Loughborough University

This item was submitted to Loughborough's Research Repository by the author.
Items in Figshare are protected by copyright, with all rights reserved, unless otherwise indicated.

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

PLEASE CITE THE PUBLISHED VERSION

https://doi.org/10.1152/japplphysiol.01126.2017

PUBLISHER

© American Physiological Society

VERSION

AM (Accepted Manuscript)

PUBLISHER STATEMENT

This work is made available according to the conditions of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) licence. Full details of this licence are available at: https://creativecommons.org/licenses/by-nc-nd/4.0/

LICENCE

CC BY-NC-ND 4.0

REPOSITORY RECORD

Mitchell, Emma A., Neil Martin, Stephen Bailey, and Richard Ferguson. 2018. “Critical Power Is Positively Related to Skeletal Muscle Capillarity and Type I Muscle Fibers in Endurance Trained Individuals”. Loughborough University. https://hdl.handle.net/2134/34592.

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

Emma A. Mitchell, Neil R. W. Martin, Stephen J. Bailey, Richard A. Ferguson

School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, LE11 3TU, United Kingdom

Running head: Critical power and skeletal muscle morphology

Corresponding author:

Richard A. Ferguson

School of Sport, Exercise and Health Sciences

Loughborough University

LE11 3TU

United Kingdom

Tel: +44 (0) 1509 226333

Fax: +44 (0) 1509 226301

Email: R.A.Ferguson@lboro.ac.uk

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

ABSTRACT

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

NEW & NOTEWORTHY

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

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

INTRODUCTION

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

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

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

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

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

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

METHODS

Participants

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

Experimental protocol

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

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

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

Performance measures

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

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

CP and W'

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

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

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

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

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

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

Muscle sampling and analysis

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

Immuno-histochemistry

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

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

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

Statistics

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

RESULTS

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

CP correlates

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

W′ correlates

There were no correlations between *W′* and any measures of fiber type composition and capillarity (Fig 3).

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

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

236 correlated with % proportion of type II fibers. $\dot{V}O_{2peak}$ 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).

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

DISCUSSION

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

CP correlates

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

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

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

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

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

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

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

W′ correlates

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

Conclusion

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

ACKNOWLEDGEMENTS

The authors would like to thank the participants for their commitment and effort. The authors also thank Xavier Frisch, Said Ibeggazene, Rosa Leftwich, Samantha Rees-Clark, Ismael Serrablo-Torrejon, Tom Smith and Robert Sullivan for their technical assistance at various points throughout the experiment.

The monoclonal antibody for MHC I developed by Blau HM, was obtained from the Developmental Studies Hybridoma Bank, created by the NICHD of the NIH and maintained at The University of Iowa, Department of Biology, Iowa City, IA 52242.

GRANTS

No funding has been received for this experiment.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

REFERENCES

1. **Allen DG**, **Lamb GD**, **Westerblad H**. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev* 88: 287–332, 2008.

2. **Altenburg TM**, **Degens H**, **van Mechelen W**, **Sargeant AJ**, **de Haan A.** Recruitment of single muscle fibers during submaximal cycling exercise. *J Appl Physiol* 103: 1752–1756, 2007.

3. **Andersen P**, **Henriksson J**. Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. *J Physiol* 270: 677–690, 1977.

4. **Broxterman RM**, **Ade CJ**, **Craig JC**, **Wilcox SL**, **Schlup SJ**, **Barstow TJ**. Influence of blood flow occlusion on muscle oxygenation characteristics and the parameters of the power-duration relationship. *J Appl Physiol* 118: 880–889, 2015.

5. **Burgomaster KA**, **Howarth KR**, **Phillips SM**, **Rakobowchuk M**, **Macdonald MJ**, **McGee SL**, **Gibala MJ**. Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *J Physiol* 586: 151–60, 2008.

6. **Burnley M**, **Vanhatalo A**, **Jones AM**. Distinct profiles of neuromuscular fatigue during muscle contractions below and above the critical torque in humans. *J Appl Physiol* 113: 215–223, 2012.

7. **Copp SW**, **Hirai DM**, **Musch TI**, **Poole DC**. Critical speed in the rat : implications for hindlimb muscle blood flow distribution and fibre recruitment. *J Physiol* 588: 5077–5087, 2010.

8. **Costill DL**, **Daniels J**, **Evans W**, **Fink W**, **Krahenbuhl G**, **Saltin B**. Skeletal muscle

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

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 conitnuous 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 O_2 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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

characteristics. *J Appl Physiol* 110: 1555–1563, 2011.

17. **Ingjer F**. Capillary supply and mitochondrial content of different skeletal muscle fiber types in untrained and endurance-trained men. A histochemical and ultrastructural study. *Eur J Appl Physiol Occup Physiol* 40: 197–209, 1979.

18. **Jenkins DG**, **Quigley BM**. Endurance training enhances critical power. *Med Sci Sport Exerc* 24: 1283–1289, 1992.

19. **Jensen L**, **Bangsbo J**, **Hellsten Y**. Effect of high intensity training on capillarization and presence of angiogenic factors in human skeletal muscle. *J Physiol* 557: 571–582, 2004.

20. **Jones AM**, **Vanhatalo A**, **Burnley M**, **Morton RH**, **Poole D**. Critical power: implications for determination of VO_{2max} and exercise tolerance. *Med Sci Sport Exerc* 42: 1876–1890, 2010.

21. **Jones AM**, **Wilkerson DP**, **Dimenna F**, **Fulford J**, **Poole DC**. Muscle metabolic responses to exercise above and below the " critical power " assessed using ^{31}P-MRS. *Am J Physiol Regul Integr Comp Physiol* 294: R585–R593, 2008.

22. **Joyner MJ**, **Coyle EF**. Endurance exercise performance: the physiology of champions. *J Physiol* 586: 35–44, 2008.

23. **Karatzaferi C**, **de Haan A**, **van Mechelen W**, **Sargeant AJ**. Metabolic changes in single human muscle fibres during brief maximal exercise. *Exp Physiol* 86: 411–415, 2001.

24. **Kordi M**, **Menzies C**, **Parker Simpson L**. Relationship between power–duration parameters and mechanical and anthropometric properties of the thigh in elite cyclists.

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

Eur J Appl Physiol 118: 637–645, 2018.

25. **Krustrup P**, **Söderlund K**, **Mohr M, Bangsbo J**. The slow component of oxygen uptake during intense, sub-maximal exercise in man is associated with additional fibre recruitment. *Pflugers Arch Eur J Physiol* 447: 855–866, 2004.

26. **Monod H**, **Scherrer J**. The work capacity of a synergic muscular group. *Ergonomics* 8: 329–338, 1965.

27. **Moritani T**, **Nagata A**, **DeVries HA**, **Muro M**. Critical power as a measure of physical work capacity and anaerobic threshold. *Ergonomics* 24: 339–50, 1981.

28. **Morton RH**. The critical power and related whole-body bioenergetic models. *Eur J Appl Physiol* 96: 339–354, 2006.

29. **Murgatroyd SR**, **Ferguson C**, **Ward SA**, **Whipp BJ**, **Rossiter HB**. Pulmonary O_2 uptake kinetics as a determinant of high-intensity exercise tolerance in humans. *J Appl Physiol* 110: 1598–1606, 2011.

30. **Olfert IM**, **Baum O**, **Hellsten Y**, **Egginton S**. Advances and challenges in skeletal muscle angiogenesis. *Am J Physiol Heart Circ Physiol* 310: H326-36, 2016.

31. **Poole DC**, **Burnley M**, **Vanhatalo A**, **Rossiter HB**, **Jones AM**. Critical power: an important fatigue threshold. *Med Sci Sport Exerc* 48: 2320–2334, 2016.

32. **Poole DC**, **Copp SW**, **Ferguson SK**, **Musch TI**. Skeletal muscle capillary function: Contemporary observations and novel hypotheses. *Exp Physiol* 98: 1645–1658, 2013.

33. **Poole DC**, **Ward SA**, **Gardner GW**, **Whipp BJ**. Metabolic and respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics* 31: 1265–1279, 1988.

34. **Poole DC**, **Ward SA**, **Whipp BJ**. The effects of training on the metabolic and

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

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 O_2 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 O_2, 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.

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

42. **Tesch A**, **Karlsson J**. Muscle fiber types and size in trained and untrained muscles of elite athletes. *J Appl Physiol* 59: 1716–1720, 1985.

43. **Vanhatalo A**, **Black MI**, **Dimenna FJ**, **Blackwell JR**, **Schmidt JF**, **Thompson C**, **Wylie LJ**, **Mohr M**, **Bangsbo J**, **Krustrup P**, **Jones AM**. The mechanistic bases of the power – time relationship : muscle metabolic responses and relationships to muscle fibre type. *J Physiol* 15: 4407–4423, 2016.

44. **Vanhatalo A**, **Doust JH**, **Burnley M**. A 3-min all-out cycling test is sensitive to a change in critical power. *Med Sci Sports Exerc* 40: 1693–1699, 2008.

45. **Vanhatalo A**, **Fulford J**, **Dimenna FJ**, **Jones AM**. Influence of hyperoxia on muscle metabolic responses and the power – duration relationship during severe-intensity exercise in humans : a ^{31}P magnetic resonance spectroscopy study. *Exp Physiol* 95: 528–540, 2010.

46. **Vanhatalo A**, **Jones AM**, **Burnley M**. Application of critical power in sport. *Int J Sports Physiol Perform* 6: 128–136, 2011.

47. **Vanhatalo A**, **Poole DC**, **Dimenna FJ**, **Bailey SJ**, **Jones AM**. Muscle fiber recruitment and the slow component of O_2 uptake : constant work rate vs . all-out sprint exercise. *Am J Physiol Regul Integr Comp Physiol* 300: R700–R707, 2011.

48. **Wagner PD**. Gas exchange and peripheral diffusion limitation. *Med Sci Sport Exerc* 24: 54–58, 1992.

49. **Whipp BJ**. Domains of aerobic function and their limiting parameters. In: *The Physiology and Pathophysiology of Exercise Tolerance*, edited by Steinacker JM, Ward SA. New York: Plenum, 1996, pp. 83–89.

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

FIGURE LEGENDS

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

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

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

TABLE LEGENDS

Table 1. Parameters of performance and skeletal muscle morphology

Table 2. Parameter estimates of the power-duration relationship

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

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

525 **TABLE 1**

526

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

538

539

540

541

542

543

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

544 **TABLE 2**

	CP	CP SE (%)	W'	W' SE (%)	R^2
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.

545

546

547

548

549

550

551

552

553

554

555

556

557

558

559

Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

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 %	*r* = 0.82 *P* = 0.002	*r* = 0.33 *P* = 0.32	*r* = 0.74 *P* = 0.002
Type II fiber %	*r* = -0.82 *P* = 0.002	*r* = -0.33 *P* = 0.32	*r* = -0.74 *P* = 0.002
CSA fiber type I (μm^{-2})	*r* = 0.81 *P* = 0.003	*r* = 0.19 *P* = 0.58	*r* = 0.69 *P* = 0.01
CSA fiber type II (μm^{-2})	*r* = 0.47 *P* = 0.14	*r* = 0.22 *P* = 0.52	*r* = 0.21 *P* = 0.46
Capillary density ($cap.mm^{-2}$)	*r* = 0.43 *P* = 0.19	*r* = 0.42 *P* = 0.20	*r* = 0.55 *P* = 0.04
Capillary-to-fiber ratio	*r* = 0.94 *P* < 0.001	*r* = 0.40 *P* = 0.23	*r* = 0.86 *P* < 0.001
CC type I	*r* = 0.95 *P* < 0.001	*r* = 0.40 *P* = 0.22	*r* = 0.92 *P* < 0.001
CC type II	*r* = 0.81 *P* = 0.003	*r* = 0.49 *P* = 0.13	*r* = 0.68 *P* = 0.01

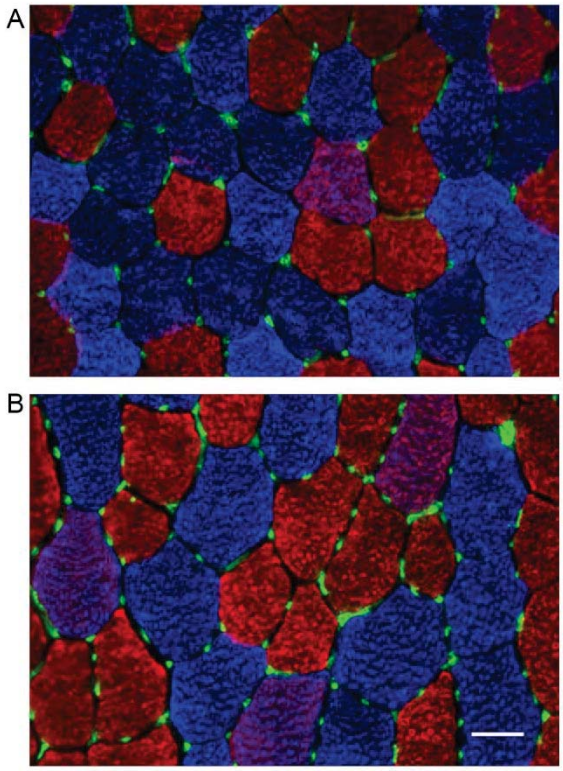
Abbreviations: CC type I, capillary contacts around type I fibers; CC type II, capillary contacts around type II fibers; CSA fiber type I, cross sectional area of type I fibers; CSA fiber type II, cross sectional area of type II fibers; Type I fiber %, % proportion of type I fibers; Type II fiber %,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

561

562

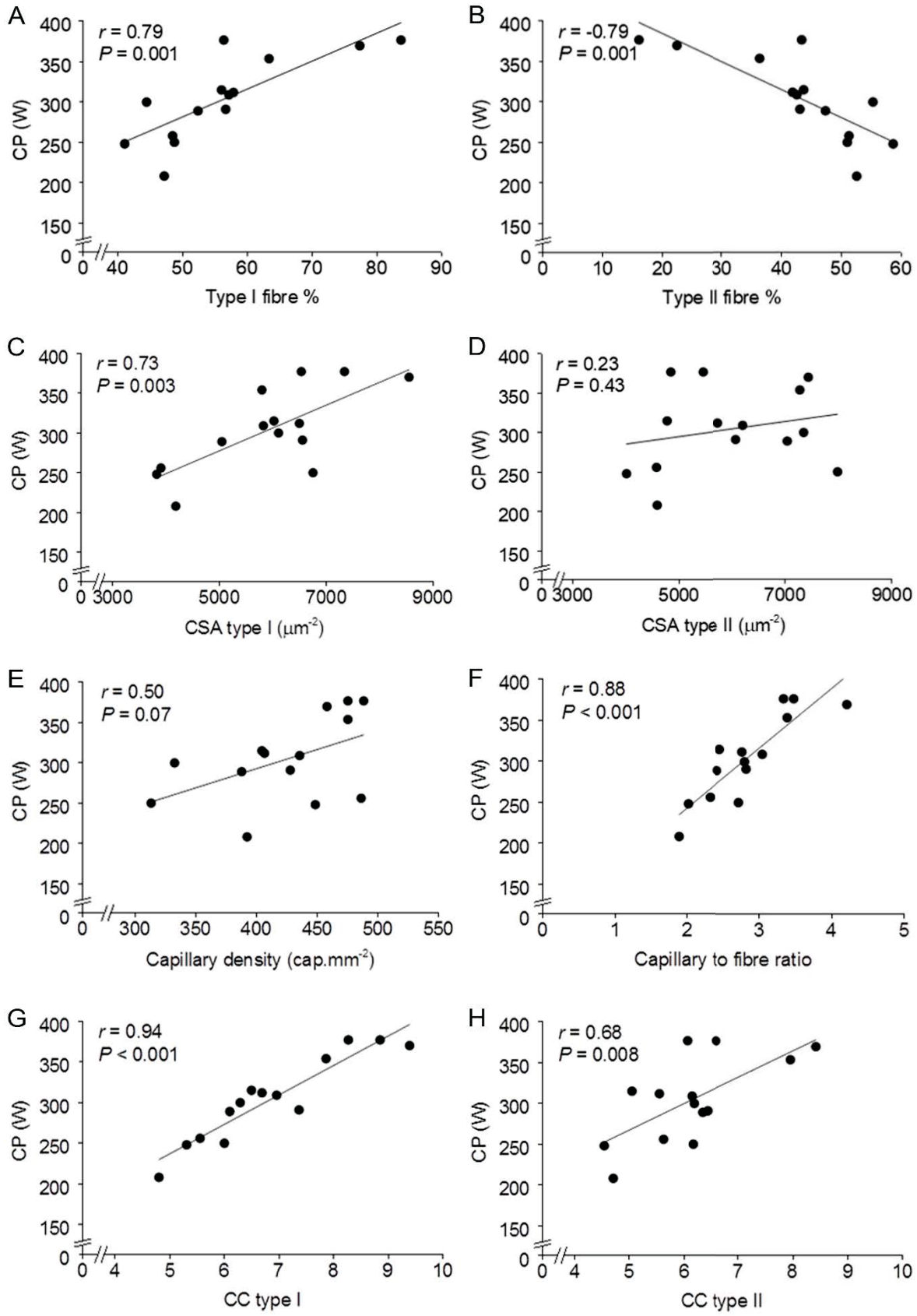
Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

FIGURE 1



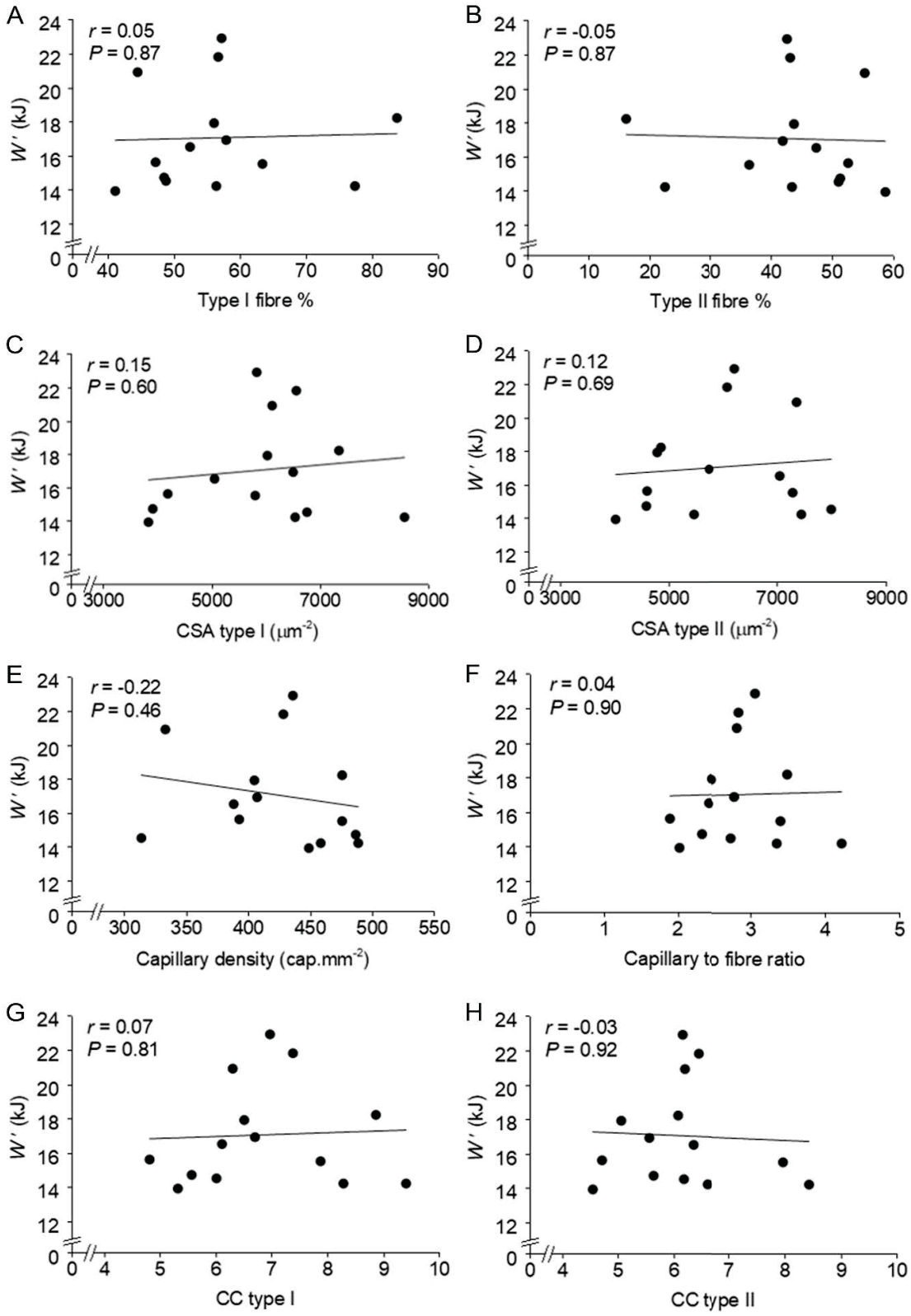
Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

FIGURE 2



Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.

FIGURE 3



Downloaded from www.physiology.org/journal/jappl by ${individualUser.givenNames} ${individualUser.surname} (131.231.070.172) on June 8, 2018.
Copyright © 2018 American Physiological Society. All rights reserved.