Accepted Manuscript

Type: Regular Article

Title: Lower leg vascular conductance in triathletes

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Number of Figures: 3

Number of Tables: 3

Running Title: Vascular conductance in triathletes

1 Abstract

2 Triathlon is an endurance sport that requires a high aerobic capacity. Previous 3 studies demonstrated that lower-leg vascular conductance (VC) was associated with maximal 4 oxygen uptake. However, it is unclear whether muscular VC is greater in triathletes versus 5 untrained individuals. To test our hypothesis that VC in skeletal muscle is greater in triathletes 6 relative to untrained peers and is associated with higher aerobic capacity in triathletes, this 7 study investigated 10 male triathletes (21 ± 1 years of age) and 8 untrained men (22 ± 1 years). 8 Triathletes exhibited greater maximal oxygen uptake than their untrained counterparts (61±6 9 vs. 39 ± 6 mL/kg/min, P < 0.01), as well as lower heart rate (54 ± 7 vs. 65 ± 9 bpm, P < 0.01) and body fat (11 \pm 2 vs. 16 \pm 6 %, P = 0.03). There were no detectable intergroup differences in 10 systolic (115 \pm 8 vs. 117 \pm 9 mmHg, P = 0.74), mean (83 \pm 6 vs. 86 \pm 8 mmHg, P = 0.30), or 11 12 diastolic (62 ± 6 vs. 65 ± 7 mmHg, P = 0.49) blood pressure. Lower-leg blood flow (4.2 ± 1.3 vs. 2.5±0.9 mL/dL/min, P = 0.01) and VC (0.05±0.01 vs. 0.03±0.01 mL/dL/min/mmHg, P = 13 14 0.02) based on venous occlusion plethysmography were greater in triathletes versus untrained 15 men. Maximal oxygen uptake was correlated with lower-leg blood flow (r = 0.70, P < 0.01) and VC (r = 0.69, P < 0.01). These results demonstrate that lower-leg VC is higher in 16 17 triathletes than in untrained individuals, and it may be associated with higher aerobic capacity 18 in triathletes relative to untrained counterparts. 19

20 Keywords: blood flow; maximal oxygen uptake; vascular resistance

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トライアスロン選手の下腿血管コンダクタンス

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トライアスロンは高い有酸素性能力を必要とする持久系スポーツである.先行研究 27 では、下腿の血管コンダクタンス(vascular conductance, VC)は最大酸素摂取量と関 28 連することが示されている.しかしながら、トライアスロン選手において骨格筋の 29 VC が非鍛錬者に比べて大きいか否かは不明である.本研究では、骨格筋の VC は非 30 鍛錬者に比べてトライアスロン選手で大きく、トライアスロン選手の高い有酸素性 31 能力と関連しているという仮説を検証するために、男性トライアスロン選手10人 32 (21±1 歳)および非鍛錬者 8人(22±1 歳)を対象に測定を行った.トライアスロン 33 34 選手は、非鍛錬者よりも最大酸素摂取量(61±6 vs 39±6 mL/kg/min, P < 0.01)は多 く、心拍数(54±7 vs 65±9 bpm, P<0.01) および体脂肪率(11±2 vs 16±6 %, P= 35 0.03) は低かった. 収縮期血圧 (115±8 vs 117±9 mmHg, P=0.74), 平均血圧 (83±6 vs 36 37 86±8 mmHg, P=0.30), 拡張期血圧(62±6 vs 65±7 mmHg, P=0.49) に両者間の有意 差は認められなかった.静脈閉塞プレチスモグラフィで測定した下腿の血流量 38 (4.2±1.3 vs 2.5±0.9 mL/dL/min, P=0.01) および VC (0.05±0.01 vs 0.03±0.01 39 mL/dL/min/mmHg, P=0.02)は、トライアスロン選手で非鍛錬者より高値を示した. 40 41 最大酸素摂取量と下肢の血流量(r=0.70, P<0.01)および VC(r=0.69, P<0.01) 42 との間に相関関係が認められた.これらの結果は、下腿 VC は非鍛錬者よりもトラ イアスロン選手で大きいことを示唆する. トライアスロン選手の下腿 VC は非鍛錬 43 者に比べて有酸素性能力が高いことに関連するかもしれない. 44

45 Introduction

Triathlon, which consists of swimming, biking, and running, is an endurance sport that requires high aerobic capacity¹⁾. Since maximal oxygen uptake, the gold standard for evaluating aerobic capacity, is strongly correlated with maximal cardiac output, blood circulation plays a crucial role in aerobic capacity²⁾. The main components of the circulatory system are the heart and blood vessels. Whereas many previous studies have investigated cardiac function in triathletes³⁾, vascular function in this population has not been fully elucidated.

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54 Vascular conductance (VC) in skeletal muscle may contribute to aerobic capacity 55 since the peak VC in the lower leg after calf exercise is correlated with maximal oxygen uptake ⁴⁻⁶). However, it is unclear whether muscular VC is greater in triathletes than in 56 57 untrained individuals. First, triathlon requires both aerobic capacity and muscular strength. Previous studies have demonstrated that triathletes have excellent aerobic capacity⁷). On the other 58 hand, Etxebarria et al.⁸⁾ reported that cycling of triathlon requires high power output; power output per 59 60 body weight during cycling of elite-level triathlon race was similar to those of hill climbers. Therefore, 61 triathletes are supposed to be adapted to both endurance and strength training. Capillary density increases with endurance training but not with strength training⁹⁾. Second, previous studies 62 reported that while systemic vascular resistance (VR, the inverse of VC) was comparable in 63 triathletes and their sedentary counterparts¹⁰, pulmonary VR was higher in triathletes¹¹. Thus 64 it has been unclear if muscular VC is greater in triathletes relative to untrained individuals. To 65 test our hypothesis that VC in skeletal muscle is greater in triathletes versus untrained 66 67 individuals and is associated with higher aerobic capacity in triathletes, we investigated 68 lower-leg VC and maximal oxygen uptake in male triathletes and their untrained counterparts.

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70 Methods

Page 4

71 Participants

72 Study participants comprised 10 male triathletes (triathlon career, 7±5 years) in university 73 triathlon clubs and 8 healthy male university students who did not regularly exercise. 74 Inclusion criteria of triathletes were intercollegiate triathletes belonging to triathlon teams 75 with at least 1 year of triathlon career and with training frequency of 5 or more days per week. 76 Five of them had satisfied the entry standards for the Japan Triathlon Championships (i.e., 77 listed in the top 40 of NTT Japan Cup Ranking or met the qualifying standard [04:38.30 for swimming of 400 m and 09:40.92 for running of 3 km]). Inclusion criteria of untrained men 78 79 were university students who had sedentary lifestyles (no regular physical activity except for 80 sport classes in a university or high school) for at least 3 years. All were non-smokers, took no 81 medications, and had no chronic diseases. The participants refrained from alcohol 82 consumption and high-intensity exercise on the day before testing, and avoided caffeine 83 consumption on the day of the study. Additionally, they did not eat or drink anything except 84 for water for 2 hours prior to the measurements. After at least 10-minute of rest in the supine 85 position in the air-conditioned laboratory, femoral-ankle pulse wave velocity (PWV) and 86 brachial and post-tibial arterial blood pressure were measured simultaneously. Next, lower-leg 87 blood flow (BF) was measured in the supine position. The resting data of vascular measurements were considered as acceptable if resting of the participants were kept during 88 89 the rest and measurement periods. Finally, a maximal exercise test was performed to measure 90 maximal oxygen uptake.

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92 This study conformed to the principles of the Declaration of Helsinki and was
93 approved by the Ethics Committee of the Ryutsu Keizai University (approval number 25). All
94 participants provided written informed consent prior to study participation.

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97 Lower-leg BF and VC measurements

98 Lower-leg BF and VC were measured using venous occlusion plethysmography with a mercury-filled strain gauge (EC6; Hokanson, Bellevue, WA, USA)¹²⁾. A strain gauge was 99 100 attached to the widest part of the lower leg. Briefly, an ankle cuff was inflated to 50 mmHg 101 above post-tibial arterial systolic blood pressure (SBP) at 1 min before BF measurements 102 began. A femoral cuff was rapidly inflated to 40–50 mmHg for 7 s during a 15-s cycle (E20; 103 Hokanson). BF was calculated as the percent change in lower-leg volume during the 104 occlusion phase. VC was calculated as BF divided by post-tibial arterial mean blood pressure 105 (MBP).

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107 Maximal oxygen uptake and body fat measurements

108 After the participants underwent a voluntary warm-up (e.g., muscular stretching), a maximal 109 exercise test (4 min at 100 W, with a 30-W increase every 2 min) was conducted using a cycle 110 ergometer (75XL; Konami Sports, Tokyo, Japan). Breath-by-breath oxygen uptake and carbon 111 dioxide production (AE300S; Minato Medical Science, Osaka, Japan), heart rate (HR) 112 calculated using 3-lead electrocardiography (LRR-03; GMS, Tokyo, Japan), and rating of 113 perceived exertion (RPE, Borg's 6-20 scale) were measured during the exercise test. We considered a participant as having achieved maximal exertion when at least 2 of the following 114 4 criteria were met; 1) a plateau in oxygen uptake with increasing exercise intensity (<100 115 mL/min); 2) achievement of age-predicted maximal HR (±10 bpm); 3) a respiratory exchange 116 ratio of at least 1.15; and 4) an RPE of at least 18 units^{13,14}). 117

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To demonstrate that the participants in this study were well-trained triathletes,
body fat was measured using a body impedance–based body composition analyzer (InBody
430; InBody, Seoul, South Korea).

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123 Femoral-ankle PWV and blood pressure measurements

124 Femoral-ankle PWV, an index of leg arterial stiffness, was measured using applanation 125 tonometry and air plethysmography. Femoral and post-tibial artery pulse waves were obtained 126 using a pulse wave analyzer (BP-203RPE II; Fukuda Colin, Tokyo, Japan). Pulse wave transit 127 time, the delay between the proximal and distal waveforms, was automatically determined by 128 the device. The distance between the femoral and post-tibial recording sites was assessed over 129 the surface of the body, and PWV was calculated as the distance divided by the transit time. Simultaneously, SBP, MBP, and diastolic blood pressures (DBP) were measured in the 130 131 brachial and post-tibial arteries using oscillometry (BP-203RPE II; Fukuda Colin). HR was 132 calculated using electrocardiography (BP-203RPE II; Fukuda Colin). 133 134 Statistical Analysis 135 Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) for 136 Windows 27 (IBM, Armonk, NY, USA). Values are presented as means ± SDs. Normal 137 distribution of variables was evaluated with the Shapiro-Wilk test. The unpaired t test was used for comparisons between the 2 groups. Relationships between 2 variables were 138 139 investigated using Pearson's correlation coefficient. P-values < 0.05 were considered statistically significant. 140

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142 **Results**

All variables were distributed normally. There were no significant intergroup differences in age, height, or body weight (Table 1). Compared to their untrained counterparts, triathletes demonstrated significantly lower body fat (Table 1) and HR (Table 2) and significantly greater lower-leg BF based on venous occlusion plethysmography (Figure 1) and maximal oxygen uptake (Figure 2). There were no detectable intergroup differences in blood pressure and femoral-ankle PWV (Table 2). Compared to untrained individuals, triathletes had significantly 149 greater lower-leg VC (Figure 3).

150

Maximal oxygen uptake was correlated with body fat, HR, and lower-leg BF and
VC based on venous occlusion plethysmography (Table 3).

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154 **Discussion**

This study measured lower-leg VC based on venous occlusion plethysmography, maximal
oxygen uptake, and other indices in male triathletes and age-matched untrained men.
Compared to their untrained counterparts, triathletes demonstrated significantly lower body

158 fat and HR, and significantly greater lower-leg VC and maximal oxygen uptake. Additionally,

159 lower-leg VC was correlated with maximal oxygen uptake. The results demonstrate that

160 lower-leg VC is greater in triathletes than in untrained individuals, and it may be associated

161 with higher aerobic capacity in triathletes relative to untrained peers.

162

163 Lower-leg VC based on venous occlusion plethysmography was higher in triathletes versus untrained men in this study. This result is inconsistent with previous studies 164 investigating systemic and pulmonary VR (the inverse of VC) in triathletes. Lakin et al.¹⁰⁾ 165 reported that there was no difference in systemic VR between triathletes and sedentary 166 individuals. However, it is possible that there are differences between systemic and peripheral 167 168 vascular adaptations to exercise, since systemic VR transiently decreased after swimming while forearm VR increased¹²). Domenech-Ximenos et al.¹¹ demonstrated that pulmonary VR 169 170 was higher in triathletes compared to sedentary peers. Again, the discrepancy between the study of Domenech-Ximenos et al.¹¹⁾ and our own is not surprising because there are 171 172 significant differences between the systemic and pulmonary circulations (e.g., blood pressure and roles in oxygen delivery). The inconsistencies between this and previous studies^{10,11} do 173 174not cast doubt on the conclusions of this study.

Page 8

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176 It is apparent that the skeletal muscles of the lower leg play an important role in 177 the bike and run portions of triathlon. In particular, the soleus is the largest muscle of the triceps surae¹⁵⁾ and contributes to upward and forward center-of-mass acceleration during 178 running more than other skeletal muscles¹⁶. Regarding biking, electromyography showed that 179 the activity of the soleus increased with increasing workload¹⁷). We measured VC in the lower 180 181 leg because previous studies showed that the peak value of lower-leg VC after calf exercise was correlated with maximal oxygen uptake during running^{4,6)} and pedaling exercise⁵⁾. This 182 183 was a cross-sectional study, but it is possible that the correlation between lower-leg VC based 184 on venous occlusion plethysmography and maximal oxygen uptake implies that high VC in 185 triathletes contributes to their significant aerobic capacity.

186

In swimmers¹⁸, cyclists^{18,19}, runners^{4,6}, and endurance athletes (sport event was 187 not described)⁵⁾, there were no significant differences in resting lower-leg VC or VR between 188 athletes and sedentary individuals. On the other hand, Hepple et al.⁴⁾ demonstrated that lower-189 190 leg VC at rest was greater in body builders versus sedentary individuals, runners, and power 191 athletes, and they speculated that high-repetition and high-intensity resistance exercise 192 training in body builders may be implicated in vascular adaptations. High-repetition and 193 intensive resistance exercise is also required in the triathlon during swimming and also during 194 biking, especially on ascending slopes, just after sharp corners, and while increasing speed⁸). 195 However, not only triathletes but also swimmers swim, and cyclists also do biking. The point 196 makes triathlon unique is conjunction of these exercises and running. Eccentric muscle 197 contractions of lower leg are involved in running whereas concentric contractions are dominant in cycling and swimming²⁰. Kano et al.²¹ reported that eccentric gastrocnemius 198 muscle contractions increased capillary luminal area in the muscles. Combination of high-199 200 repetition and intensive resistance exercise during swimming and biking and eccentric muscle

201 contractions during running may be associated with greater VC in triathletes.

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| 203 | Resting HR was lower in triathletes versus sedentary men. Generally, endurance- |
|-----|---|
| 204 | trained athletes with lower resting HR have eccentrically remodeled left ventricle and higher |
| 205 | stroke volume ²²⁾ . Additionally, previous studies demonstrated eccentric remodeling of left |
| 206 | ventricle in triathletes ^{11,23,24}). We can speculate that triathletes in this study also had |
| 207 | eccentrically remodeled heart and higher stroke volume. Greater stroke volume increases |
| 208 | maximal oxygen uptake in endurance-trained athletes ²⁾ . It is not surprising that resting HR |
| 209 | was correlated with maximal oxygen uptake in this study. |
| 210 | |
| 211 | According to the two-element Windkessel model, VC and arterial stiffness are the |
| 212 | major vessel factors affecting hemodynamics. VC is mainly determined by internal diameter |
| 213 | of peripheral arteries, and higher VC indicates superior blood transport capability. Arterial |
| 214 | stiffness is an inverse of distensibility of arterial wall and associated with ability to buffer |
| 215 | fluctuations of blood pressure and flow. Therefore, we measured not only lower-leg VC but |
| 216 | also femoral-ankle PWV, an index of leg arterial stiffness. However, there was no detectable |
| 217 | difference in femoral-ankle PWV between triathletes and sedentary counterparts. Previous |
| 218 | studies on femoral arterial stiffness in runners and swimmers ²⁵⁾ and cyclists ²⁶⁾ reported |
| 219 | conflicting results. The result of this study is consistent with the study of Nualnim <i>et al.</i> ²⁵⁾ that |
| 220 | there were no significant differences in femoral arterial stiffness among runners, swimmers, |
| 221 | and sedentary peers. Leg artery is a muscular artery with less compliant walls; contribution to |
| 222 | blood flow and pressure is greater in VC versus arterial stiffness in peripheral muscular |
| 223 | arteries. Adaptation of leg arterial stiffness may be non-essential for triathletes. |
| 224 | |

This study demonstrated that triathletes have greater lower-leg VC based on venous occlusion plethysmography and that the higher VC is associated with higher aerobic

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| 227 | capacity. The lower-leg VC could be used as an index of conditioning of triathletes and to |
|-----|--|
| 228 | discover a talented triathlete. A decrease in VC with aging increases blood pressure. Again, |
| 229 | aerobic capacity is a predictor of cardiovascular events ²⁷⁾ . Investigating vascular adaptations |
| 230 | to triathlon could potentially demonstrate the beneficial effects of triathlon as a lifelong sport. |
| 231 | |
| 232 | This study has several limitations. First, this was a cross-sectional study with a |
| 233 | small sample size. Second, we did not investigate the mechanisms underlying the greater VC |
| 234 | in triathletes. Third, we demonstrated that VC is correlated with maximal oxygen uptake but |
| 235 | did not conduct multivariate analysis due to small sample size. It is difficult to ascertain |
| 236 | whether the correlation between VC and maximal oxygen uptake suggests a causal |
| 237 | relationship. Further studies are warranted to establish the effects of triathlon on vascular |
| 238 | function. |
| 239 | |
| 240 | Conclusions |

Triathletes have higher VC of the lower leg compared to untrained individuals, and it may beassociated with higher aerobic capacity relative to untrained counterparts.

Conflict of interests

None.

Author contributions

HN and TO conceived, designed, and performed the experiments; HN analyzed data, prepared figures, and drafted the manuscript; HT, SM, and TO revised the manuscript; and all authors approved the final version of the manuscript.

Funding

This research was supported by JSPS KAKENHI (23K10638) and a grant from Advanced Research Initiative for Human High Performance (ARIHHP), University of Tsukuba.

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| | Triathletes | Untrained | P value |
|-------------|--|--|---------|
| п | 10 | 8 | |
| Age, years | $20.7 \hspace{0.2cm} \pm \hspace{0.2cm} 1.3$ | $21.5 \hspace{0.2cm} \pm \hspace{0.2cm} 1.1$ | 0.17 |
| Height, cm | 169 ± 6 | 171 ± 5 | 0.49 |
| Weight, kg | $63.3 \hspace{0.2cm} \pm \hspace{0.2cm} 7.6$ | $64.2 \hspace{0.2cm} \pm \hspace{0.2cm} 8.9$ | 0.82 |
| Body fat, % | 11.4 ± 2.1 | 16.2 ± 6.0 | 0.03 |

 Table 1. Characteristics of participants.

Values are means \pm SDs.

| | | Triathletes | Untrained | P value |
|---------------|--------------|---|----------------|---------|
| Brachial | SBP, mmHg | 115 ± 8 | 117 ± 9 | 0.74 |
| | MBP, mmHg | 83 ± 6 | 86 ± 8 | 0.30 |
| | DBP, mmHg | 62 ± 6 | 65 ± 7 | 0.49 |
| Post-tibial | SBP, mmHg | 133 ± 10 | 131 ± 12 | 0.74 |
| | MBP, mmHg | 87 ± 6 | 86 ± 9 | 0.77 |
| | DBP, mmHg | 66 ± 6 | $67 \ \pm \ 8$ | 0.91 |
| Heart rate, b | pm | 54 ± 7 | 65 ± 9 | 0.01 |
| Femoral-ank | tle PWV, m/s | $7.9 \hspace{0.2cm} \pm \hspace{0.2cm} 0.9$ | 7.6 ± 0.7 | 0.47 |

Table 2. Blood pressure, heart rate, and arterial stiffness.

Values are means \pm SDs. SBP, systolic blood pressure; MBP, mean blood pressure; DBP, diastolic blood pressure; and PWV, pulse wave velocity.

| | Maximal oxygen uptake | |
|--------------|-----------------------|---------|
| | r | P value |
| Body fat | - 0.53 | 0.02 |
| Heart rate | - 0.56 | 0.01 |
| Lower-leg BF | 0.70 | < 0.01 |
| Lower-leg VC | 0.69 | < 0.01 |

 Table 3. Correlation with maximal oxygen uptake.

BF, blood flow and VC, vascular conductance.

Figure Legends

Figure 1. Lower-leg blood flow (BF) in male triathletes and untrained men. Values are means \pm SDs.

Figure 2. Aerobic capacity in male triathletes and untrained men. Values are means \pm SDs.

Figure 3. Lower-leg vascular conductance (VC) in male triathletes and untrained men. Values are means \pm SDs.

Figure 1 (Namatame et al.)



Figure 2 (Namatame et al.)



Figure 3 (Namatame et al.)

