies for CD-31 (ab119339, abcam, Cambridge, UK)
184 diluted 1:100 and MHC II (ab91506, abcam, Cambridge, UK) diluted 1:1000 in PBS-2%

185 BSA or MHC I (A4.951, DSHB, Iowa, USA) diluted 1:500 in PBS-2% BSA for 1 h at room
186 temperature. Sections were then incubated for 2 h at room temperature with the appropriate
187 secondary antibodies; goat anti-mouse Alexa Fluor 488, (CD-31, MHC I) and goat anti-rabbit
188 Alexa Fluor 594 (MHC II) diluted 1:500 in PBS-2% BSA. Following incubation cover slips
189 were mounted with fluoromount aqueous mounting medium (F4680, Sigma-Aldrich, Dorset,
190 UK). Specificity of staining was assessed with no primary antibody negative controls.

191 Images were captured using a fluorescence microscope (Leica DM2500) at 20x
192 magnification. A minimum of 7 images were taken from across the entire cross-sectional area
193 of the sample to avoid bias towards smaller fibers, with at least 6 cryo-sections per participant
194 analyzed. The number of fibers counted equated to nearly 200 per participant (type I = $93 \pm$
195 18 and type II; 80 ± 33). Images analysis was undertaken using Fiji (ImageJ) software and the
196 investigator was blinded to the participant code of each sample. Only transverse fibers were
197 included in the analysis which was assessed primarily by the presumption of circularity. Any
198 fibers which were clearly oblique or not transverse to the long axis of the fiber were excluded
199 from analysis. Cross-sectional area (CSA) of fibers was assessed by manually drawing
200 around the perimeter of each fiber and was calculated as the cumulative area of each fiber
201 type divided by the number of fibers analyzed. Although absolute fiber size may be over-
202 estimated due to fiber swelling during thawing of frozen sections, this should be consistent
203 between participants. Fiber type composition was expressed as a percentage of the number of
204 fibers of each type relative to the total number of fibers counted. Capillarity was expressed
205 as; capillary density, capillary-to-fiber ratio, number of capillaries in contact with type I (CC
206 type I) and type II fibers (CC type II) and sharing index of type I and type II fibers
207 (calculated as CC/capillary-to-fiber ratio).

208 *Statistics*

209 Data were initially checked for normality using Shapiro-Wilk tests and relationships were
210 analyzed using the Pearson's product-moment correlation coefficient. Data are displayed as
211 mean \pm SD unless otherwise stated. Significance was accepted at $P \leq 0.05$ and a statistical
212 trend as $P \leq 0.10$.

213 RESULTS

214 The parameter estimates of the power-duration relationship for all three equations are
215 displayed in Table 2. The linear inverse relationship produced the lowest combined standard
216 error for CP and W' and was therefore used for further analyses. The ranges of times-to-
217 exhaustion for the shortest and longest trials were 127 – 218 s and 512 – 1050 s, respectively.
218 Power-duration relationship parameters were established from; 3 trials $n = 8$, 4 trials $n = 5$,
219 and 5 trials $n = 1$. Representative images for immuno-histochemical staining are displayed in
220 Fig. 1 Performance and skeletal muscle morphology characteristics are displayed in Table 1.

221 *CP correlates*

222 CP was positively correlated with the % proportion of type I fibers, and inversely correlated
223 with the % proportion of type II fibers. The correlation remained when type I fibers were
224 expressed as CSA but was eliminated when type II fibers were expressed as CSA (Fig 2). CP
225 was positively correlated with capillary-to-fiber ratio and CC type I and CC type II. There
226 was a modest (non-significant, $P = 0.07$) correlation between CP and capillary density (Fig
227 2). CP was negatively correlated with the sharing index of type II fibers ($r = -0.69$, $P =$
228 0.006), but was not correlated with the sharing index of type I fibers ($r = -0.16$, $P = 0.59$).

229 *W' correlates*

230 There were no correlations between W' and any measures of fiber type composition and
231 capillarity (Fig 3).

232 *$\dot{V}O_{2peak}$ correlates*

233 Absolute $\dot{V}O_{2peak}$ was positively correlated with capillary-to-fiber ratio and CC type I and CC
234 type II. In contrast, there was no correlation between $\dot{V}O_{2peak}$ and capillary density. $\dot{V}O_{2peak}$
235 was also positively correlated with % proportion and CSA of type I fibers and negatively

236 correlated with % proportion of type II fibers. $\dot{V}O_{2\text{peak}}$ was not correlated with CSA of type II
237 fibers (Table 3).

238 *Maximal aerobic power correlates*

239 Maximal aerobic power was positively correlated with all measures of capillarity; capillary
240 density, capillary-to-fiber ratio, CC type I and CC type II. Maximal aerobic power was also
241 positively correlated with % proportion and CSA of type I fibers and negatively correlated
242 with % proportion of type II fibers. Maximal aerobic power was not correlated with CSA of
243 type II fibers (Table 3).

244 **DISCUSSION**

245 The novel findings of this study are the very strong positive correlations between CP and
246 indices of capillarity in a homogenous group of endurance trained individuals. The findings
247 of the current study also confirm previous observations, undertaken on recreationally active
248 individuals, of a positive association between the proportion of type I skeletal muscle fibers
249 and CP and extend these observations by indicating that CP is also positively associated with
250 type I muscle fiber CSA. In contrast, there were no correlations between W' and any index of
251 skeletal muscle fiber type or capillarity. These observations improve our understanding of
252 the physiological mechanisms that underpin CP and, by extension, endurance exercise
253 performance and the maximum sustainable rate of oxidative metabolism.

254 CP correlates

255 In the current study CP was positively correlated with indices of skeletal muscle capillarity,
256 in particular the number of capillary contacts with type I fibers, which displayed a correlation
257 coefficient >0.9 . These novel findings extend previous observations of significant
258 correlations between capillary-to-fiber ratio and time to task failure during exercise trials
259 lasting ~2-20 minutes (16), which span the spectrum of the tolerable duration of exercise
260 within the severe exercise intensity domain (20, 31). The strong positive correlations between
261 CP and capillarity in the group of endurance trained individuals who participated in the
262 present study imply that skeletal muscle capillary supply is an important determinant of CP.

263 A high capillary supply is likely to be beneficial to CP, and therefore the ability to sustain
264 high rates of oxidative phosphorylation, through enhancing oxygen extraction, via improved
265 muscle oxygen diffusion capacity (37, 48), and an enhanced ability to remove metabolites
266 considered to be involved in skeletal muscle fatigue, such as H^+ and K^+ (1). In support of the
267 latter, Iaia *et al.* (16) demonstrated positive correlations between capillary supply and the rate

268 of plasma K^+ accumulation and muscle pH recovery. In contrast to the other markers of
269 capillarity, there was only a modest (non-significant) correlation between CP and capillary
270 density. This is perhaps not surprising as it is important to note that capillary density is also a
271 function of muscle fiber CSA (30) which was also positively correlated with CP, at least in
272 type I fibers. Given that oxygen extraction is primarily determined by the number of red
273 blood cells in contact with the contracting skeletal muscle fibers (10, 37, 48) this would
274 suggest that the number of capillaries as opposed to capillary density per se would be
275 important to oxygen extraction. This interpretation is supported by previous observations by
276 Hepple *et al.* (13) which demonstrated that an increase in capillary density following short-
277 term immobilization did not increase muscle oxygen diffusing capacity, and supports the
278 premise that a high capillary-to-fiber ratio and capillary contacts are more important
279 determinants of CP.

280 We have also demonstrated a positive correlation between CP and % proportion of type I
281 fibers in endurance trained athletes, which is consistent with previous observations in
282 recreationally-active participants (43). We have further extended this observation by
283 demonstrating that CP was also positively related to the CSA of type I fibers. These
284 observations are in keeping with the notion that CP is parameter largely dictated by facets of
285 oxidative metabolism since type I fibers possess characteristics that facilitate high rates of
286 oxidative metabolism including higher mitochondrial content, density and enzyme activity
287 and a higher capillary supply, as well as greater fatigue resistance compared to type II fibers
288 (39, 41).

289 Whilst correlation does not specifically imply causation, our data is supported by a significant
290 body of experimental evidence demonstrating CP to be a parameter of aerobic function. For
291 example, CP is negatively correlated with the fundamental time constant of the oxygen
292 uptake response to constant load exercise within the severe intensity domain (29). Moreover,

293 CP is sensitive to changes in oxygen delivery and has been demonstrated to decrease under
294 systemic hypoxia (9) and with blood flow restriction (4). Furthermore, CP has been shown to
295 increase with both continuous (11, 18) and high intensity interval training (11, 34, 44), which
296 increase both skeletal muscle capillarity (3, 19) and oxidative capacity (5). Further direct
297 support could be obtained by establishing the relationship between CP and mitochondrial
298 content and functional parameters.

299 We also demonstrated a negative correlation between CP and % proportion of type II fibers
300 which supports the observation by Vanhatalo *et al.* (43). In contrast, we have demonstrated
301 that there was no correlation when type II fiber composition was expressed relative to CSA,
302 however, it is important to acknowledge that we did not distinguish between type IIa and IIx
303 fibers. It has been demonstrated that type IIa fibers are larger than type IIx and type I fibers
304 (3, 17, 39) and therefore the relative proportions of type IIa and type IIx fibers may affect this
305 relationship. Furthermore, given that type IIa fibers possess greater oxidative capacity
306 relative to type IIx fibers (41), it could also be speculated that type IIx but not type IIa fiber
307 proportion and CSA would be negatively correlated with CP. Indeed, Vanhatalo *et al.* (43)
308 demonstrated a negative correlation only between CP and type IIx fiber proportion, whereas
309 CP was not related to type IIa fibers. Moreover, the mean CSA area of type I and type II
310 fibers were similar in the present study. The endurance trained status of our participants is
311 likely to explain this observation which is consistent with previous reports that fiber CSA is
312 comparable between type I and type II fibers in a group of well-trained middle- and long-
313 distance runners (42) supporting the notion that preferential hypertrophy of type I fibers may
314 occur with prolonged endurance training.

315 W' correlates

316 There were no correlations between W' and any of the skeletal muscle morphological
317 measurements in the present study. The lack of correlation with the % proportion or CSA of
318 type II muscle fibers may perhaps seem surprising given that type II fibers possess a greater
319 resting content of PCr (38, 40) and glycolytic capacity (12, 41), both of which has been
320 suggested to play an integral role in determining W' i.e. the so-called “anaerobic capacity”
321 (27, 28). Moreover, individuals with a greater proportion of type II fibers possess a greater
322 $\dot{V}O_2$ slow-component (35), which has been demonstrated to have a strong relationship with
323 the magnitude of W' (29, 47). However, Vanhatalo *et al.* (43) also demonstrated that there
324 was no significant correlation between the magnitude of W' and the proportion of either type
325 IIa or type IIx muscle fibers, suggesting that the determinants of W' are not specific to type II
326 muscle fibers per se. It could be speculated that the absence of a correlation between W' and
327 muscle morphology is attributable to the muscle fiber recruitment patterns that would be
328 observed during exhaustive exercise in the severe intensity domain. For example, maximal
329 sprint exercise has been shown to activate all muscle fiber types, as demonstrated by large
330 reductions in PCr concentrations in type I and II fibers (including IIA and IIX hybrid fibers;
331 23, 40), whereas during submaximal exercise at 75% of $\dot{V}O_{2max}$ muscle fiber recruitment was
332 shown to reach a steady state during 45 minutes of exercise, with only around 55% of type II
333 fibers recruited (2). Therefore, it is possible that during whole body exhaustive exercise in the
334 severe intensity domain not all type II fibers would be fully recruited when task failure and
335 complete W' utilization occur. This might account for the lack of a correlation between W'
336 and skeletal muscle fibre type reported in the current study and elsewhere (43) and suggests
337 that W' might be more closely linked to other physiological events. For example, a recent
338 study has demonstrated, in a group of elite track cyclists, that W' is positively correlated with
339 the maximum force generating capacity of the knee extensors and gross thigh volume (24).
340 Clearly, further research is required to resolve the physiological bases of W' .

341 Conclusion

342 This investigation has demonstrated very strong positive correlations between critical power
343 (CP) and skeletal muscle capillarity, particularly in relation to type I fibers, in endurance
344 trained individuals. Moreover, CP was positively correlated with type I skeletal muscle fiber
345 proportion and CSA. In contrast, there were no correlations between W' and capillarity or
346 fiber type. Collectively, these results add support to the notion that CP is a parameter of
347 aerobic function and is largely influenced by physiological processes that support oxidative
348 metabolism.

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357

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360

361 **DISCLOSURES**

362 No conflicts of interest, financial or otherwise, are declared by the author(s).

363

364 **AUTHOR CONTRIBUTIONS**

365 E.A.M., S.J.B., and R.A.F. conceived and designed research; E.A.M., and R.A.F. performed
366 experiments; E.A.M. analyzed data; E.A.M., N.R.W.M., S.J.B., and R.A.F. interpreted results
367 of experiments; E.A.M. prepared figures; E.A.M., and R.A.F. drafted manuscript; E.A.M.,
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369 approved final version of manuscript.

370 **REFERENCES**

- 371 1. **Allen DG, Lamb GD, Westerblad H.** Skeletal muscle fatigue: cellular mechanisms.
372 *Physiol Rev* 88: 287–332, 2008.
- 373 2. **Altenburg TM, Degens H, van Mechelen W, Sargeant AJ, de Haan A.**
374 Recruitment of single muscle fibers during submaximal cycling exercise. *J Appl*
375 *Physiol* 103: 1752–1756, 2007.
- 376 3. **Andersen P, Henriksson J.** Capillary supply of the quadriceps femoris muscle of
377 man: adaptive response to exercise. *J Physiol* 270: 677–690, 1977.
- 378 4. **Broxterman RM, Ade CJ, Craig JC, Wilcox SL, Schlup SJ, Barstow TJ.** Influence
379 of blood flow occlusion on muscle oxygenation characteristics and the parameters of
380 the power-duration relationship. *J Appl Physiol* 118: 880–889, 2015.
- 381 5. **Burgomaster KA, Howarth KR, Phillips SM, Rakobowchuk M, Macdonald MJ,**
382 **McGee SL, Gibala MJ.** Similar metabolic adaptations during exercise after low
383 volume sprint interval and traditional endurance training in humans. *J Physiol* 586:
384 151–60, 2008.
- 385 6. **Burnley M, Vanhatalo A, Jones AM.** Distinct profiles of neuromuscular fatigue
386 during muscle contractions below and above the critical torque in humans. *J Appl*
387 *Physiol* 113: 215–223, 2012.
- 388 7. **Copp SW, Hirai DM, Musch TI, Poole DC.** Critical speed in the rat : implications
389 for hindlimb muscle blood flow distribution and fibre recruitment. *J Physiol* 588:
390 5077–5087, 2010.
- 391 8. **Costill DL, Daniels J, Evans W, Fink W, Krahenbuhl G, Saltin B.** Skeletal muscle

- 392 enzymes and fiber composition in male and female track athletes. *J Appl Physiol* 40:
393 149–154, 1976.
- 394 9. **Dekerle J, Mucci P, Carter H.** Influence of moderate hypoxia on tolerance to high-
395 intensity exercise. *Eur J Appl Physiol* 112: 327–335, 2012.
- 396 10. **Federspiel WJ, Popel AS.** A theoretical analysis of the effect of the particulate nature
397 of blood on oxygen release in capillaries. *Microvasc Res* 32: 164–189, 1986.
- 398 11. **Gaesser GA, Wilson LA.** Effects of continuous and interval training on the
399 parameters of power-endurance time relationship for high-intensity exercise. *Int J*
400 *Sport Med* 9: 417–421, 1988.
- 401 12. **Greenhaff PL, Soderlund K, Ren J-M, Hultman E.** Energy metabolism in single
402 human muscle fibres during intermittent contraction with occluded circulation. *J*
403 *Physiol* 460: 443–453, 1993.
- 404 13. **Hepple RT, Hogan MC, Stary C, Bebout DE, Mathieu-Costello O, Wagner PD.**
405 Structural basis of muscle O₂ diffusing capacity: evidence from muscle function in
406 situ. *J Appl Physiol* 88: 560–566, 2000.
- 407 14. **Hermansen L, Wachtlova M.** Capillary density of skeletal muscle in well-trained and
408 untrained men. *J Appl Physiol* 30: 860–863, 1971.
- 409 15. **Hill DW, Smith JC.** A method to ensure the accuracy of estimates of anaerobic
410 capacity derived using the critical power concept. *J Sports Med Phys Fitness* 34: 23–
411 37, 1994.
- 412 16. **Iaia FM, Perez-Gomez J, Thomassen M, Nordsborg NB, Hellsten Y, Bangsbo J.**
413 Relationship between performance at different exercise intensities and skeletal muscle

- 414 characteristics. *J Appl Physiol* 110: 1555–1563, 2011.
- 415 17. **Ingjer F.** Capillary supply and mitochondrial content of different skeletal muscle fiber
416 types in untrained and endurance-trained men. A histochemical and ultrastructural
417 study. *Eur J Appl Physiol Occup Physiol* 40: 197–209, 1979.
- 418 18. **Jenkins DG, Quigley BM.** Endurance training enhances critical power. *Med Sci Sport*
419 *Exerc* 24: 1283–1289, 1992.
- 420 19. **Jensen L, Bangsbo J, Hellsten Y.** Effect of high intensity training on capillarization
421 and presence of angiogenic factors in human skeletal muscle. *J Physiol* 557: 571–582,
422 2004.
- 423 20. **Jones AM, Vanhatalo A, Burnley M, Morton RH, Poole D.** Critical power:
424 implications for determination of $\text{VO}_{2\text{max}}$ and exercise tolerance. *Med Sci Sport Exerc*
425 42: 1876–1890, 2010.
- 426 21. **Jones AM, Wilkerson DP, Dimenna F, Fulford J, Poole DC.** Muscle metabolic
427 responses to exercise above and below the “critical power” assessed using ^{31}P -MRS.
428 *Am J Physiol Regul Integr Comp Physiol* 294: R585–R593, 2008.
- 429 22. **Joyner MJ, Coyle EF.** Endurance exercise performance: the physiology of
430 champions. *J Physiol* 586: 35–44, 2008.
- 431 23. **Karatzafieri C, de Haan A, van Mechelen W, Sargeant AJ.** Metabolic changes in
432 single human muscle fibres during brief maximal exercise. *Exp Physiol* 86: 411–415,
433 2001.
- 434 24. **Kordi M, Menzies C, Parker Simpson L.** Relationship between power–duration
435 parameters and mechanical and anthropometric properties of the thigh in elite cyclists.

- 436 *Eur J Appl Physiol* 118: 637–645, 2018.
- 437 25. **Krustrup P, Söderlund K, Mohr M, Bangsbo J.** The slow component of oxygen
438 uptake during intense, sub-maximal exercise in man is associated with additional fibre
439 recruitment. *Pflugers Arch Eur J Physiol* 447: 855–866, 2004.
- 440 26. **Monod H, Scherrer J.** The work capacity of a synergic muscular group. *Ergonomics*
441 8: 329–338, 1965.
- 442 27. **Moritani T, Nagata A, DeVries HA, Muro M.** Critical power as a measure of
443 physical work capacity and anaerobic threshold. *Ergonomics* 24: 339–50, 1981.
- 444 28. **Morton RH.** The critical power and related whole-body bioenergetic models. *Eur J*
445 *Appl Physiol* 96: 339–354, 2006.
- 446 29. **Murgatroyd SR, Ferguson C, Ward SA, Whipp BJ, Rossiter HB.** Pulmonary O₂
447 uptake kinetics as a determinant of high-intensity exercise tolerance in humans. *J Appl*
448 *Physiol* 110: 1598–1606, 2011.
- 449 30. **Olfert IM, Baum O, Hellsten Y, Egginton S.** Advances and challenges in skeletal
450 muscle angiogenesis. *Am J Physiol Heart Circ Physiol* 310: H326–36, 2016.
- 451 31. **Poole DC, Burnley M, Vanhatalo A, Rossiter HB, Jones AM.** Critical power: an
452 important fatigue threshold. *Med Sci Sport Exerc* 48: 2320–2334, 2016.
- 453 32. **Poole DC, Copp SW, Ferguson SK, Musch TI.** Skeletal muscle capillary function:
454 Contemporary observations and novel hypotheses. *Exp Physiol* 98: 1645–1658, 2013.
- 455 33. **Poole DC, Ward SA, Gardner GW, Whipp BJ.** Metabolic and respiratory profile of
456 the upper limit for prolonged exercise in man. *Ergonomics* 31: 1265–1279, 1988.
- 457 34. **Poole DC, Ward SA, Whipp BJ.** The effects of training on the metabolic and

- 458 respiratory profile of high-intensity cycle ergometer exercise. *Eur J Appl Physiol*
459 *Occup Physiol* 59: 421–429, 1990.
- 460 35. **Pringle JSM, Doust JH, Carter H, Tolfrey K, Campbell IT, Sakkas GK, Jones**
461 **AM.** Oxygen uptake kinetics during moderate, heavy and severe intensity
462 “submaximal” exercise in humans: the influence of muscle fibre type and
463 capillarisation. *Eur J Appl Physiol* 89: 289–300, 2003.
- 464 36. **Richardson RS, Poole DC, Knight DR, Kurdak SS, Hogan MC, Grassi B,**
465 **Johnson EC, Kendrick KF, Erickson BK, Wagner PD.** High muscle blood flow in
466 man: is maximal O₂ extraction compromised? *J Appl Physiol* 75: 1911–1916, 1993.
- 467 37. **Roca J, Agusti AGN, Alonso A, Poole DC, Viegas C, Barbera JA, Rodriguez-**
468 **Roisin R, Ferrer A, Wagner PD.** Effects of training on muscle O₂ transport at
469 $\dot{V}O_{2max}$. *J Appl Physiol* 73: 1067–1076, 1992.
- 470 38. **Sahlin K, Soderlund K, Tonkonogi M, Hirakoba K.** Phosphocreatine content in
471 single fibers of human muscle after sustained submaximal exercise. *Am J Physiol* 273:
472 C172–C178, 1997.
- 473 39. **Saltin B, Henriksson J, Nygaard E, Andersen P, Jansson E.** Fiber types and
474 metabolic potentials of skeletal muscles in sedentary man and endurance runners. *Ann*
475 *N Y Acad Sci* 301: 3–29, 1977.
- 476 40. **Sant’ Ana Pereira JAA, Sargeant AJ, Rademaker ACHJ, Haan A De, Mechelen**
477 **W Van.** Myosin heavy chain isoform expression and high energy phosphate content in
478 human muscle fibres at rest and post-exercise. *J Physiol* 496: 583–588, 1996.
- 479 41. **Schiaffino S, Reggiani C.** Fiber types om mammalian skeletal muscles. *Physiol Rev*
480 91: 1447–1531, 2011.

- 481 42. **Tesch A, Karlsson J.** Muscle fiber types and size in trained and untrained muscles of
482 elite athletes. *J Appl Physiol* 59: 1716–1720, 1985.
- 483 43. **Vanhatalo A, Black MI, Dimenna FJ, Blackwell JR, Schmidt JF, Thompson C,**
484 **Wylie LJ, Mohr M, Bangsbo J, Krstrup P, Jones AM.** The mechanistic bases of
485 the power – time relationship : muscle metabolic responses and relationships to muscle
486 fibre type. *J Physiol* 15: 4407–4423, 2016.
- 487 44. **Vanhatalo A, Doust JH, Burnley M.** A 3-min all-out cycling test is sensitive to a
488 change in critical power. *Med Sci Sports Exerc* 40: 1693–1699, 2008.
- 489 45. **Vanhatalo A, Fulford J, Dimenna FJ, Jones AM.** Influence of hyperoxia on muscle
490 metabolic responses and the power – duration relationship during severe-intensity
491 exercise in humans : a ³¹P magnetic resonance spectroscopy study. *Exp Physiol* 95:
492 528–540, 2010.
- 493 46. **Vanhatalo A, Jones AM, Burnley M.** Application of critical power in sport. *Int J*
494 *Sports Physiol Perform* 6: 128–136, 2011.
- 495 47. **Vanhatalo A, Poole DC, Dimenna FJ, Bailey SJ, Jones AM.** Muscle fiber
496 recruitment and the slow component of O₂ uptake : constant work rate vs . all-out
497 sprint exercise. *Am J Physiol Regul Integr Comp Physiol* 300: R700–R707, 2011.
- 498 48. **Wagner PD.** Gas exchange and peripheral diffusion limitation. *Med Sci Sport Exerc*
499 24: 54–58, 1992.
- 500 49. **Whipp BJ.** Domains of aerobic function and their limiting parameters. In: *The*
501 *Physiology and Pathophysiology of Exercise Tolerance*, edited by Steinacker JM,
502 Ward SA. New York: Plenum, 1996, pp. 83–89.

503 **FIGURE LEGENDS**

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505 Figure 1. Representative images of immuno-histochemical staining of muscle samples from
506 two participants; A) CP = 207 W, $\dot{V}O_{2peak} = 3.93 \text{ l}\cdot\text{min}^{-1}$, C:F = 1.90; B) CP = 353 W, $\dot{V}O_{2peak}$
507 = $5.38 \text{ l}\cdot\text{min}^{-1}$, C:F = 3.40. Type I fibers = red, type II fibers = blue, and capillaries = green.
508 Scale Bar = 50 μm .

509 Figure 2. Correlations between CP and; A) % proportion of type I fibers, B) % proportion of
510 type II fibers, C) CSA of type I fibers, D) CSA of type II fibers, E) capillary density, F)
511 capillary-to-fiber ratio, G) capillary contacts around type I fibers, H) capillary contacts
512 around type II fibers.

513 Figure 3. Correlations between W' and; A) % proportion of type I fibers, B) % proportion of
514 type II fibers, C) CSA of type I fibers, D) CSA of type II fibers, E) capillary density, F)
515 capillary-to-fiber ratio, G) capillary contacts around type I fibers, H) capillary contacts
516 around type II fibers.

517

518 **TABLE LEGENDS**

519

520 Table 1. Parameters of performance and skeletal muscle morphology

521 Table 2. Parameter estimates of the power-duration relationship

522 Table 3. Correlations between $\dot{V}O_{2peak}$ and maximal aerobic power and markers of skeletal
523 muscle capillarization and muscle fiber composition

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525 **TABLE 1**

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	Mean \pm SD	Range
Participant characteristics		
Age (yr)	25 \pm 6	20 – 41
Height (m)	1.82 \pm 0.06	1.70 – 1.95
Mass (kg)	76.5 \pm 9.0	64.2 – 90.4
Performance parameters		
$\dot{V}O_{2peak}$ (ml.min ⁻¹ .kg ⁻¹)	63.2 \pm 4.1	58.7 – 72.2
$\dot{V}O_{2peak}$ (l.min ⁻¹)	4.86 \pm 0.68	3.93 – 5.86
Maximal aerobic power (W)	406 \pm 63	295 – 485
CP (W)	303 \pm 52	207 – 376
W' (kJ)	17.0 \pm 3.0	13.9 – 22.9
Skeletal muscle morphology		
Type I fiber %	56.6 \pm 11.9	41.2 – 83.9
Type II fiber %	43.4 \pm 11.9	16.1 – 58.8
CSA fiber type I (μm^2)	5937 \pm 1333	3835 – 8568
CSA fiber type II (μm^2)	5967 \pm 1294	4024 – 7997
Capillary density (cap.mm ⁻²)	424 \pm 55	314 – 489
Capillary-to-fiber ratio	2.84 \pm 0.63	1.90 – 4.22
CC type I	6.9 \pm 1.4	4.8 – 9.4
CC type II	6.1 \pm 1.1	4.6 – 8.4
Sharing index type I	2.43 \pm 0.16	2.20 – 2.64
Sharing index type II	2.19 \pm 0.24	1.74 – 2.62

Abbreviations: CC type I, capillary contacts around type I fibers; CC type II, capillary contacts around type II fibers; CP, critical power; CSA fiber type I, cross sectional area of type I fibers; CSA fiber type II, cross sectional area of type II fibers; Sharing index type I, sharing index of type I fibers; Sharing index type II, sharing index of type II fibers; Type I fiber %, % proportion of type I fibers; Type II fiber %, % proportion of type II fibers %; $\dot{V}O_{2peak}$, peak oxygen uptake; W' , curvature constant.

544 **TABLE 2**

	CP	CP SE (%)	W'	W' SE (%)	R²
Inverse model	303 ± 52	1.8 ± 1.0	17.0 ± 3.0	8.9 ± 5.2	0.982 ± 0.019
Work-time model	302 ± 52	1.8 ± 1.0	17.4 ± 2.3	14.5 ± 8.5	0.999 ± 0.001
Hyperbolic model	299 ± 52	1.8 ± 1.3	19.0 ± 3.0	16.8 ± 10.1	-

Abbreviations: CP, critical power; SE, standard error; W', curvature constant.

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560 **TABLE 3**

	$\dot{V}O_{2peak}$ (l.min ⁻¹)	$\dot{V}O_{2peak}$ (ml.min ⁻¹ .kg ⁻¹)	Maximal aerobic power (W)
Type I fiber %	<i>r</i> = 0.82 <i>P</i> = 0.002	<i>r</i> = 0.33 <i>P</i> = 0.32	<i>r</i> = 0.74 <i>P</i> = 0.002
Type II fiber %	<i>r</i> = -0.82 <i>P</i> = 0.002	<i>r</i> = -0.33 <i>P</i> = 0.32	<i>r</i> = -0.74 <i>P</i> = 0.002
CSA fiber type I (μm ²)	<i>r</i> = 0.81 <i>P</i> = 0.003	<i>r</i> = 0.19 <i>P</i> = 0.58	<i>r</i> = 0.69 <i>P</i> = 0.01
CSA fiber type II (μm ²)	<i>r</i> = 0.47 <i>P</i> = 0.14	<i>r</i> = 0.22 <i>P</i> = 0.52	<i>r</i> = 0.21 <i>P</i> = 0.46
Capillary density (cap.mm ⁻²)	<i>r</i> = 0.43 <i>P</i> = 0.19	<i>r</i> = 0.42 <i>P</i> = 0.20	<i>r</i> = 0.55 <i>P</i> = 0.04
Capillary-to-fiber ratio	<i>r</i> = 0.94 <i>P</i> < 0.001	<i>r</i> = 0.40 <i>P</i> = 0.23	<i>r</i> = 0.86 <i>P</i> < 0.001
CC type I	<i>r</i> = 0.95 <i>P</i> < 0.001	<i>r</i> = 0.40 <i>P</i> = 0.22	<i>r</i> = 0.92 <i>P</i> < 0.001
CC type II	<i>r</i> = 0.81 <i>P</i> = 0.003	<i>r</i> = 0.49 <i>P</i> = 0.13	<i>r</i> = 0.68 <i>P</i> = 0.01

Abbreviations: CC type I, capillary contacts around type I fibers; CC type II, capillary contacts around type II fibers; CSA fiber type I, cross sectional area of type I fibers; CSA fiber type II, cross sectional area of type II fibers; Type I fiber %, % proportion of type I fibers; Type II fiber %, % proportion of type II fibers %; $\dot{V}O_{2peak}$, peak oxygen uptake.

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FIGURE 1

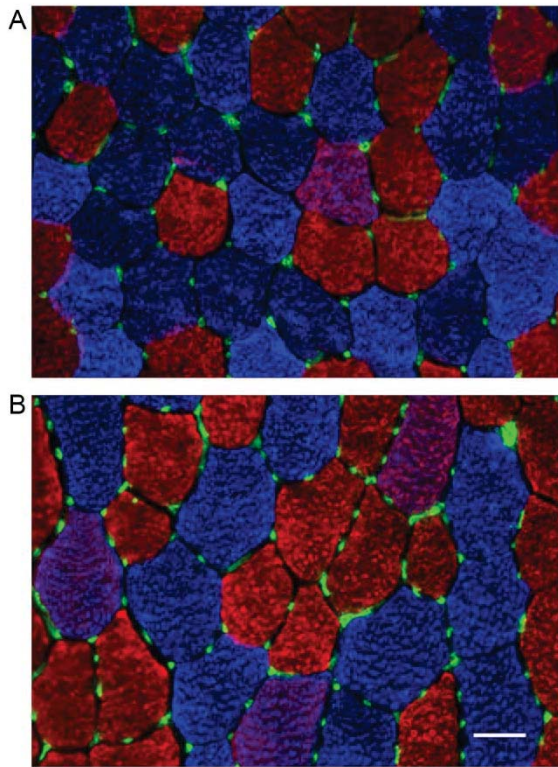


FIGURE 2

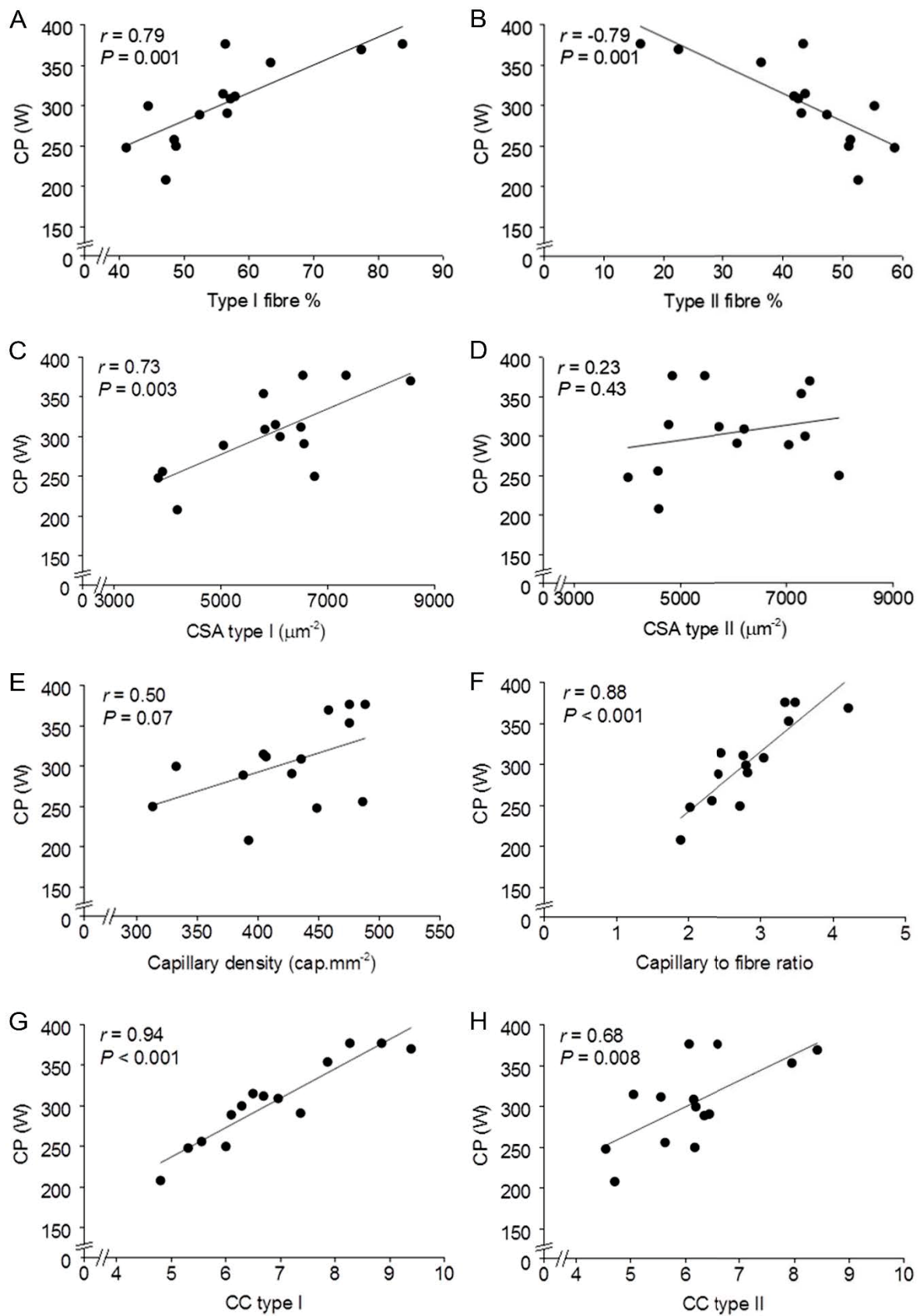


FIGURE 3

