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Changes in arterial stiffness after eccentric versus concentric cycling

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Short title: Arterial stiffness changes after cycling

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ABSTRACT

This study compared changes in brachial-ankle pulse wave velocity (baPWV) after concentric (CON) versus eccentric cycling (ECC). It was hypothesized that baPWV would increase after the first ECC bout (ECC1) because of muscle damage, but not after the second ECC bout (ECC2), and would decrease after CON. Fifteen young (20-30 y) men performed two bouts of 30-min ECC (ECC1 and ECC2) at 60% of maximal CON power output and two bouts of 30-min CON at the same intensity as that of ECC (CON1), and at the same oxygen consumption as that of ECC (CON2) every two weeks. Oxygen uptake during the cycling was recorded, and baPWV was measured before and 0.5, 1, 24 and 48 h after each cycling. Maximal voluntary contraction (MVC) torque and muscle soreness of the knee extensors were assessed before, 24 and 48 h after each cycling. Changes in these variables over time were compared among the four cycling bouts by two-way repeated measured ANOVA. baPWV decreased ($P<0.05$) 8% from the baseline (1119 ± 116 cm/s) at 0.5 h after CON1 (1028 ± 126 cm/s), but no significant changes were evident after ECC1, ECC2 and CON2. MVC torque decreased 10% from the baseline at 24 h after ECC1, but no significant changes were evident after CON1, CON2 and ECC2. These results did not support the hypothesis, and suggest that minor muscle damage induced by eccentric cycling does not affect arterial stiffness.

Key words: pulse wave velocity, muscle damage, muscle soreness, repeated bout effect, aerobic exercise, muscle contraction mode

INTRODUCTION

Arterial stiffness can be assessed by pulse wave velocity (PWV) (Sugawara et al. 2005), and it has been reported that PWV increases with ageing (Tomiyama et al. 2003). Arterial stiffness is also changed after an acute exercise bout (Heffernan et al. 2007; Kingwell et al. 1997; Kobayashi et al. 2017, 2018; Okamoto et al. 2018; Sugawara et al. 2005). Several studies reported that aerobic exercises such as conventional (concentric) cycling in which knee extensor muscles undergo active shortening or concentric contractions, decreased systemic arterial stiffness indicated by carotid-femoral (cf), femoral-ankle (fa) or brachial-ankle (ba) PWV. For example, Kobayashi et al. (2017) reported that a single bout 30-min of conventional cycling exercise at 65% of peak oxygen uptake (VO_2 peak) decreased cfPWV and faPWV by 7% and 4% at 30 min post-exercise, respectively. Similarly, Kobayashi et al. (2018) recently reported that two sets of 15-min of conventional cycling at 65% of VO_2 peak with a 20-min rest between sets decreased cfPWV and faPWV at 20 min post-exercise by 13% and 11%, respectively.

On the other hand, some studies reported increases in arterial stiffness after exercise. Burr et al. (2015) reported 10% increase in cfPWV at 48 h after 45-min downhill running performed by healthy young adults. They showed an association between the magnitude of increase in cfPWV and the magnitude of delayed-onset muscle soreness (DOMS). Similarly, Lin et al. (2017) showed 17% increase in cfPWV at 48 h after 30-min downhill running in healthy

young adults. It appears that muscle damage induced by eccentrically-biased aerobic exercise increases arterial stiffness. However, no previous study has systematically compared the acute effect of muscle contractions (i.e., concentric versus eccentric contractions) performed in exercise on changes in arterial stiffness.

In eccentric cycling, the knee extensor muscles undergo active lengthening when resisting to the backward crank movements, and eccentric cycling requires less oxygen than concentric cycling for the same workload (Bigland-Ritchie and Woods 1976; Knuttgen et al. 1982; Penailillo et al. 2013; Perrey et al. 2001). Penailillo et al. (2013) showed that eccentric cycling required 50% less oxygen than concentric cycling for the same workload (approximately 160 W), and only eccentric cycling resulted in decreases in knee extension strength lasting for three days post-exercise and development of DOMS. However, the second bout of the same eccentric cycling performed two weeks later did not induce such symptoms of muscle damage. If muscle damage causes an increase in arterial stiffness as shown in the previous studies (Burr et al. 2015; Lin et al. 2017), it seems reasonable to assume that arterial stiffness would increase greater after the first than the second bout of eccentric cycling. If eccentric cycling increases arterial stiffness, caution is necessary when it is applied for elderly and clinical populations. Thus, it is important to compare between eccentric and concentric cycling, and between the first and second bouts of eccentric cycling for changes in arterial stiffness in relation to muscle

76 damage.

77 The present study therefore compared eccentric and concentric cycling matched for
78 external workload as well as matched for oxygen consumption during exercise to investigate
79 changes in baPWV, and changes in baPWV between the first and second eccentric cycling bouts.
80 It was hypothesized that the first bout of eccentric cycling would increase baPWV, but this would
81 be attenuated after the second bout, and baPWV would decrease after concentric cycling,
82 especially when its intensity was greater than that in ECC cycling.

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METHODS

Participants

The sample size was calculated by an estimated effect size of 0.25 for a potential difference in the baPWV changes over time (before, and 0.5, 1, 24 and 48 h after cycling) among the four conditions (CON1, CON2, ECC1, ECC2), with $\alpha = 0.05$ and $\beta = 0.8$ using G*power (version 3.1.4, Heinrich-Heine University, Dusseldorf, Germany). It was found that at least 12 participants were necessary, thus considering a possible estimation error, 15 participants were recruited. All participants had normal blood pressure (BP) ($<120/80$ mmHg) and no signs, symptoms, or history of overt chronic diseases. They were non-smoking, non-obese men, and none of them were engaging in any form of aerobic and/or resistance exercise training in the past one year, and none had previously performed eccentric cycling. Body composition was determined using a bioelectrical impedance analysis device (InBody, Biospace Co., Ltd., Seoul, Korea). Their mean $\pm$ standard deviation (SD) age, height, body mass and percent body fat were 22.7 ± 2.8 y, 170.9 ± 5.6 cm, 63.6 ± 6.4 kg, and $15.8 \pm 4.3\%$, respectively. This study did not include female participants to eliminate possible effects of the menstrual cycle on changes in arterial stiffness after acute exercise (Okamoto et al. 2017). Participants were instructed not to perform any exercises, apply any treatments (e.g., massage or stretching) or intake any medicine during the experimental periods. They were also instructed to fast and not to intake fluid except

water for 10 - 12 h before being tested. All potential risks were explained in detail to the participants, and a written informed consent was obtained from each participant before the experiment began. The present study was approved by the Nippon Sport Science University Ethics Committee.

Study design

To avoid possible effects of diurnal variation on the measures, all measurements were taken at the same time of day (08:00 - 11:00 h) without breakfast after overnight fasting to eliminate potential effects of food intake on the baPWV and other measures. In the first session, peak oxygen uptake ($\dot{V}O_2$ peak) and maximal concentric cycling power output were determined. The second to fifth sessions consisted of three days, and for each day, after a 20-min rest period, baPWV, brachial BP, and heart rate (HR) were simultaneously measured, then visual analogue scale (VAS) scores for muscle soreness and maximum voluntary isometric contraction (MVC) torque of the knee extensors were assessed as indirect markers of muscle damage. On the first day of each session, the participants performed either 30-min concentric (CON) cycling or 30-min eccentric (ECC) cycling. They performed CON cycling (CON1) in the second session followed by two bouts of ECC cycling (ECC1, ECC2) in the third and fourth sessions, and the second CON cycling (CON2) in the last session. This order was chosen because it was

considered that the carry-over effects of CON on ECC cycling would be minimal, but the first bout of ECC cycling would confer a protective effect against muscle damage in the second ECC cycling bout due to the repeated bout effect (Clarkson and Hubal 2002; McHugh 2003; Nosaka and Clarkson 1995; Penailillo et al. 2013). CON2 was included to examine the effects of CON cycling on baPWV, in which the oxygen consumption was matched with that of ECC cycling, because it was possible that changes in baPWV were more affected by exercise intensity than exercise mode.

Oxygen uptake (VO_2) and heart rate (HR) were recorded during the 30-min cycling and baPWV, systolic and diastolic blood pressure (BP) and HR were measured before, and at 0.5, 1, 24, and 48 h after each cycling. The time points of the measurements were based on several studies (Burr et al. 2015; Kingwell et al. 1997; Kobayashi et al. 2017; Lin et al. 2017) that reported acute changes in arterial stiffness after exercise. Muscle soreness and MVC torque were also measured before and 0.5, 24 and 48 h after each cycling. All measurements were recorded in a quiet room at a constant temperature (23–25°C). Participants were instructed not to perform any exercises, apply any treatments (e.g., massage or stretching), nor intake any medicines during the experimental period, and were asked not to change their diet.

Cycling exercises

137 Peak oxygen uptake (VO_2 peak) and maximal CON power output were determined one
138 week before CON1 using a recumbent ergometer (StrengthErgo 240, Mitsubishi Electric
139 Engineering Co, Ltd, Nagoya, Japan). This test consisted of incremental cycling starting at 30 W
140 for two min followed by a 20-W increment every minute until volitional exhaustion. HR during
141 cycling was monitored using an electrocardiograph (Life Scope BSM-2400; Nihon Kohden, Co.,
142 Ltd., Tokyo, Japan). VO_2 was continuously measured using an open-circuit spirometry and
143 analyzed using a metabolic measurement cart (AE-100i; Minato Medical Science CO., Ltd.,
144 Kyoto, Japan). Average HR and VO_2 during the last 20-min cycling were calculated for the four
145 cycling sessions. CON1, ECC1, and ECC2 were performed at 60% of maximal CON power
146 output (PO_{max}) based on the graded exercise test. Penailillo et al. (2013) reported that 60% of
147 the concentric PO_{max} was close to the highest concentric power output that could be maintained
148 for 30 min by healthy young men. As the feedback to the participants, a line indicating the 60%
149 PO_{max} was shown on the ergometer screen, and the participants were instructed to maintain the
150 target power output for 30 min. In the bout of ECC cycling, the participants were instructed to
151 resist the backward pedaling movement without pulling upwards against the pedal. Their waist
152 was stabilized by a seat belt to minimize the movement of the sitting position. In addition,
153 participants were instructed not to grip the handles of the cycling machine during CON and ECC
154 cycling. Immediately before the first bout of ECC cycling, the participants were familiarized with

ECC cycling, which consisted of five min of cycling at ~50 W. Participants achieved the targeted work and torque production during ECC cycling. All participants performed CON2 for 30 min at the same intensity (based on VO_2) as that in the ECC2 trial in the last session. All cycling was performed on the same ergometer as that was used for the VO_2 peak test at 60 rpm for 30 min. Each participant performed the four cycling exercises at the same time of day to minimize possible diurnal changes in the dependent variables.

Resting HR and BP

Resting values of HR and systolic and diastolic BP were simultaneously measured using electrocardiography and an automatic oscillometric device (Form PWV/ABI, Omron-Colin Co. Ltd.). Data were recorded in triplicate from participants while in a supine position. The pressure signal obtained by plethysmography was calibrated by equating systolic and diastolic BP values, and then used to calculate mean arterial pressure.

baPWV

baPWV was measured using an automatic volume-plethymographic device (Form PWV/ABI, Omron-Colin Co., Ltd., Komaki, Japan) that records PWV, BP, electrocardiogram, and heart sounds simultaneously (Suzuki et al. 2001). The measurements were taken after

participants rested in a supine position for a minimum of 20 min, according to the procedure described in a previous study (Okamoto et al. 2017). Electrocardiogram electrodes placed on both wrists, a microphone for detecting heart sounds placed on the left edge of the sternum, and cuffs wrapped on both the brachia and ankles. The cuffs were connected to a plethymographic sensor that determines volume pulse form and an oscillometric pressure sensor that measures BP. The pulse volume waveforms were recorded using a semiconductor pressure sensor. Volume waveforms for the brachium and ankle were stored, and the sampling time was 10 s with automatic gain analysis and quality adjustment (Sugawara et al. 2005).

The path length from the suprasternal notch to the measuring point in the brachial region (L_b) was calculated using the following equation: $L_b = 0.2195 \times \text{height of the participant (cm)} - 2.0734$ (Yamashina et al. 2002). The path length from the suprasternal notch to the ankle (L_a) was obtained from body surface measurements using the equation: $L_a = 0.8129 \times \text{height of the participant (cm)} + 12.328$ (Yamashina et al. 2002). The distance between the two baPWV recording sites was calculated based on the height of the participant and anthropomorphic data for the Japanese population using the following formula.

$\text{baPWV} = (L_a - L_b) / T_{ba}$ (Yamashina et al. 2002).

In the present study, the coefficient of variation for the test-retest reliability of baPWV measured at a two-week interval was $3 \pm 1\%$.

191

192 **MVC torque of the knee extensors**

193 MVC torque of the knee extensors was measured using an isokinetic dynamometer

194 (Biodex System 3, Biodex Medical Systems Inc., Shirley, NY, USA) after other measurements.

195 While seated on the isokinetic dynamometer, the trunk, waist, and thigh of each participant were

196 fixed with a seatbelt. MVC peak torque of the knee extensors was measured for the dominant leg

197 (i.e., the kicking leg) at 70° of knee flexion. Before testing, warm-up was performed with three

198 submaximal contractions at 50%, 50% and 80% of self-perceived maximum intensity for three s.

199 Participants were then instructed to perform maximal isometric knee extension at a fixed knee

200 joint angle of 70°, with full anatomical extension of the knee regarded as 0°. MVC was measured

201 twice with a one-min rest between trials, and the higher value was used for further analysis.

202

203 **Muscle soreness**

204 Muscle soreness was quantified by a 0–100-mm visual analog scale VAS with 0

205 indicating no pain and 100 indicating the worst pain imaginable. The participants were asked to

206 rate the level of perceived pain of the quadriceps femoris muscles on the VAS, while completely

207 squatting.

208

209 **Statistical analysis**

210 Statistical analyses were performed using a software (SPSS ver. 24; Chicago, IL, USA).
211 One-way analysis of variance (ANOVA) was used to compare the four cycling bouts for mean
212 cycling power output, VO_2 , and HR during cycling. Two-way repeated-measures ANOVA
213 (condition $\times$ time) was used to compare changes in baPWV, HR, BP, MVC and VAS scores over
214 time among four conditions. When a significant interaction effect was found, a Bonferroni–Dunn
215 post hoc test was used to identify significant differences between mean values for each time point
216 and trial. Test-retest reliability was confirmed by performing the same test on the same
217 participants on two separate occasions. As a measure of effect size, eta-squared [η^2] was used for
218 one-way ANOVA, and its value was interpreted as 0.01: small, 0.06: medium and 0.14: large
219 (Cohen 1988). Relative effect size for performance data was calculated using Cohen's d and were
220 defined as small ($d = 0.2$), medium ($d = 0.5$), or large ($d = 0.8$) (Cohen 1988). Statistical
221 significance was set at $P < 0.05$. The results are shown in mean $\pm$ standard deviation (SD), and
222 95% confidence intervals (CI) are also provided.

RESULTS

HR, BP and VO₂

Pre-exercise HR and BP were similar among the four cycling sessions (Table 1). BP did not change significantly after exercise from the baseline for any of the cycling bouts. HR was higher than the baseline at 0.5 h ($P = 0.005$, $d = 1.29$, CI = 66 – 80 bpm) and one h after CON1 ($P = 0.018$, $d = 0.78$, CI = 60 – 74 bpm), but this was not the case for CON2, ECC1 and ECC2.

Figure 1 shows average HR and VO₂ during the last 20-min exercise for the four cycling bouts. HR (CIs: CON1: 162 – 180 bpm; CON2: 91 – 101 bpm; ECC1: 94 – 106 bpm; and ECC2: 88 – 102 bpm) and VO₂ (CIs: CON1: 34.0 – 38.4 mL/kg/min; CON2: 11.2 – 14.0 mL/kg/min; ECC1: 11.0 – 16.4 mL/kg/min; and ECC2: 9.6 – 15.0 mL/kg/min) were greater for CON1 ($P < 0.001$, HR: $\eta^2 = 0.86$, VO₂: $\eta^2 = 0.85$) than others, without a significant difference among CON2, ECC1 and ECC2. During ECC (ECC1 and ECC2), HR was 62-66% lower and VO₂ was 41-44% lower when compared with those during CON1.

baPWV

No significant ($P = 0.724$, $\eta^2 = 0.00$) difference in baPWV was evident at baseline among four exercise bouts (CON1: 1054 – 1184 cm/s, CON2: 1048 – 1176 cm/s, ECC1: 1040 – 1160 cm/s, ECC2: 1045 – 1183 cm/s). As shown in Figure 2, no significant changes were

observed after CON2 ($\eta^2 = 0.00$), ECC1 ($\eta^2 = 0.02$) and ECC2 ($\eta^2 = 0.06$). However, baPWV decreased by 8% at 0.5 h ($P = 0.02$, $d = 0.75$, $CI = 958 - 1098$ cm/s), returning to the baseline at 1 h after CON1 ($CI = 1017 - 1157$ cm/s).

MVC torque

As shown in Figure 3, MVC torque at baseline was not significantly different among four conditions (CON1: 163.8 – 204.2 Nm, CON2: 169.7 – 215.1 Nm, ECC1: 171.9 – 217.9 Nm, ECC2: 170.8 – 219.8 Nm). MVC torque decreased 13% from baseline at 24 h ($P = 0.019$, $d = 0.64$, $CI = 152.1 - 198.1$ Nm), but returned to the baseline at 48 h after ECC1. No significant changes in MVC torque were seen after CON1, CON2 and ECC2.

Muscle soreness

No significant muscle soreness was evident before exercise for any of the cycling bouts (Figure 4). VAS scores significantly increased at 24 h after ECC1 ($P < 0.001$, $d = 4.21$, $CI = 3.8 - 5.6$ mm) and ECC2 ($P < 0.001$, $d = 2.09$, $CI = 2.0 - 4.2$ mm), but still elevated at 48 h after ECC1 only ($P < 0.001$, $d = 2.33$, $CI = 3.4 - 5.8$ mm). VAS scores were significantly higher after ECC1 than ECC2 at 24 h ($P = 0.001$, $d = 0.83$) and 48 h ($P = 0.002$, $d = 0.93$). No significant changes in VAS were found after CON1 and CON2.

DISCUSSION

The key finding of this study was that neither the first nor second bout of ECC cycling significantly changed baPWV at any time points, but baPWV decreased at 0.5 h after the first CON cycling in which oxygen consumption was almost two-fold greater than that of other cycling bouts (Figure 2). These results did not support the hypothesis that the first but not second bout of ECC cycling would increase baPWV.

Some of the previous studies (Okamoto et al. 2014; Okamoto et al. 2017; Sugawara et al. 2005) used baPWV to investigate the acute effect of exercise on arterial stiffness, and baPWV reflects both central (aortic) and peripheral (brachial and leg) arterial stiffness (Sugawara et al. 2005). Changes in baPWV have been reported to be strongly associated with changes in cfPWV, suggesting that the arterial stiffness of central elastic arteries is a major determinant of baPWV (Sugawara et al. 2005). Sugawara et al. (2005) reported that after aerobic cycling, decreases in cfPWV were significantly associated with decreases in baPWV ($r=0.74$). It seems likely that baPWV provides qualitatively similar information to that derived from central arterial stiffness. Therefore, it is assumed that baPWV represented acute effect of exercise on arterial stiffness in a similar way to cfPWV or other parameters in relation to muscle damage induced by eccentric cycling.

Several studies reported that aerobic exercise acutely reduced arterial stiffness assessed

by PWV (Heffernan et al. 2007; Kingwell et al. 1997; Wang et al. 2014). For example, Kingwell et al. (1997) reported that cfPWV and faPWV were decreased by 4% and 10%, respectively, at 30 min after a single 30-min bout of aerobic cycling at 65% of $\dot{V}O_2$ peak. Sugawara et al. (2015) also reported that a 60-min bout of moderate intensity (65–75% HR reserve) aerobic cycling, decreased cfPWV by 6% at 20 min and 8% at 50 min post-exercise in healthy young men. In the present study, the exercise intensity was assumed to be approximately 70% of $\dot{V}O_2$ peak, thus the decreased baPWV by 8% at 0.5 h post-CON1 was in line with the finding of Kingwell et al. (1997) and Sugawara et al. (2015). No significant changes in arterial stiffness were observed at 30 min after CON2 in which $\dot{V}O_2$ was approximately 40% of that of CON1 (Fig. 1). Wang et al. (2014) reported that a 30-min bout of low-intensity (35% HR reserve) aerobic exercise acutely decreased cardio-ankle vascular index by 10%. However, the exercise intensity of CON2 was estimated to be 27% HR reserve, thus it may be that the exercise intensity in CON2 was too low to affect arterial stiffness.

Several studies reported that eccentrically-biased aerobic exercises acutely increased arterial stiffness. Burr et al. (2015) reported 10% increase in cfPWV at 48 h after 40-min downhill running at 60% of $\dot{V}O_2$ max. Lin et al. (2017) also showed that cfPWV increased by 17% at 48 h after 30-min downhill running at 70% of $\dot{V}O_2$ max. The present study was the first to examine changes in a marker of arterial stiffness after eccentric cycling. As shown in Figure 2, no

changes in baPWV were observed after the first and second bouts of ECC cycling. It is important to note that metabolic demand was approximately 40% less for ECC1 than CON1 cycling as indicated by $\dot{V}O_2$ (Figure 1). It is possible that lower metabolic demand of ECC cycling led to the lack of changes in baPWV after the exercise.

Unaccustomed eccentric exercise has been shown to induce muscle damage that is characterized by a prolonged loss of muscle function and DOMS (Barnes et al. 2010; Burr et al. 2015; Lin et al. 2017; Penailillo et al. 2015; Penailillo et al. 2013). In the present study, MVC torque decreased 11% at 24 h after ECC1 (Figure 3), and VAS scores for DOMS increased at 24 and 48 h after ECC1 (Figure 4); however, MVC torque and VAS scores for DOMS did not increase at 24 and 48 h after CON1. As expected, changes in the MVC and VAS scores at 24 and 48 h after ECC2 were minimal (Figures 3 & 4), suggesting that ECC2 did not induce muscle damage due to the repeated bout effect (Penailillo et al. 2015; Penailillo et al. 2013). After ECC2, no significant changes in baPWV were evident at any time point, similarly to ECC1. These suggest that the muscle damage induced ECC1 did not affect the changes in baPWV. As described above, previous studies have shown that arterial stiffness increased after eccentric exercise that induced muscle damage (Barnes et al. 2010; Burr et al. 2015; Choi et al. 2016; Lin et al. 2017). However, no significant changes in baPWV were evident at any time point after ECC1. This might be related to the magnitude of muscle damage such that the decrease in MVC

torque was smaller in the present study (13%) when compared with the study by Barnes et al. (2010) who reported 25% decrease in muscle strength and 20% increase in cfPWV at 48 h after eccentric exercise of the elbow flexors. Burr et al. (2015) found 10% increase in cfPWV at 48 h after downhill running that induced severe DOMS. Transient acute increases in arterial stiffness after eccentric exercise is probably mediated by acute inflammation responses (Lin et al. 2017). No increases in baPWV after ECC cycling in the present study were probably due to little or minor inflammation induced by the ECC cycling. It may be that arterial stiffness is unaffected when the magnitude of muscle damage induced by ECC exercise is minor.

According to the American College of Sports Medicine (ACSM) guideline, healthy adults need to perform aerobic exercise at a moderate intensity to maintain and increase health (Chodzko-Zajko et al. 2009). In ECC1 and ECC2, VO_2 was much lower than that recommended by the ACSM. ECC cycling exercise can be performed with a lower oxygen cost than CON cycling exercise for the same workload (Knuttgen et al. 1982; Penailillo et al. 2013; Perrey et al. 2001), and the present study confirmed it. We observed that ECC cycling induced minor muscle damage but did not increase arterial stiffness. In the present study, participants were healthy young men, which precludes generalizing our findings to middle-aged, elderly or female individuals. It should be noted that all participants completed the four trials (CON1, ECC1, ECC2 and CON2) in the same order with separated by two weeks between sessions. However, to

minimize possible effects of eccentric cycling on concentric cycling, this order was important (Penailillo et al. 2013). In addition, we believe that there was no residual effect by performing each trial at two week intervals from the baseline value on each trials (e.g baPWV, MVC or VAS).

In conclusion, the first bout of CON cycling reduced arterial stiffness, but the first bout of ECC cycling induced moderate muscle damage, and did not affect arterial stiffness. In addition, the second bout of ECC cycling attenuated muscle damage but did not affect arterial stiffness. These results suggest that ECC cycling does not produce negative effect on arterial stiffness. In future studies, the effects of ECC cycling on arterial stiffness in regard to exercise intensity and duration need to be investigated.

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349

350 **Conflict of interest**

351 The authors have no conflict of interest to declare.

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432

Table 1. Changes (mean $\pm$ SD, n=15) in heart rate (HR), systolic (SBP) and diastolic blood pressure (DBP) before, and 0.5, 1, 24 and 48 hours after the first and second bouts of concentric cycling (CON1, CON2) and eccentric cycling (ECC1, ECC2).

	Before	0.5h after	1h after	24h after	48h after
HR (beats/min)					
CON1	58 $\pm$ 10	73 $\pm$ 13*, †	67 $\pm$ 13*, †	57 $\pm$ 8	57 $\pm$ 7
CON2	59 $\pm$ 9	58 $\pm$ 10	57 $\pm$ 10	60 $\pm$ 10	60 $\pm$ 10
ECC1	57 $\pm$ 10	56 $\pm$ 8	55 $\pm$ 8	59 $\pm$ 12	58 $\pm$ 8
ECC2	57 $\pm$ 7	56 $\pm$ 8	56 $\pm$ 7	58 $\pm$ 9	59 $\pm$ 9
SBP (mmHg)					
CON1	109 $\pm$ 5	106 $\pm$ 6	107 $\pm$ 6	109 $\pm$ 6	109 $\pm$ 8
CON2	110 $\pm$ 9	109 $\pm$ 7	109 $\pm$ 7	112 $\pm$ 9	111 $\pm$ 6
ECC1	110 $\pm$ 7	110 $\pm$ 7	110 $\pm$ 9	109 $\pm$ 7	111 $\pm$ 6
ECC2	110 $\pm$ 9	109 $\pm$ 8	109 $\pm$ 8	111 $\pm$ 8	110 $\pm$ 7
DBP (mmHg)					
CON1	62 $\pm$ 5	62 $\pm$ 7	63 $\pm$ 6	60 $\pm$ 7	63 $\pm$ 6
CON2	63 $\pm$ 6	61 $\pm$ 7	63 $\pm$ 7	64 $\pm$ 8	64 $\pm$ 6
ECC1	64 $\pm$ 6	63 $\pm$ 6	64 $\pm$ 9	63 $\pm$ 7	63 $\pm$ 6
ECC2	63 $\pm$ 8	64 $\pm$ 6	64 $\pm$ 9	64 $\pm$ 8	62 $\pm$ 6

*: significantly (P<0.05) different from the baseline value (Before). †: significantly (P<0.05) from others.

Figure captions

Figure 1. Comparison of average (mean $\pm$ SD, n=15) oxygen uptake during 30-min exercise among the first concentric (CON1) and second concentric cycling (CON2), and the first eccentric (ECC1) and second eccentric (ECC2) cycling bouts. *: significantly ($P<0.01$) different from CON1.

Figure 2. Changes (mean $\pm$ SD, n=15) in brachial-ankle pulse wave velocity (baPWV) before and 0.5, 1, 24 and 48 hours after the first concentric (CON1: ●) and second concentric cycling (CON2: ○), and the first eccentric (ECC1: ■) and second eccentric (ECC2: □) cycling bouts. *: significantly ($P<0.05$) different from others; †: significantly ($P<0.05$) vs. pre-exercise value.

Figure 3. Changes (mean $\pm$ SD, n=15) in maximum voluntary isometric contraction (MVC) torque of the knee extensors before and 24 and 48 hours after the first concentric (CON1: ●), second concentric cycling (CON2: ○), and the first eccentric (ECC1: ■) and second eccentric (ECC2: □) cycling bouts. *: significantly ($P<0.05$) different from others. †: significantly ($P<0.05$) different from the pre-exercise value.

Figure 4. Changes (mean $\pm$ SD, n=15) in visual analog scale (VAS) of muscle soreness before

456 and 24 and 48 hours after the first concentric (CON1: ●) and second concentric cycling (CON2:
457 ○), and the first eccentric (ECC1: ■) and second eccentric (ECC2: □) cycling bouts. *:
458 significantly ($P<0.01$) different from CON1 and CON2, #: significantly ($P<0.05$) different from
459 ECC1, †: significantly ($P<0.01$) different from the pre-exercise value.

Draft

Figure 1

Figure 1. Comparison of average (mean $\pm$ SD, $n=15$) oxygen uptake during 30-min exercise among the first concentric (CON1) and second concentric cycling (CON2), and the first eccentric (ECC1) and second eccentric (ECC2) cycling bouts. *: significantly ($P<0.01$) different from CON1.

95x127mm (300 x 300 DPI)

Figure 2

Figure 2. Changes (mean $\pm$ SD, n=15) in brachial-ankle pulse wave velocity (baPWV) before and 0.5, 1, 24 and 48 hours after the first concentric (CON1: ●) and second concentric cycling (CON2: ○), and the first eccentric (ECC1: ■) and second eccentric (ECC2: □) cycling bouts. *: significantly ($P<0.05$) different from others; †: significantly ($P<0.05$) vs. pre-exercise value.

95x127mm (300 x 300 DPI)

Figure 3

Figure 3. Changes (mean $\pm$ SD, $n=15$) in maximum voluntary isometric contraction (MVC) torque of the knee extensors before and 24 and 48 hours after the first concentric (CON1: ●), second concentric cycling (CON2: ○), and the first eccentric (ECC1: ■) and second eccentric (ECC2: □) cycling bouts. *: significantly ($P<0.05$) different from others. †: significantly ($P<0.05$) different from the pre-exercise value.

95x127mm (300 x 300 DPI)

Figure 4

Figure 4. Changes (mean $\pm$ SD, $n=15$) in visual analog scale (VAS) of muscle soreness before and 24 and 48 hours after the first concentric (CON1: ●) and second concentric cycling (CON2: ○), and the first eccentric (ECC1: ■) and second eccentric (ECC2: □) cycling bouts. *: significantly ($P<0.01$) different from CON1 and CON2, #: significantly ($P<0.05$) different from ECC1, †: significantly ($P<0.01$) different from the pre-exercise value.

95x127mm (300 x 300 DPI)