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ORIGINAL ARTICLE

Changes in oxidative stress, inflammation and muscle damage markers following eccentric versus concentric cycling in older adults

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Abstract

Purpose To compare concentric and eccentric cycling performed by older adults for metabolic demand and post-exercise oxidative stress, inflammation and muscle damage.

Methods Eight male and two female healthy older adults (60.4 ± 6.8 years) performed 30 min of moderate-intensity concentric (CONC-M: 50% maximum power output; PO_{max}) and eccentric cycling (ECC-M: 50% PO_{max}) and high-intensity eccentric cycling (ECC-H: 100% PO_{max}) in a randomized order. Average power output (PO), oxygen consumption (VO_2), heart rate (HR) and rate of perceived exertion were recorded during cycling. Some indirect markers of muscle damage were assessed before, and immediately, 24 and 48 h after cycling. Markers of oxidative stress (malondialdehyde: MDA, protein carbonyl), antioxidant (total antioxidant capacity, glutathione peroxidase activity: GPx) and inflammation (IL-6, TNF-α) were measured before and 5 min after cycling.

Results PO in ECC-H (202.6 ± 78.5 W) was > 50% greater ($P < 0.05$) than that of CONC-M (98.6 ± 33.1 W) and ECC-M (112.0 ± 42.1 W). VO_2 and HR were also greater ($P < 0.05$) for ECC-H than CONC-M (50% and 17%, respectively) and ECC-M (40% and 23%, respectively). Muscle strength loss at 1 day post-exercise (8–22%), peak soreness (10–62 mm) and creatine kinase activity (30–250 IU/L) after ECC-H were greater ($P < 0.05$) than those after ECC-M and CONC-M. MDA decreased ($P < 0.05$) after CONC-M (− 28%) and ECC-M (− 22%), but not after ECC-H. GPx activity increased after all exercises similarly (20–27%). IL-6 increased ($P < 0.05$) only after ECC-H (18%).

Conclusion Oxidative stress was minimal after eccentric cycling, but high-intensity eccentric cycling induced moderate muscle damage and inflammation, which is not desirable for older individuals.

Keywords Lengthening muscle action · Muscle soreness · Creatine kinase · TNF alpha · TBARS · Total antioxidant capacity · Interleukin 6

Introduction

A good balance between reactive oxygen species and antioxidants is necessary to maintain physiological processes (Powers et al. 2016), but the balance is impaired with ageing (Powers et al. 2011). Oxidant substances interact with various macromolecules of the body, and cause damage to lipids and structural proteins, inducing damage to deoxyribonucleic acid (Powers et al. 2011, 2016). It has been shown that older individuals have less tolerance for acute oxidative stress than young counterparts (Done and Traustadottir 2016), which is likely due to decreased antioxidant factors, poor cellular signalling and high baseline state of oxidative stress with ageing. This makes older adults more vulnerable

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to cell damage than young individuals when performing exercises (Nordin et al. 2014).

Furthermore, aging is characterised by a chronically elevated level of systemic inflammation that is associated with an increased risk of developing many chronic diseases (Crossley et al. 2014; Lane-Cordova et al. 2016). For instance, plasma interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α) are two to fourfold greater in elderly when compared with young individuals (Woods et al. 2012). This elevated systemic inflammatory condition in the elderly is also related to an accelerated loss of muscle mass (Costamagna et al. 2015; Granic et al. 2017). An elevated inflammation and oxidative stress negatively affect elderly individuals, thus they should be minimised (Moylan and Reid 2007).

Acute exercise elevates the level of oxidative stress, which could negatively affect muscle function in old individuals (Nikolaidis 2017; Powers et al. 2016; Webb et al. 2017). It has been reported that unaccustomed exercise, especially exercise consisting of eccentric contractions (lengthening muscle actions), induces muscle damage, which elevates circulating inflammatory cytokines such as interleukins 6 (IL-6) and 1 (IL-1) (Toft et al. 2002). Pokora et al. (2014) reported that plasma IL-6 concentration increased immediately (46%) and 24 h (206%) after downhill running in healthy young men. Lee and Park (2015) showed that an initial bout of downhill running induced greater increase in muscle damage markers when compared with flat running or after a secondary bout of downhill running. In addition, they showed that the total antioxidant capacity (TAC) levels decreased 29% after the first bout of downhill running, which returned to the baseline values at 48 h after the running, while TAC levels returned to the baseline at 24 h after flat running (Park and Lee 2015). Sacheck et al. (2003) reported that TAC decreased more in elderly (12%) than in young individuals (8%) after 45-min downhill running performed at 75% of peak oxygen consumption (VO_{2peak}), and that plasma levels of malondialdehyde (MDA) did not increase in the young, but increased 25% immediately after downhill running in the elderly.

There are several eccentric exercise modalities such as resistance training (Chen et al. 2017b), stair descending (Chen et al. 2017a; Theodorou et al. 2013) and eccentric cycling (Lastayo et al. 2003) that have been shown to effectively improve health and fitness parameters of older adults. As mentioned above, it seems that eccentric exercise induces greater inflammation and oxidative stress in older than young adults. However, most of the previous studies used high-intensity eccentric exercise protocols (e.g., downhill running at 75–100% of VO_{2peak}), which have been shown to induce moderate to severe muscle damage (Nikolaidis et al. 2013; Sacheck et al. 2003). No previous study has investigated changes in inflammation and oxidative stress after eccentric cycling that are performed at much lower intensity (e.g., less than 50% of VO_{2peak}) by older adults. Eccentric cycling has been shown to be efficient in increasing muscle strength and mass in older adults (Lastayo et al. 1999, 2014). Furthermore, eccentric cycling is not affected by walking ability that older adults often have, and the intensity of the exercise can be adjusted precisely, thus considered to be safer for old or fragile individuals. This may give more benefits and advantages over walking or running exercise protocols. However, no previous study has investigated acute effects of eccentric cycling on oxidative stress and inflammation markers in middle-aged and older individuals.

Thus, the aim of this study was to compare changes in oxidative stress and systemic inflammation after a moderate-intensity concentric cycling, a moderate- and high-intensity eccentric cycling in healthy middle-aged and elderly participants. Additionally, this study compared metabolic demand and changes in indirect markers of muscle damage between the three cycling bouts. We hypothesized that moderate-intensity eccentric cycling would induce similar systemic inflammation and oxidative stress to those after concentric cycling, but high-intensity eccentric cycling would induce greater inflammation and oxidative stress due to greater muscle damage in comparison to the concentric and moderate-intensity eccentric cycling.

Methods

Participants

Ten healthy individuals (eight men and two women) over 50 years old (range 51–73 years old) were recruited for this study, and their physical characteristics are shown in Table 1. The sample size was estimated using the data from a previous study (Sacheck et al. 2003) in which a 23.6% increase in MDA immediately after high-intensity downhill running (75% VO_{2max}) was found in older (> 60 years

Table 1 Physical characteristics (mean ± SD) of the participants: sex, age, body mass, height, body mass index (BMI), maximum oxygen uptake (VO_{2peak}), maximal power output (PO_{max}), and physical activity in metabolic equivalents (METS)

Variable	Mean ± SD
Sex	Eight men/two women
Age (years)	60.4 ± 6.8
Body mass (kg)	78.5 ± 14.7
Height (cm)	170.4 ± 7.5
BMI (kg/cm^2)	27.2 ± 3.7
VO_{2peak} (ml/kg/min)	25.8 ± 6.5
PO_{max} (W)	197.3 ± 66.1
Physical activity (METS/week)	2,967 ± 1.417

old) individuals. The sample size estimation showed that eight participants would be sufficient for the effect size of 1.2, with α level of 0.05, and β level of 0.2. None of the participants had performed lower limb resistance training, smoked for at least 6 months prior to the study, and had musculoskeletal or neurological injuries of the lower limbs. They were instructed not to consume any antioxidant supplementation or be in any hormonal treatment. All participants completed a medical questionnaire to confirm that they were able to perform physical activity. This study was conducted in accordance with the Declaration of Helsinki and all participants signed an informed consent approved by the Institutional Ethics Committee prior to the commencement of the study. Participants were given a food diary to monitor their diet for 3 days, and to determine food consumption with antioxidant properties. Analyses of the food intake diaries with the Food Processor Nutrition and Fitness Software V11.3.285 are shown in Table 2. They were advised to reproduce the same diet during all three blocks of testing.

Study design

Participants reported to the laboratory 13 times, including 4 familiarisation sessions of eccentric cycling separated by 2 days between sessions. The familiarisation sessions were conducted to demonstrate the exercise, improve coordination and induce adaptations to eccentric cycling to avoid severe muscle damage (Fig. 1). During the first visit, the participants performed a concentric cycling incremental test to determine the maximal concentric power output (PO_{max}) and peak oxygen consumption ($\dot{V}O_{2peak}$). This was followed by the first familiarisation session for eccentric cycling (5 min), and in the second to fourth visits, three additional familiarisation sessions for the eccentric cycling were performed (5–20 min) as detailed below.

At least 4 days after the fourth familiarisation session, the participants visited the laboratory in three blocks of 3 days to perform a moderate-intensity concentric cycling (CONC-M) using a recumbent cycle ergometer (Livestrong, LS 5.0 R model, USA), a moderate-intensity eccentric cycling (ECC-M) and high-intensity eccentric cycling (ECC-H) on the eccentric recumbent ergometer (Eccentric Trainer, Metitur, Finland) in a randomized order, separated by 2 weeks between bouts.

All sessions were performed under normal laboratory conditions (22–23 °C and 40–50% relative humidity). During cycling, oxygen consumption ($\dot{V}O_2$), heart rate (HR) and rate of perceived exertion (RPE) were monitored and recorded. Maximum voluntary knee extension isometric contraction (MVC) strength, and muscle soreness of the knee extensor muscles were measured before, immediately after, and 24 and 48 h after each cycling bout. Plasma CK activity was measured before, 24 and 48 h after cycling. A 10-ml blood sample was withdrawn from the antecubital vein before and 5 min after cycling to measure inflammatory and oxidative stress markers from plasma and serum samples according to the ELISA kit preference.

Table 2 Analysis of daily energy and vitamins intake (mean ± SD) during all three bouts

Variable	Mean ± SD
Energy (kcal/day)	2263.3 ± 648.9
Carbohydrate (% of energy)	45.5 ± 8.3
Fat (% of energy)	36.9 ± 6.7
Protein (% of energy)	16.5 ± 4.5
Vitamin A (mg/day)	0.5 ± 0.3
Vitamin C (mg/day)	108.9 ± 81.8
Vitamin E (α-tocopherol equivalents, mg/day)	11.8 ± 4.8
Beta carotene (μg/day)	1769.9 ± 2319.3
Selenium (μg/day)	89.1 ± 47.9

Maximal incremental test

On the first visit to the laboratory, the participants performed a maximal incremental test on an electromagnetically braked ergometer (Livestrong, LS 5.0 R model, USA). The test started with the participants cycling at 50 watts (W) for 2 min, after which the intensity increased 20 W every minute

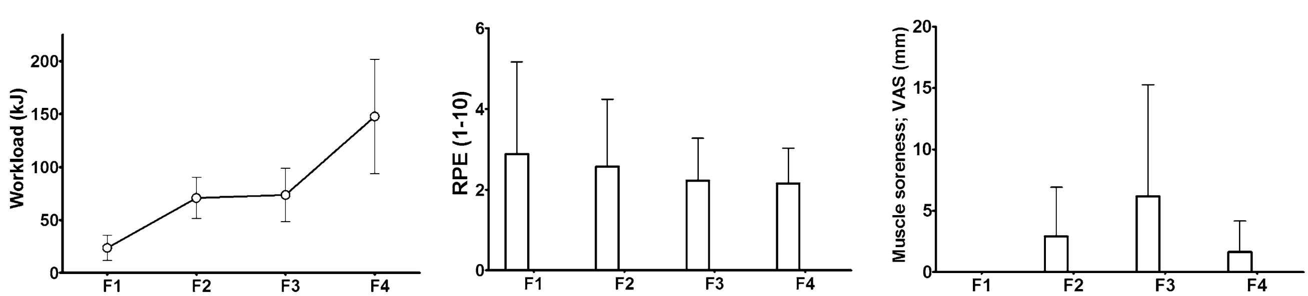

Fig. 1 **a** Workload (kJ), **b** rate of perceived exertion (RPE: 1–10 scale), and **c** muscle soreness (VAS: 0–100 mm) of the four familiarisation sessions (F1–4)

until voluntary exhaustion or until each participant was unable to maintain the required cadence of 60 rpm (Penailillo et al. 2013). All participants received verbal encouragement during the test. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured during the test using an open circuit breath-by-breath respiratory gas analyser (Ergocard, Medisoft, Belgium). The gas analyser was calibrated before each testing using alpha gases of known concentrations, and the airflow was calibrated using a 3-l syringe (Hans Rudolph, Kansas, MO, USA). Determination of PO_{max} and VO_{2peak} was performed as previously described (Penailillo et al. 2013).

Familiarisation sessions

Four familiarisation sessions (F1–F4) were performed on the eccentric cycle ergometer before the experimental cycling bouts to improve coordination and reduce the possibility of severe muscle damage. The first familiarisation began with 5 min and increased to 20 min over the fourth session (additional 5 min from session to session), in which the participants were asked to reach 50% of their concentric PO_{max} for 20 min. The interval between the familiarisation sessions was 2 days. Muscle soreness of the knee extensor muscles during sitting to and standing from a chair three times was assessed using a visual analogue scale (VAS) before every familiarisation session to assess any residual soreness from previous familiarisation session. During each familiarisation session, the total workload and RPE (0–10 scale) were monitored. The workload was incrementally increased as shown in Fig. 1a, and the RPE ratings were low (F1: 2.8 ± 2.2, F2: 2.5 ± 1.6, F3: 2.2 ± 1.0, F4: 2.1 ± 0.89) as shown in Fig. 1b. Minimal soreness induced after each session was minor as shown in Fig. 1c.

Cycling sessions

The three cycling bouts consisted of 30 min of either moderate-intensity concentric cycling on a recumbent cycle ergometer at 50% of concentric PO_{max} (CONC-M), moderate-intensity eccentric cycling at 50% of concentric PO_{max} (ECC-M) or high-intensity eccentric cycling at 50% of VO_{2peak} (ECC-H; ~ 100% concentric PO_{max}), performed at 60 rpm. During CONC-M, the same recumbent cycle ergometer used for the incremental test was used. The eccentric cycling bouts were performed on the recumbent cycle ergometer (Eccentric Trainer, Metitur, Finland), in which participants were instructed to resist the backward movement of the pedals inducing eccentric contractions of the knee extensor muscles mainly (Elmer et al. 2010). Participants were asked to maintain a constant power level displayed on a screen.

Average power output was recorded during each cycling bout. The VO_2 consumed and VCO_2 produced were continuously measured during each 30-min cycling bout using an open circuit breath-by-breath metabolic cart (Medisoft, Belgium). Furthermore, HR (Polar 625 ×, Finland) and RPE (0–10 Borg's scale) were monitored at 10, 20 and 29 min of each cycling bout. The average of these three time points was used for further analyses.

Indirect markers of muscle damage

MVC strength

The MVC strength of the knee extensors of the dominant leg (i.e., kicking leg) was measured at 90° of knee flexion using a force plate (Tesys 1000, Globus System, Italy) attached to a footplate of a standard leg press machine (James et al. 2014). Participants performed a specific warm-up prior to the MVC testing, which consisted of 3-min cycling at 50 W and three submaximal isometric contractions at 50%, 70% and 80% of each participant's self-perceived MVC with a 30-s rest between contractions. After warm-up, participants performed three attempts of 3-s MVC with a 1-min rest between contractions. Participants were instructed to perform the MVC as fast and hard as possible, and verbal encouragement and visual feedback were provided simultaneously. The largest value of three contractions was used for further analysis.

Muscle soreness

Muscle soreness level was quantified using a 100-mm visual analogue scale (VAS). Participants were asked to rate their perceived muscle soreness of the knee extensor muscles from 0 (no pain) to 100 (worst pain imaginable) while sitting and standing three times on a 42-cm chair (Penailillo et al. 2013).

Plasma CK activity

A 35 µl capillary blood sample was taken by a finger prick, and plasma CK activity was measured by a Reflotron (Roche Diagnosis, Germany) using standard procedures.

Blood sampling for inflammatory and oxidative stress markers

Venous blood samples were taken at rest at the same time of the day (± 1 h) for each cycling bout. Blood samples were taken before and ~ 5 min after each of the three cycling bouts. Samples of whole blood were taken in two EDTA tubes for plasma collection and two Vacutainer© tubes with pro-coagulant for serum collection. The EDTA tubes were kept in a refrigerator (4 °C) while the serum tubes coagulated for 30 min at room temperature. All blood samples were centrifuged for 10 min at 4 °C and 4000 rpm. Plasma and serum obtained were aliquoted in Eppendorf tubes with 500 µl of sample and were stored at − 80 °C until analysis.

Inflammatory and oxidative stress markers

All inflammation and oxidative stress markers were analysed by commercially available ELISA or colorimetric kits (Cayman, USA) and were read on a plate reader Multiskan FC (Thermo Scientific, China). All samples were analysed in duplicate. From plasma, IL-6 and TNF-α were measured in plasma by the "sandwich" technique with a specific monoclonal antibody for human IL-6 and TNF-α, respectively, and read at 450 nm. Intra-assay CV was 4.2 ± 0.7% for IL-6, and 3.6 ± 2.0% for TNF-α. Thiobarbituric acid reactive substances (TBARS, MDA) were measured in plasma through the reaction between MDA and TBA (tertiary butyl alcohol) under high temperatures (90–100 °C) and read at 530 nm. Protein carbonyl was measured in plasma through the reaction with 2,4-dinitrophenylhydrazine (DNHP), which binds to the carbonyls of the proteins forming hydrazone that can be analysed spectrophotometrically (read at 385 nm) and the carbonyl content was standardized according to the total concentration of proteins in the plasma read at 270 nm. The activity of glutathione peroxidase (GPx activity) was measured in plasma indirectly by a coupled reaction with glutathione reductase (GR) and read at 340 nm. Total antioxidant capacity (TAC) in serum was analysed with an assay that is based on the antioxidant capacity of the sample to inhibit the oxidation of ABTS® (2,2′-azino-di-[3-ethylbenzothiazoline-sulphonic acid]), and read at 750 nm. Intra-assay CV was 2.2 ± 0.9% for MDA, 5.0 ± 2.2% for protein carbonyls, 3.8 ± 2.3% for GPx, and 2.8 ± 1.4% for TAC.

Statistical analysis

The Shapiro–Wilk test was used to verify the normality of the data, which showed that all were normally distributed. A one-way analysis of variance (ANOVA) was used to compare the average power output, HR, VO_2 and RPE between the three cycling bouts. A two-way repeated-measures ANOVA was used to compare the changes in indirect markers of muscle damage (MVC strength, muscle soreness, plasma CK activity) over time between CONC-M, ECC-M, and ECC-H. A paired Student's *t* test was used to compare the before and after each cycling bout for the concentrations of oxidative stress and systemic inflammation markers. A statistical significance was set at $P < 0.05$. All statistical analyses were performed in the PASW 21.0 software (IBM Company, USA).

Results

Cycling performance

The training workload was progressively increased from F1 to F4 (Fig. 1a), which resulted in minimal RPE (2.2–2.4; Fig. 1b) and muscle soreness (peak = 5.1 ± 8.9 mm; Fig. 1c).

The average power output was similar ($P = 0.83$) between CONC-M (98.6 ± 33.1 W) and ECC-M (112.0 ± 42.1 W), but that of ECC-H (192.8 ± 84.9 W) was 48.8 ± 14.3% greater when compared with CONC-M ($P < 0.001$) and 41.8 ± 15.4% greater than that of ECC-M ($P = 0.001$) as shown in Fig. 2a. The average VO_2 during ECC-M (8.2 ± 2.2 ml/kg/min) was 49.7 ± 14.7% smaller than that of CONC-M (16.3 ± 3.5 ml/kg/min; $P < 0.0001$) and 40.1 ± 12.2% smaller than that of ECC-H (13.7 ± 4.1 ml/kg/min; $P = 0.0002$), without a significant difference between CONC-M and ECC-H (Fig. 2b). The average HR during ECC-M (99.9 ± 12.4 bpm) was 16.7 ± 9.7% lower than that of CONC-M (119.9 ± 17.1 bpm; $P = 0.001$) and 23.1 ± 10.7% lower than that of ECC-H (129.9 ± 15.3 bpm; $P = 0.0002$) as shown in Fig. 2c. The average RPE during ECC-M (1.6 ± 0.7) was similar to that of CONC-M (2.6 ± 0.7; $P = 0.11$), but was 57.9 ± 23.4% lower than that of ECC-H (3.8 ± 2.1; $P = 0.01$) as shown in Fig. 2d.

MVC strength

Baseline MVC strength was similar ($P > 0.05$) between CONC-M (663.5 ± 191.2 N), ECC-M (747.4 ± 157.6 N) and ECC-H (732.7 ± 162.3 N). A significant group × time interaction effect ($P = 0.001$) was found for the changes in MVC strength. As shown in Fig. 3a, MVC strength did not change after CONC-M from the baseline value ($P = 0.63$), but showed a 7.9 ± 8.0% decrease only at immediately after ECC-M ($P = 0.008$). However, ECC-H showed a 22.1 ± 10.1% and 8.3 ± 6.9% decrease at immediately ($P < 0.0001$) and 24 h ($P = 0.003$) post-exercise, respectively.

Muscle soreness

The two-way ANOVA showed a significant group × time interaction effect ($P = 0.001$) for the changes in muscle soreness. As shown in Fig. 3c, muscle soreness did not change after CONC-M ($P > 0.05$), but increased at 24 h ($P = 0.02$) and 48 h ($P = 0.03$) after ECC-M, and at 24 h ($P = 0.004$) and 48 h ($P = 0.01$) after ECC-H. Muscle soreness was 94.2 ± 20.2% ($P = 0.007$) and 51.6 ± 60.4% ($P = 0.03$) greater at 24 h after ECC-H when compared with CONC-M and ECC-M, respectively.

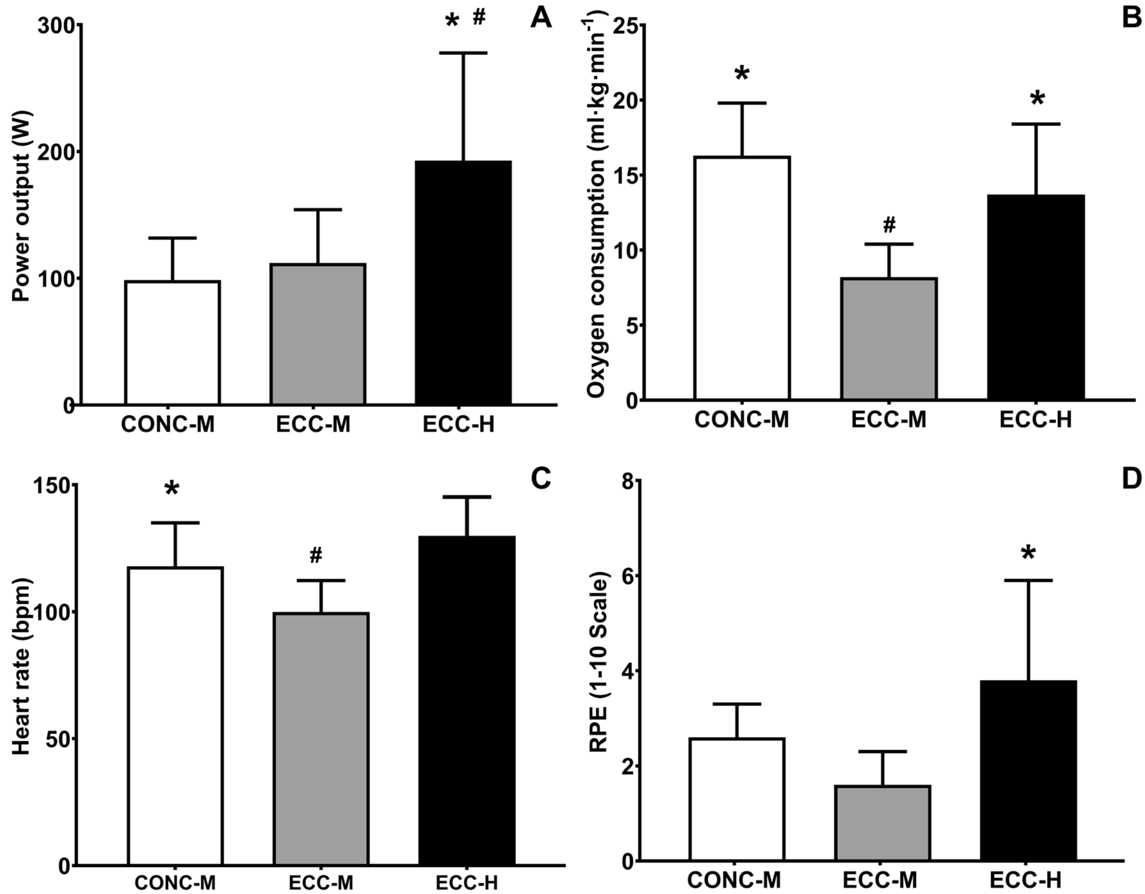

Fig. 2 Average (mean ± SD, $n = 10$) power output (**a**), oxygen consumption (**b**), heart rate (**c**) and rate of perceived exertion (RPE) (**d**) during exercise for moderate-intensity concentric cycling (CONC-M: white), moderate-intensity eccentric cycling (ECC-M: grey) and high-intensity eccentric cycling (ECC-H, black). [&]Significantly different ($P < 0.05$) to ECC-M; [#]significantly different ($P < 0.05$) to CONC-M

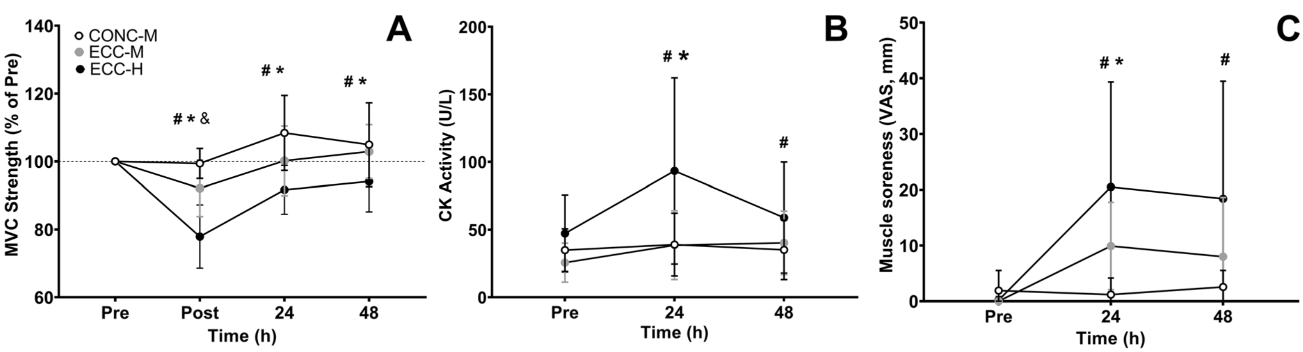

Fig. 3 Changes (mean ± SD, $n = 10$) in maximal voluntary isometric contraction strength of the knee extensors (**a**), plasma CK activity (**b**) and muscle soreness (**c**) before (pre), immediately after and 24 and 48 h after moderate-intensity concentric cycling (CONC-M: white), moderate-intensity eccentric cycling (ECC-M: grey) and high-intensity eccentric cycling (ECC-H, black). [&]Significantly different ($P < 0.05$) between CONC-M and ECC-M; [#]significantly different ($P < 0.05$) between CONC-M and ECC-H. *Significantly different ($P < 0.05$) between ECC-M and ECC-H

Plasma CK activity

A significant group × time interaction effect ($P = 0.002$) was found for the changes in plasma CK activity. As shown in Fig. 3b, CK activity did not change after CONC-M ($P > 0.05$), but increased to 33.5 ± 31.5% ($P = 0.02$) and 36.4 ± 29.6% ($P = 0.01$) from the baseline at 24 and 48 h after ECC-M and ECC-H, respectively. Plasma CK activity increased to 49.4 ± 26.3% ($P = 0.03$) at 24 h after ECC-H from the baseline, which was 58.2 ± 34.4% ($P = 0.007$) greater than that of CONC-M and 58.7 ± 20.7% ($P = 0.0005$) greater than that of ECC-M.

Oxidative stress markers

Figure 4 shows the mean changes in MDA, protein carbonyl, GPx activity and TAC before and after each cycling bout. MDA concentration decreased 28.0 ± 15.1% after CONC-M (6.79 ± 0.52 to 4.89 ± 0.51 nmol/ml; $P < 0.0001$), 21.8 ± 33.8% after ECC-M (6.92 ± 0.93 to 5.41 ± 1.69 nmol/ml; $P = 0.005$), but no significant change after ECC-H was observed (Fig. 4a–c). There were no changes in protein carbonyl concentration after any exercise bout (Fig. 4d–f). GPx activity increased to 31.7 ± 31.7% after CONC-M (50.89 ± 18.34 to 67.02 ± 11.15 nmol/min/ml; $P = 0.04$), 42.7 ± 53.7% after ECC-M (47.07 ± 13.65 to 67.17 ± 26.14 nmol/min/ml; $P = 0.05$) and 41.4 ± 29.7% after ECC-H (47.60 ± 10.96 to 67.31 ± 18.37 nmol/min/ml; $P = 0.04$) (Fig. 4g–i). No significant changes in TAC concentration were found after any exercise bout (Fig. 4j–l).

Systemic inflammation markers

As shown in Fig. 5, IL-6 increased 35.0 ± 40.6% after ECC-H (4.23 ± 2.19 to 5.71 ± 1.29 pg/ml; $P = 0.04$), but no significant changes were observed after CONC-M and ECC-M (Fig. 5a–c). No significant changes in TNF-α were found after any exercise bout (Fig. 5d–f).

Discussion

We confirmed the lower metabolic demand of eccentric cycling when compared with concentric cycling at the same workload in older individuals (ECC-M vs. CONC-M). We also found that the high-intensity eccentric cycling (ECC-H) induced moderate muscle damage, but no muscle damage was induced after other cycling bouts, and a 35% increase in interleukin-6 (IL-6) concentration after ECC-H only. However, MDA decreased after the moderate concentric (CONC-M; − 28%) and eccentric (ECC-M; − 22%) cycling, but did not change after ECC-H. On the contrary, the enzymatic antioxidant capacity (i.e., GPx activity) increased after all three cycling bouts similarly. These results support our initial hypothesis that muscle damage, oxidative stress and inflammation would be similar between moderate-intensity eccentric cycling and moderate-intensity concentric cycling, but high-intensity eccentric cycling would induce greater muscle damage, oxidative stress and inflammation when compared with the other cycling bouts in middle and old age individuals.

It has been previously shown that oxygen consumption is ~ 50% lower in eccentric than concentric cycling at the same workload in healthy young individuals (Penailillo et al. 2013), in patients with chronic heart failure (Chasland et al. 2017) and in elderly (Lastayo et al. 2003). The present study also showed approximately 50% lower oxygen consumption during ECC-M when compared with CONC-M, accompanied by a 16.8 ± 9.7% lower HR and 38.2 ± 47.1% lower RPE (Fig. 2c, d). Penailillo et al. (2017) reported that the less motor unit recruitment of agonist and antagonist muscles and ATP independency during eccentric than concentric cycling were the principal factors involved in this smaller metabolic demand in eccentric cycling. Interestingly, when metabolic intensity (VO_2) was matched between concentric and eccentric cycling (i.e., CONC-M vs. ECC-H), the power output produced during cycling was 48.8 ± 14.3% greater for ECC-H than CONC-M, with similar increase in HR and RPE. Therefore, moderate-intensity eccentric cycling could be safely performed in elderly, inducing less metabolic stress than concentric cycling for the same workload.

We found that ECC-M induced a small strength loss only immediately after cycling, whereas ECC-H induced larger and prolonged decrease in MVC strength (recovered at 48 h post-exercise) after cycling (Fig. 3a). Furthermore, ECC-H induced larger increase in muscle soreness and plasma CK activity when compared with ECC-M and CONC-M, which peaked 24 h after cycling (Fig. 3b, c). Similar eccentric cycling protocols performed in young men showed greater decrease in muscle strength, increase in muscle soreness and plasma CK activity (Penailillo et al. 2013), when compared with those in the present study. This seems to be consistent with previous study showing smaller strength loss, muscle soreness and CK activity in old than young adults after six sets of five submaximal isokinetic eccentric contractions of the elbow flexors (Lavender and Nosaka 2006). They speculated that the changes in joint stiffness and tendon–aponeurosis structure with ageing could reduce the strain on the muscles making the muscle less susceptible to muscle damage. They also speculated that the smaller responses to the eccentric exercise in the older group could be attributed to a smaller proportion of type II fibres (Lavender and Nosaka 2006). Our results confirm that elderly seems to be less susceptible to eccentric exercise-induced muscle damage than young individuals. However, it is also possible that our familiarisation sessions (Fig. 1) conferred adaptations

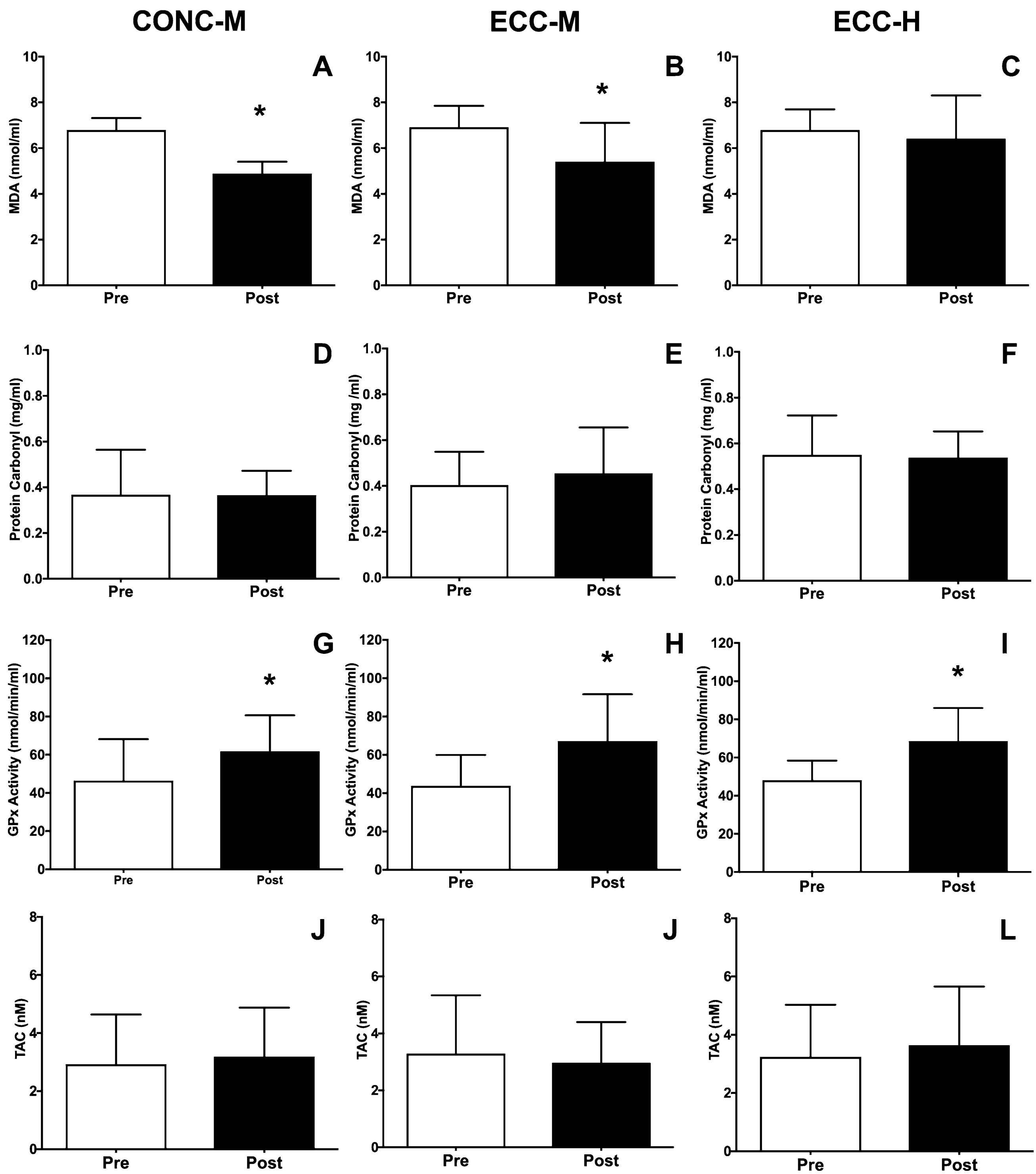

Fig. 4 Average (mean ± SD, $n=10$) malondialdehyde (MDA), protein carbonyl, glutathione peroxidase (GPx) and total antioxidant capacity (TAC) before (pre) and 5 min after (post) moderate-intensity concentric cycling (CONC-M), moderate-intensity eccentric cycling (ECC-M) and high-intensity eccentric cycling (ECC-H). *Significantly different from pre-value

against muscle damage in the elderly, since the magnitude of muscle damage in the present study seems to be lower than that previously reported in which participants were naïve to eccentric exercise (Lavender and Nosaka 2006). Familiarisation sessions were conducted in all participants and carried out progressively from 5 to 20 min, to improve coordination

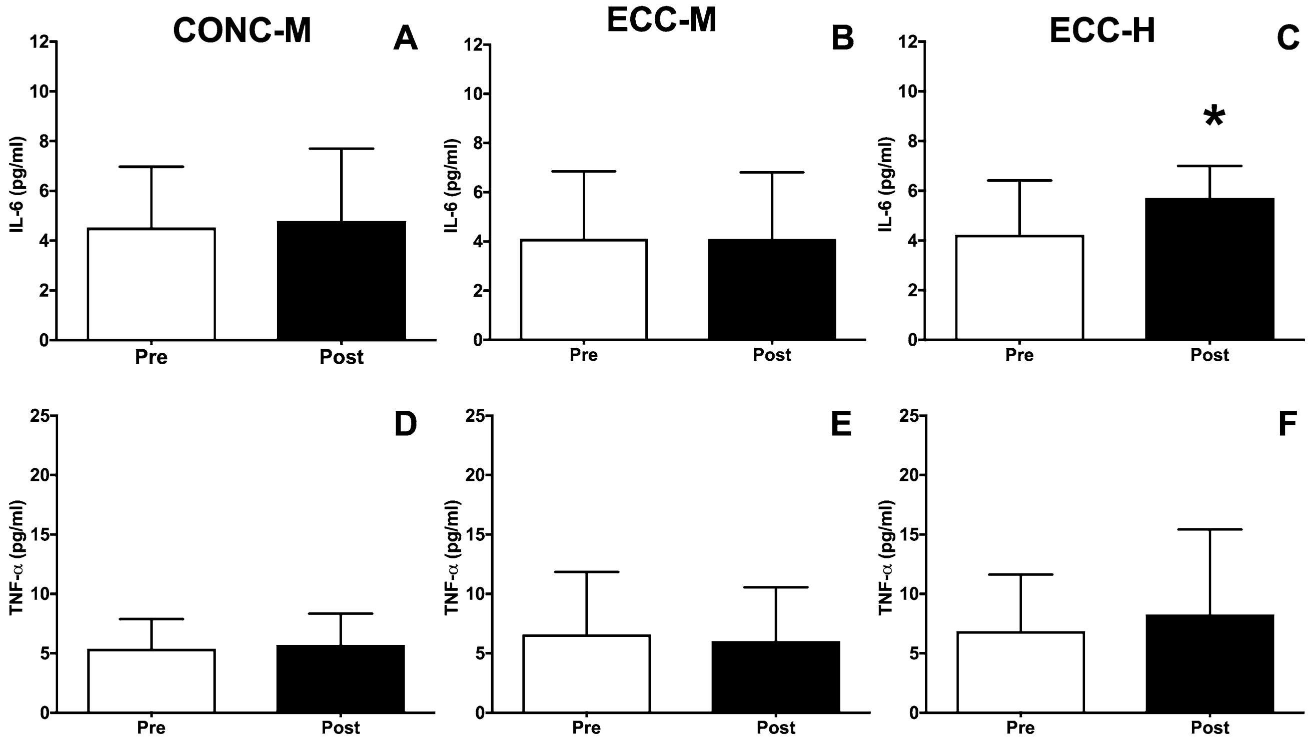

Fig. 5 Average (mean ± SD, n = 10) interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α) before (pre) and 5 min after (post) moderate-intensity concentric cycling (CONC-M: white), moderate-intensity eccentric cycling (ECC-M: grey) and high-intensity eccentric cycling (ECC-H, black). *Significantly different than pre-value

and avoid severe muscle damage. It is noteworthy to observe that, even after the ECC-H, only moderate muscle damage was induced in the present study. Effects of preconditioning exercise with low-intensity eccentric contractions to attenuate symptoms of muscle damage have been reported (Chen et al. 2012; Lavender and Nosaka 2008). It appears that the progressive introduction of eccentric cycling over four familiarisation sessions was effective to confer protective effect against muscle damage induced by ECC-M, but this was not sufficient enough to protect muscle damage after ECC-H. It is possible that inclusion of higher-intensity eccentric cycling during familiarisation sessions could attenuate the magnitude of muscle damage further after ECC-H. Thus, several familiarisation sessions with progressively increasing intensity are important to introduce eccentric cycling to elderly individuals or clinical populations to minimise or avoid muscle damage.

Previous studies reported that eccentric exercise induced inflammation and oxidative stress when performed at high intensity. Furthermore, increase in both markers was greater in older than in young individuals (Nikolaidis et al. 2013; Sacheck et al. 2003). Nikolaidis et al. (2007) reported that MDA increased at 72 h after an initial bout of 5 sets of 15 maximal isokinetic eccentric contractions of the knee flexors, but MDA did not increase at any time points after a secondary bout was performed several weeks later. Similarly, Sacheck et al. (2003) reported that unaccustomed eccentric exercise (45 min of downhill running at 75% of VO_{2max}) increased concentrations of MDA (44%) after exercise in elderly. We found that CONC-M and ECC-M decreased MDA concentrations (−28% and −22%, respectively), but MDA did not change after the high-intensity eccentric cycling (Fig. 4). However, protein carbonyl concentration did not change after any cycling bout. Although several studies have reported increase in MDA levels after acute exercise, the present study found a decrease (Fig. 4). A decrease of MDA levels after acute exercise has also been previously reported (Spirlandeli et al. 2014; Vincent et al. 2002). It may be that changes in MDA levels are affected by age, exercise mode, intensity and duration. Thus, a possibility exists that moderate-intensity exercise (CONC-C and ECC-M) induced decrease in MDA levels by increasing antioxidants' response (Spirlandeli et al. 2014; Vincent et al. 2002). Furthermore, it appears that the familiarisation sessions diminished muscle damage induced by moderate-intensity eccentric cycling, but they were not enough to protect the muscle damage against a high-intensity eccentric cycling (i.e., ECC-H). Interestingly, all three cycling bouts induced an increase in the enzymatic antioxidant (i.e., GPx activity) after cycling; this was independent of total antioxidant capacity (TAC; Fig. 4), which includes non-enzymatic antioxidants in blood (e.g., bilirubin, urea). In line with our findings, Nikolaidis et al. (2013) also found that five sets of eight maximal eccentric isokinetic contractions induced a 35% increase in GPx activity in healthy elderly adults 24 h after exercise. Furthermore, Berzosa et al. (2011) reported that GPx activity increased 8.7% immediately after 30 min of concentric cycling at 70% of PO_{max}. It has been proposed that GPx and other antioxidant enzymes interact with oxidants to maintain the redox balance (Berzosa et al. 2011). Thus, it is probable that ECC-H caused damage to the membrane of the muscle cells and the lipids of these damaged membranes were peroxidised by oxidizing agents that are normally elevated when performing physical exercise. However, the increase in GPx activity could act as a buffer and controlled the increase of MDA after ECC-H. In addition, it is possible that in CONC-M and ECC-M, where muscle damage was lesser, lipid peroxidation was lower and GPx had a greater buffer effect and decreased MDA concentration in both bouts.

Previous studies have shown an inflammation reflected in an increase of IL-6 concentrations after eccentric exercise-induced muscle damage (Douglas et al. 2017; Park and Lee 2015; Paulsen et al. 2012; Toft et al. 2002). McKay et al. (2009) reported a twofold increase in serum IL-6 after 300 maximal eccentric isokinetic contractions in young healthy men. This is consistent with the greater increase in muscle damage and IL-6 concentration (35%) found after ECC-H in the present study. Conversely, TNF-α did not change after any cycling bout in the present study, despite some studies showing elevations of TNF-α after acute exercise (Bernecker et al. 2013; Liu and Timmons 2016). It is possible that TNF-α is already elevated in the elderly and that eccentric cycling was not sufficiently intense to increase this marker further (Bernecker et al. 2013; Monteiro-Junior et al. 2018). We speculate that muscle inflammation was related to the unchanged MDA concentrations after ECC-H. Thus, it is possible that high-intensity eccentric cycling induces inflammation and blunted antioxidant effects of exercise, which is not desirable in elderly due to the already elevated systemic inflammation in elderly.

There are some limitations in the present study, which should be considered in future studies. The present study did not include a group of young adults. Although young adults were investigated for their responses to eccentric cycling in previous studies (Penailillo et al. 2013, 2017), no previous study has compared between younger and older adults for changes in oxidative stress and inflammation markers after eccentric cycling. Although plasma samples have been used in previous studies (Berzosa et al. 2011; Nikolaidis et al. 2007), plasma samples have greater variability in concentrations of oxidative stress markers (Margaritelis et al. 2018). The use of the oxidative stress markers such as MDA and GPx in blood have limitations evaluating what occurs in a biological complex like lipid peroxidation (Cobley et al. 2017). Thus, other more adequate and precise methodologies should be considered in future studies in this area (Cobley et al. 2017; Nikolaidis et al. 2015). Furthermore, it is possible that we have missed potential changes in oxidative stress and inflammation markers due to the limited time points included in the study. Michailidis et al. (2007) reported that concentrations of MDA in plasma increased (46%) immediately after running at 70% of VO_{2max}, but also found that protein carbonyl levels increased (32%) at 30 min post-exercise. It seems that many factors affect the status of oxidative stress and inflammation markers in the blood, and it is difficult to control all. In the present study, we tested our participants at the same time of the day across the different exercise sessions and asked them to record their nutritional intake and to maintain the same diet. Thus, we assume that our results well represent what would happen after eccentric cycling performed by older individuals.

In conclusion, the metabolic demand was less during eccentric than concentric cycling at moderate intensity and did not induce increase in muscle damage, inflammation or oxidative stress markers after exercise in the elderly. Moderate-intensity cycling (concentric and eccentric) induced increase in the enzymatic antioxidant capacity and decreased oxidative stress markers. However, high-intensity eccentric-cycling induced greater muscle damage, inflammation and oxidative stress than other cycling bouts. Thus, the intensity of eccentric cycling should be kept at moderate to avoid any inflammation and oxidative stress in older adults. More research is warranted in this area to design the best exercise intervention for elderly and fragile individuals.

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Compliance with ethical standards

Conflict of interest There were no conflicts of interest.

References

Bernecker C, Scherr J, Schinner S, Braun S, Scherbaum WA, Halle M (2013) Evidence for an exercise induced increase of TNF-alpha and IL-6 in marathon runners. Scand J Med Sci Sports 23:207–214

Berzosa C, Cebrian I, Fuentes-Broto L, Gomez-Trullen E, Piedrafita E, Martinez-Ballarin E, Lopez-Pingarron L, Reiter RJ, Garcia JJ (2011) Acute exercise increases plasma total antioxidant status and antioxidant enzyme activities in untrained men. J Biomed Biotech 2011:540458

Chasland LC, Green DJ, Maiorana AJ, Nosaka K, Haynes A, Dembo LG, Naylor LH (2017) Eccentric cycling: a promising modality for patients with chronic heart failure. Med Sci Sports Exer 49:646–651

Chen TC, Chen HL, Pearce AJ, Nosaka K (2012) Attenuation of eccentric exercise-induced muscle damage by preconditioning exercises. Med Sci Sports Exer 44:2090–2098

Chen TC, Hsieh CC, Tseng KW, Ho CC, Nosaka K (2017a) Effects of descending stair walking on health and fitness of elderly obese women. Med Sci Sports Exer 49:1614–1622

Chen TC, Tseng WC, Huang GL, Chen HL, Tseng KW, Nosaka K (2017b) Superior effects of eccentric to concentric knee extensor resistance training on physical fitness, insulin sensitivity and lipid profiles of elderly men. Front Physiol 8:209

Cobley JN, Close GL, Bailey DM, Davison GW (2017) Exercise redox biochemistry: conceptual, methodological and technical recommendations. Redox biol 12:540–548

Costamagna D, Costelli P, Sampaolesi M, Penna F (2015) Role of inflammation in muscle homeostasis and myogenesis. Mediators Inflamm 2015:805172

Crossley E, Seri S, Stern JS, Robertson MM, Cavanna AE (2014) Premonitory urges for tics in adult patients with Tourette syndrome. Brain Dev 36:45–50

Done AJ, Traustadottir T (2016) Aerobic exercise increases resistance to oxidative stress in sedentary older middle-aged adults. A pilot study. Age 38:505–512

Douglas J, Pearson S, Ross A, McGuigan M (2017) Eccentric exercise: physiological characteristics and acute responses. Sports Med 47:663–675

Elmer SJ, Madigan ML, Lastayo PC, Martin JC (2010) Joint-specific power absorption during eccentric cycling. Clin Biomech 25:154–158

Granic A, Davies K, Martin-Ruiz C, Jagger C, Kirkwood TBL, von Zglinicki T, Aihie Sayer A (2017) Grip strength and inflammatory biomarker profiles in very old adults. Age Ageing 46:976–982

James DA, Simjanovic M, Leadbetter R, Wearing S (2014) Design and test of a custom instrumented leg press for injury and recovery intervention. Procedia Eng 72:38–43

Lane-Cordova AD, Phillips SA, Baynard T, Woods JA, Motl RW, Fernhall B (2016) Effects of ageing and physical activity on blood pressure and endothelial function during acute inflammation. Exp Physiol 101:962–971

Lastayo P, Marcus RL, Dibble L, Frajacomo F, Lindstedt SL (2014) Eccentric exercise in rehabilitation: safety, feasibility and application. J Appl Physiol 116:1426–1434

Lastayo PC, Ewy GA, Pierotti DD, Johns RK, Lindstedt S (2003) The positive effects of negative work: increased muscle strength and decreased fall risk in a frail elderly population. J Gerontol 58:M419–424

Lastayo PC, Reich TE, Urquhart M, Hoppeler H, Lindstedt SL (1999) Chronic eccentric exercise: improvements in muscle strength can occur with little demand for oxygen. Am J Physiol 276:R611–R615

Lavender AP, Nosaka K (2006) Comparison between old and young men for changes in makers of muscle damage following voluntary eccentric exercise of the elbow flexors. Appl Physiol Nutr Metab 31:218–225

Lavender AP, Nosaka K (2008) A light load eccentric exercise confers protection against a subsequent bout of more demanding eccentric exercise. J Sci Med Sport 11:291–298

Liu M, Timmons BW (2016) The effect of acute exercise on neutrophil reactive oxygen species production and inflammatory markers in healthy prepubertal and adult males. Pediatric Exer Sci 28:55–63

Margaritelis NV, Theodorou AA, Paschalis V, Veskoukis AS, Dipla K, Zafeiridis A, Panayiotou G, Vrabas IS, Kyparos A, Nikolaidis MG (2018) Adaptations to endurance training depend on exercise-induced oxidative stress: exploiting redox interindividual variability. Acta Physiol 22(2):e12898

McKay BR, De Lisio M, Johnston AP, O'Reilly CE, Phillips SM, Tarnopolsky MA, Parise G (2009) Association of interleukin-6 signalling with the muscle stem cell response following muscle-lengthening contractions in humans. PloS one 4:e6027

Michailidis Y, Jamurtas AZ, Nikolaidis MG, Fatouros IG, Koutedakis Y, Papassotiriou I, Kouretas D (2007) Sampling time is crucial for measurement of aerobic exercise-induced oxidative stress. Med Sci Sports Exerc 39:1107–1113

Monteiro-Junior RS, de Maciel-Pinheiro Tarso P, da Matta Mello Portugal E, da Silva Figueiredo LF, Terra R, Carneiro LSF, Rodrigues VD, Nascimento OJM, Deslandes AC, Laks J (2018) Effect of exercise on inflammatory profile of older persons: systematic review and meta-analyses. J Phys Act Health 15:64–71

Moylan JS, Reid MB (2007) Oxidative stress, chronic disease, and muscle wasting. Muscle Nerve 35:411–429

Nikolaidis M (2017) The effects of resistance exercise on muscle damage, position sense, and blood redox status in young and elderly individuals. Geriatrics 2(3):20

Nikolaidis MG, Kyparos A, Spanou C, Paschalis V, Theodorou AA, Panayiotou G, Grivas GV, Zafeiridis A, Dipla K, Vrabas IS (2013) Aging is not a barrier to muscle and redox adaptations: applying the repeated eccentric exercise model. Exp Gerontol 48:734–743

Nikolaidis MG, Margaritelis NV, Paschalis V et al (2015) Common questions and tentative answers on how to assess oxidative stress after antioxidant supplementation and exercise. In: Lamprecht M (ed) Antioxidants in sport nutrition, chap 14. CRC Press/Taylor & Francis, Boca Raton (FL)

Nikolaidis MG, Paschalis V, Giakas G, Fatouros IG, Koutedakis Y, Kouretas D, Jamurtas AZ (2007) Decreased blood oxidative stress after repeated muscle-damaging exercise. Med Sci Sports Exer 39:1080–1089

Nordin TC, Done AJ, Traustadottir T (2014) Acute exercise increases resistance to oxidative stress in young but not older adults. Age 36:9727

Park KS, Lee MG (2015) Effects of unaccustomed downhill running on muscle damage, oxidative stress, and leukocyte apoptosis. J Exerc Nutr Biochem 19:55–63

Paulsen G, Mikkelsen UR, Raastad T, Peake JM (2012) Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? Exerc Immunol Rev 18:42–97

Penailillo L, Blazevich A, Numazawa H, Nosaka K (2013) Metabolic and muscle damage profiles of concentric versus repeated eccentric cycling. Med Sci Sports Exer 45:1773–1781

Penailillo L, Blazevich AJ, Nosaka K (2017) Factors contributing to lower metabolic demand of eccentric compared with concentric cycling. J Appl Physiol 123:884–893

Pokora I, Kempa K, Chrapusta SJ, Langfort J (2014) Effects of downhill and uphill exercises of equivalent submaximal intensities on selected blood cytokine levels and blood creatine kinase activity. Biol sport 31:173–178

Powers SK, Nelson WB, Hudson MB (2011) Exercise-induced oxidative stress in humans: cause and consequences. Free Radic Biol Med 51:942–950

Powers SK, Radak Z, Ji LL (2016) Exercise-induced oxidative stress: past, present and future. J Physiol 594:5081–5092

Sacheck JM, Milbury PE, Cannon JG, Roubenoff R, Blumberg JB (2003) Effect of vitamin E and eccentric exercise on selected biomarkers of oxidative stress in young and elderly men. Free Radic Biol Med 34:1575–1588

Spirlandeli AL, Deminice R, Jordao AA (2014) Plasma malondialdehyde as biomarker of lipid peroxidation: effects of acute exercise. Int J Sports Med 35:14–18

Theodorou AA, Panayiotou G, Paschalis V, Nikolaidis MG, Kyparos A, Mademli L, Grivas GV, Vrabas IS (2013) Stair descending exercise increases muscle strength in elderly males with chronic heart failure. BMC Res Notes 6:87

Toft AD, Jensen LB, Bruunsgaard H, Ibfelt T, Halkjaer-Kristensen J, Febbraio M, Pedersen BK (2002) Cytokine response to eccentric exercise in young and elderly humans. Am J Physiol Cell Physiol 283:C289–295

Vincent KR, Vincent HK, Braith RW, Lennon SL, Lowenthal DT (2002) Resistance exercise training attenuates exercise-induced lipid peroxidation in the elderly. Eur J Appl Physiol 87:416–423

Webb R, Hughes MG, Thomas AW, Morris K (2017) The ability of exercise-associated oxidative stress to trigger redox-sensitive signalling responses. Antioxidants (Basel, Switzerland) 6:63

Woods JA, Wilund KR, Martin SA, Kistler BM (2012) Exercise, inflammation and aging. Aging Dis 3:130–140