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ORIGINAL ARTICLE

Eccentric exercise-induced muscle damage of pre-adolescent and adolescent boys in comparison to young men

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Abstract
Purpose This study compared changes in indirect muscle damage markers after maximal eccentric exercise of the elbow flexors (EF) among pre-adolescent (9–10 years), adolescent (14–15 years) and post-adolescent (20–25 years) men to test the hypothesis that the magnitude of muscle damage would increase with increase in age.
Methods Thirteen untrained men of each age group performed two bouts (ECC1, ECC2) of 30 maximal EF eccentric contractions. Several indirect muscle damage markers were measured from the exercised arm before, immediately after, and 1–5 days post-exercise. Changes in maximal voluntary concentric contraction torque of the EF (MVC), range of motion of the elbow joint, upper arm circumference (CIR), muscle passive stiffness, muscle soreness, plasma creatine kinase activity and myoglobin concentration after ECC1 and ECC2 were compared amongst groups by a mixed-design two-way ANOVA.
Results MVC before exercise was smaller ($P < 0.05$) for pre-adolescent (8.9 ± 1.9 Nm) than adolescent (25.1 ± 3.9 Nm) and adult (35.3 ± 4.6 Nm), and for adolescent than adult. Changes in all variables after ECC1 were smaller ($P < 0.05$) for pre-adolescent and adolescent when compared with adult, and all except CIR changes were smaller ($P < 0.05$) for pre-adolescent than adolescent. After ECC2, changes in all variables were smaller ($P < 0.05$) than those after ECC1 for all groups, but the magnitude of the changes was different among groups ($P < 0.05$) in the same way as that after ECC1.
Conclusion These results indicate that the magnitude of muscle damage is increased from pre-adolescent, adolescent to post-adolescent men.

Keywords Elbow flexors · Puberty · Lengthening contraction · Maximal voluntary contraction · Delayed onset muscle soreness · Repeated bout effect

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Abbreviations

ANOVA	Analysis of variance
AUC	The area under the curve
BMI	Body mass index
CIR	Upper arm circumference
CK	Creatine kinase
CV	Coefficient of variation
DOMS	Delayed onset muscle soreness
EDTA	Ethylenediaminetetraacetic acid
ECC1	The first bout of maximal eccentric exercise
ECC2	The second bout of maximal eccentric exercise
Mb	Myoglobin
MPS	Muscle passive stiffness
MVC	Maximal voluntary contraction
MVC–EE	Maximal voluntary isokinetic concentric contraction torque of the elbow extensors

MVC–EF	Maximal voluntary isokinetic concentric contraction torque of the elbow flexors
PTA	Peak torque angle
R	An intraclass correlation coefficient
RM	Repetition maximum
ROM	Range of motion
SOR	Muscle soreness
VAS	Visual analogue scale

Introduction

Eccentric exercise-induced muscle damage characterised by a prolonged loss of muscle function, delayed onset muscle soreness (DOMS) and increase in intramuscular proteins in the blood such as creatine kinase (CK) activity has been investigated by many studies. However, a limited number of studies have examined the muscle damage in children. To the best of our knowledge, six studies have reported eccentric exercise-induced muscle damage of children after either downhill running (Webber et al. 1989), bench press exercise (Soares et al. 1996), stepping exercise (Duarte et al. 1999), exhaustive low-intensity resistance exercise (Pullinen et al. 2011), or plyometric jumps (Marginson et al. 2005; Gorianovas et al. 2013). Three of these studies (Gorianovas et al. 2013; Marginson et al. 2005; Soares et al. 1996) showed that muscle damage was less for children when compared with adults.

Duarte et al. (1999) reported that muscle damage was induced to 13-year-old boys after a stepping exercise to exhaustion, indicated by increase in muscle soreness and plasma CK activity, and decrease in maximal voluntary isometric contraction (MVC) strength, but no comparison to adults was made. Webber et al. (1989) compared between children (10.4 years, 9 boys and 7 girls) and adults (27.1 years, 8 men and 7 women) for their responses to 30-min downhill (−10 %) running, and found no significant differences in muscle soreness and increase in serum CK activity at 1 day post-exercise between groups. Adult men (31 years) and adolescent boys (14 years) were compared in the study by Pullinen et al. (2011) for their responses to two bouts of low-load (40 % of one repetition maximum = 1RM) bilateral knee extension exercise separated by 2 days, and no significant differences between the groups were found for changes in MVC strength, serum CK activity and muscle soreness. In contrast, Soares et al. (1996) reported that increase in muscle soreness and serum CK activity, and decrease in MVC strength were significantly smaller for boys (12.1 years, $n = 10$) than adult men (28.3 years, $n = 10$) after bench press exercise with 80 % of 1RM load until exhaustion. Marginson et al. (2005) compared between boys (9–10 years) and adult men (20–29 years) for their responses to two bouts of 80 plyometric jumps performed 2 weeks apart using muscle soreness, MVC strength of the knee extensors, countermovement and squat jump height. They reported that changes in all variables after both exercise bouts were significantly smaller for boys compared with men. Gorianovas et al. (2013) have recently reported that changes in plasma CK activity, muscle soreness, and voluntary and electrically evoked torque of the knee extensors after the first bout of 100 drop jumps are significantly smaller for boys (11.8 years) and elderly men (63.2 years) than the adult men (20.8 years), but the protective effect against the muscle damage conferred by the first exercise bout is less for boys and elderly men than adult men. Thus, controversy appears to exit concerning the susceptibility of children to exercise-induced muscle damage in comparison to adults.

Only one of the aforementioned studies (Soares et al. 1996) used an exercise of upper limb muscles, and others used leg muscles. It has been reported that muscle damage profile is different between arm and leg muscles, and leg muscles are less susceptible to eccentric exercise-induced muscle damage than arm muscles for adults (Jamurtas et al. 2005). It is assumed that body weight affects the intensity of eccentric contractions of lower limb muscles in downhill running, stepping exercise, drop jumps and plyometric jumps that were used in the previous studies (Webber et al. 1989; Duarte et al. 1999; Marginson et al. 2005; Gorianovas et al. 2013). Therefore, it is necessary to compare the magnitude of muscle damage between children and adults using an arm eccentric exercise. It should be also noted that the previous studies did not clarify the maturity of children. Thus, to understand the muscle damage profile of children in comparison to adults, their maturity should be clarified.

Three of the previous studies (Marginson et al. 2005; Pullinen et al. 2011; Gorianovas et al. 2013) included the second bout of eccentric exercise for the comparison between children and adults, and two studies reported less protective effect for boys than adults (Marginson et al. 2005; Gorianovas et al. 2013), suggesting less adaptation of muscles of children to eccentric exercise. Although the second bout was included in the study by Pullinen et al. (2011), it was performed only 2 days after the first bout, and the changes in muscle damage markers were small and were not significantly different between bouts. It is known that the magnitude of the protective effect is dependent on the magnitude of muscle damage in the initial bout, and the greater the damage in the initial bout, the greater the protective effect (Chen et al. 2007). Thus, it may be that the less protective effect found for boys than adults in the previous studies (Marginson et al. 2005; Gorianovas et al. 2013) was a reflection of the less muscle damage after the first bout. It is interesting to examine whether this is also the case for the elbow flexors that could generally result in greater muscle damage than leg muscle eccentric exercises.

The present study therefore compared between pre-adolescent, adolescent and adult men for their responses to the initial and secondary bouts of maximal eccentric exercise of the elbow flexors using several indirect markers of muscle damage. The main hypotheses underpinning of the aims were that (1) the magnitude of muscle damage would be smaller for pre-adolescent boys followed by adolescent boys compared with adult men, and (2) the magnitude of protective effect would be less for pre-adolescent boys followed by adolescent boys compared with adult men.

Methods

Subjects

A group of 9- to 10-, 14- to 15- and 20- to 25-year-old men was recruited from local elementary schools, junior high schools and universities, respectively. The maturation was assessed by an experienced nurse based on the pubic and genital development scales described by Tanner (1962). Plasma testosterone concentration was also measured to ensure that the testosterone level was low (<0.87 nmol L^{-1}) for pre-adolescent (Martha et al. 1989). As shown in Table 1, all boys in the pre-adolescent group were in the Tanner's stage I, and their serum testosterone concentrations obtained in the morning (7 a.m.) were less than 0.3 nmol L^{-1}. For the adolescent group, all boys were in the Tanner's stage III–IV, and their serum testosterone concentrations ranged 6.0–12.8 nmol L^{-1}. All adult men were in the Tanner's stage V, and their serum testosterone concentrations were greater than 15.9 nmol L^{-1}. As shown in Table 1, significant differences in age, height, body mass and serum testosterone concentration among the groups were evident.

Each group consisted of 13 subjects who had not been involved in regular resistance, aerobic or flexibility training in the past 1 year. It was asked whether they regularly carried heavy objects, and they were excluded if they did. The participants signed informed consent document before participating in this study that had been approved by the University Institutional Ethics Committee. For the pre-adolescent and adolescent boys, their guardians also read the informed consent document and signed. The study was conducted in conformity with the policy statement regarding the use of human subjects by the Declaration of Helsinki. The subjects were screened to confirm that they had no joint, bone, neuromuscular diseases and musculoskeletal problems for the upper extremities.

The sample size was estimated using the data from the previous study in which 9- to 10-year-old boys and young adults were compared for muscle damage after plyometric

Table 1 Physiological characteristics [age, height, body mass, body mass index (BMI)], resting plasma testosterone concentration and baseline values of dependent variables

Pre-adolescent	Adolescent	Adult
Age (year)		
9.4 ± 0.5*, #	14.3 ± 0.4*	22.6 ± 2.0
9–10	14–15	20–25
Height (cm)		
134.5 ± 4.3*, #	165.2 ± 4.7*	172.2 ± 4.8
127.5–141.0	158.0–170.0	163.0–180.0
Body mass (kg)		
33.1 ± 6.1*, #	60.5 ± 10.6*	67.2 ± 9.3
28.0–40.5	46.0–70.0	54.0–83.0
BMI (kg/m^2)		
18.3 ± 2.4*, #	22.1 ± 2.9	22.6 ± 2.8
15.7–22.9	18.1–25.0	19.6–26.4
Testosterone (nmol/L)		
0.14 ± 0.07*, #	9.37 ± 2.33*	25.02 ± 3.47
0.07–0.28	6.00–12.77	15.92–27.76
MVC–EF (Nm)		
8.9 ± 1.9*, #	25.1 ± 3.9*	35.3 ± 4.6
6–13	17–33	26–44
MVC–EE (Nm)		
11.7 ± 2.1*, #	26.5 ± 4.3*	34.1 ± 4.5
9–16	18–34	27–45
Angle (°)		
79.9 ± 8.0*	82.0 ± 9.0	84.2 ± 7.2
68–87	70–90	76–92
ROM (°)		
142.6 ± 4.2*	140.3 ± 6.7	139.4 ± 5.8
139–150	128–148	131–145
CIR (mm)		
193.8 ± 19.0*, #	246.2 ± 19.9*	276.1 ± 19.8
170–220	195–286	234–324
MPS (mm/kg)		
18.5 ± 2.6*	17.5 ± 2.5*	15.5 ± 2.7
14.4–22.2	13.4–20.9	11.2–19.2
CK (IU/L)		
110.8 ± 5.7*	120.5 ± 6.8	131.8 ± 9.8
85.0–108.8	108.0–133.0	110.0–159.0
Mb (µg/L)		
21.0 ± 0.1*	22.7 ± 1.9	26.1 ± 2.7
20.8–21.0	21.0–25.2	21.0–31.0

Maximal voluntary isokinetic concentric contraction torque of the elbow flexors (MVC–EF) and extensors (MVC–EE), elbow joint angle at peak MVC–CON (Angle), range of motion (ROM), upper arm circumference (CIR), and muscle passive stiffness (MPS), plasma creatine kinase (CK) activity and myoglobin (Mb) concentration for pre-adolescent, adolescent and adult groups. Mean (±SD) and ranges of each variable for each group are shown

* Significantly ($P < 0.05$) different from the adult group

Significantly ($P < 0.05$) different from the adolescent group

jump exercise (Marginson et al. 2005), and our previous study (Chen et al. 2010) provided the changes in muscle damage markers after eccentric exercise of the elbow flexors of adult men. We estimated that at least 10 % difference would exist for the changes in muscle strength between boys and adult men. Based on this, the effect size was assumed to be 1, with the alpha level of 0.05 and power (1−β) of 0.80, and it was estimated that 12 subjects per group were necessary. We recruited 13 subjects for each group considering a possible drop out.

The subjects were asked and reminded to refrain from unaccustomed exercise and/or vigorous physical activity, to maintain their normal dietary habits, and not to take any anti-inflammatory drugs or nutritional supplements during the experimental period including 2 weeks prior to the first eccentric exercise bout (ECC1), and between ECC1 and second eccentric exercise (ECC2) bouts. The subjects were instructed to drink enough water (more than 1 L a day) after exercise to avoid a possible risk of acute renal failure due to rhabdomyolysis, and not to have any treatments (e.g. massage, stretching) of the exercised muscles during the study.

Experimental protocol

All subjects were familiarised with the testing procedures 3 days before ECC1. In the familiarisation session, the investigator took the measurements of height and body mass, and range of motion (ROM) of the elbow joint, upper arm circumference and muscle soreness. The subjects performed five submaximal (about 50 %) isokinetic concentric contractions of elbow flexors at 60° s^{-1} on the isokinetic dynamometer described below. The investigator demonstrated the eccentric exercise of the elbow flexors to the subjects, but they did not perform any eccentric contractions of the elbow flexors in the familiarisation session.

All subjects performed two bouts of maximal isokinetic eccentric exercise of the elbow flexors separated by 3 weeks using their non-dominant arm as detailed in the next section. The dependent variables consisted of maximal voluntary isokinetic (60° s^{-1}) concentric contraction torque of the elbow flexors (MVC–EF) and elbow extensors (MVC–EE), and the angle at peak torque (peak torque angle) of MVC–EF, ROM of the elbow joint, upper arm circumference, muscle passive stiffness, muscle soreness, and plasma CK activity and myoglobin (Mb) concentration. These measurements were taken before, immediately after, and 1, 2, 3, 4 and 5 days after exercise for MVC torque, ROM, upper arm circumference and muscle passive stiffness. Muscle soreness was assessed before and 1, 2, 3, 4 and 5 days after exercise, and plasma CK activity and Mb concentration were measured before, and 2 and 4 days post-exercise. Immediately before exercise, maximal voluntary isometric contraction torque of the elbow flexors was measured at 90° elbow flexion to set the target torque of eccentric contractions for each subject. The blood sample was taken from the non-exercised arm, but other measurements were taken from the exercised arm only, in the assumption that no or little changes in the variables would be seen for the contralateral limb (e.g. Barnes et al. 2010).

The baseline measurements were taken at 2 days and immediately before ECC1, and the test–retest reliability of the dependent variable measurements was determined by an intraclass correlation coefficient (R) and coefficient of variation (CV) using the two baseline data. The R and CV (shown in parentheses) values of the pre-adolescent group were 0.98 (13.5 %) for MVC–EF torque, 0.98 (8.0 %) for peak torque angle, 0.97 (2.9 %) for ROM, 0.99 (9.8 %) for upper arm circumference, 0.97 (0.8 %) for muscle passive stiffness, 0.84 (10.4 %) for plasma CK activity, and 0.99 (0.5 %) for plasma Mb concentration. These values were similar for the adolescent and adult groups.

Eccentric exercise

Each subject was seated upright on a chair of an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical Systems, Inc., Shirley, New York, USA), and his chest and waist were immobilised by straps, placing the upper arm on a padded support that secured the shoulder joint angle at 45° flexion and 0° abduction. The forearm was fully supinated, and the wrist was strapped to the lever attachment of the dynamometer. The eccentric exercise consisted of five sets of six maximal eccentric contractions of the elbow flexors at an angular velocity of 90° s^{-1} from 90° to an extended position (0°). Each eccentric contraction was preceded by 1-s maximal isometric contraction at 90°, and this was repeated every 10 s during which the isokinetic dynamometer passively returned the elbow joint to the flexed position at the velocity of 9° s^{-1}. The rest period between sets was 2 min. Subjects were verbally encouraged to resist against the lengthening action of the dynamometer for the whole range of motion (90°), and were given visual feedback of generated torque on a computer screen indicating the target torque that was set at 10 % greater than the maximal voluntary isometric contraction torque. The torque, work and elbow joint angle position signals of all contractions during the exercise were saved to a desktop computer (Acer Power FH, Acer Inc., Taiwan), and peak torque and work of each contraction were obtained using a software of the Biodex Medical Systems (Systems 3 Application Software for Window XP) as described in a previous study (Chen et al. 2013).

Dependent variables

MVC torque and optimum angle

MVC torque was measured by the same isokinetic dynamometer in the same position as that described for the eccentric exercise. The angular velocity was set at 60° s^{-1}, the range of motion was 140° for the elbow flexors (0–140°) and extensors (140–0°), and three continuous contractions were performed for both directions (Chen et al. 2013). Verbal encouragement was provided during the tests. Raw torque data were filtered and smoothed using an "isokinetic windowing," and the angle of peak torque for each contraction was assessed by a Biodex Medical Systems software (Chen et al. 2013). The highest value of the three trials for the elbow flexor and extensor MVC torque, respectively, was used for further analysis.

Elbow joint angles and ROM

ROM of the elbow joint for the exercised arm was determined as the difference between the elbow joint angles of maximal voluntarily flexion (FANG) and extension (EANG) measured by a manual goniometer, when each subject was standing (Chen et al. 2013). Three measurements were taken for each angle, and the mean of the three measurements was used to calculate ROM.

Upper arm circumference

While the subject was relaxing and letting the arm hang down by his side, the upper arm circumference was measured at the mid-portion between the acromion process of the clavicle and the lateral epicondyle of the humerus (Hawes and Martin 2001), using a Gulick tape measure (Creative Health Products, Plymouth, Michigan, USA). The measurements were taken three times by the same investigator, and the mean of the three measures was used for statistical analysis.

Muscle passive stiffness

Previous studies (Hung et al. 2010; Leonard et al. 2004) showed that a Myotonometer® (Neurogenic Technologies, Inc, Missoula, MT, USA), which is a computerised, electronic tissue compliance meter, was valid for assessing muscle tone or muscle passive stiffness of a relaxed muscle. The head of the myotonometer probe was placed along the longitudinal axis of the biceps brachii muscles at 50 % of the distance from the acromion process of the clavicle to the lateral epicondyle of the humerus (the same site as the circumference measurement), while each subject was sitting on a chair with the forearm being relaxed and placed on a padded table at the shoulder angle of 80° and the elbow joint angle of 10° flexion. The probe pressure was increased automatically from 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 to 2.0 kg, and this was repeated three times according to the standardised method used in the previous study (Hung et al. 2010). The software to operate the myotonometer recorded tissue displacement in response to each pressure, and the relationship between the pressure and displacement was obtained, providing a force–displacement curve, and the area under the curve (AUC) was computed. The AUC between 0.25 and 2.00 kg was used as an indicator of muscle passive stiffness (Leonard et al. 2004). In this method, the greater the value (displacement per kg), the smaller is the muscle passive stiffness.

Muscle soreness

Muscle soreness of the elbow flexors was quantified using a visual analogue scale (VAS) of a 100-mm continuous line with "no pain" on one end (0 mm) and "unbearable pain" on the other end (100 mm). The subjects were asked to rate their perceived soreness on the scale when the elbow joint was passively extended and flexed, respectively, for the same range of motion as that was used for the MVC torque measures (Chen et al. 2007).

Plasma CK activity, Mb and testosterone concentrations

Approximately 5 mL of venous blood was drawn from the antecubital vein of the dominant arm using a standard venipuncture technique, and collected to an EDTA-contained vacutainer tube (Becton–Dickinson, Co., Franklin Lakes, NJ, USA). The blood samples were centrifuged at 3,000 rpm for 10 min at 4 °C to separate the plasma, and plasma samples were stored at −80 °C for later analyses. Plasma CK activity was assayed by an automated clinical chemistry analyser (Model 7080, Hitachi, Co. Ltd., Tokyo, Japan) with an enzymatic method at 37 °C, using a test kit (Roche Diagnostics, Indianapolis, Indiana, USA). Plasma Mb concentration was measured by another automated clinical chemistry analyser (Model Elecsys 2010, Roche Diagnostics GmbH, Mannheim, Germany) using a test kit (Roche Diagnostics, Indianapolis, IN, USA). Each sample was analysed in duplicate, and the average value of two measures was used for subsequent statistical analysis. If the two values of the same sample were different by more than 10 %, one more measurement was added, and the average of the three values was used for further analysis. The intra-assay CV established in our laboratory for CK and myoglobin measures was 5.1 and 4.9 %, respectively, and the inter-assay CV was 3.8 % for CK and 3.1 % for Mb. The normal reference range for adult men for these methods was 38–174 IU L^{-1} for CK activity (Tietz 1995), and

10–68 μg L^{-1} for Mb concentration (Rosano and Kenny 1977), but no specific normal ranges of CK activity and Mb concentration are indicated for pre-adolescent and adolescent men. Plasma testosterone concentration was measured by an automated clinical chemistry analyser (Model Elecsys 2010, Roche Diagnostics GmbH, Mannheim, Germany) using a test kit (Roche Diagnostics, GmbH, D-68298 Mannheim, Indianapolis, IN, USA). Samples were analysed in duplicate, and the average of the two was used for statistical analysis. The intra- and inter-assay CV for this assay were 4.3 and 4.6 %, respectively. The normal reference range for adult men is 10.4–34.7 nmol L^{-1} (Larsen et al. 2002; Wang et al. 2004), and the value to distinguish between pre-adolescent and adolescent boys is 0.87 nmol L^{-1} (Martha et al. 1989). No specific value to distinguish between adolescent and adult is available, but it was assumed around 10.0 nmol L^{-1}, based on Larsen et al. (2002) and Wang et al. (2004).

Statistical analyses

Data were assessed by a Shapiro–Wilk test for the normality and a Levene test for the homogeneity of variance assumption. These tests showed that the data of all dependent variables were normally distributed, and the variance was homogenous. Baseline values of the dependent variables were compared amongst groups by a one-way analysis of variance (ANOVA). A mixed-design two-way ANOVA was used to compare amongst the groups for normalised changes in the dependent variables following ECC1 and ECC2 separately. When a significant interaction effect was found, a series of mixed-design two-way ANOVAs were performed to compare between the two groups for ECC1 and ECC2, respectively. When the ANOVA found a significant interaction effect, a Tukey's post hoc test was performed for the comparison between groups for each time point. The magnitude of protective effect conferred by ECC1 against ECC2 was assessed using the values at 1 day post-exercise for MVC torque, peak torque angle, ROM and muscle passive stiffness, at 4 days post-exercise for upper arm circumference, and highest values for muscle soreness and plasma CK activity and Mb concentration using the following equation of the protection index (Chen et al. 2007); (ECC1–ECC2)/ECC1 × 100. The 1 day post-exercise time point was chosen for MVC torque, peak torque angle, ROM and muscle stiffness, because it was thought to indicate muscle damage better by eliminating acute fatigue effect by exercise. The 4-day post-exercise point for upper arm circumference was thought to indicate the magnitude of swelling better, and peak values of muscle soreness and plasma CK activity and Mb concentration were thought to be better indicative of muscle damage. The index of each criterion measure was compared amongst the groups using a one-way ANOVA. Statistical significance was set at $P \leq 0.05$. Data were presented as mean ± SD, unless otherwise stated.

Results

Dependent variables at baseline

Significant differences in the baseline measures between groups were evident for MVC torque, peak torque angle, ROM, upper arm circumference, muscle passive stiffness, and plasma CK activity and Mb concentration (Table 1). MVC torque and upper arm circumference were significantly different across groups (Adult > Adolescent > Pre-adolescent), but only a significant difference between pre-adolescent/adolescent and adult groups was evident for peak torque angle, ROM and muscle passive stiffness. Plasma CK activity and Mb concentration were significantly smaller for pre-adolescent group compared with adult group.

Eccentric exercise

Peak torque produced during exercise decreased significantly over sets for ECC1 and ECC2 similarly, and the magnitude of decline was not significantly different among groups (Fig. 1). The average peak torque generated during exercise was greater ($P < 0.05$) for adult group (ECC1: 40.7 ± 4.5 Nm, ECC2: 41.1 ± 5.1 Nm) than adolescent (ECC1: 30.8 ± 4.1 Nm, ECC2: 30.5 ± 4.7 Nm) and pre-adolescent groups (ECC1: 17.2 ± 1.8 Nm, ECC2: 17.9 ± 2.0 Nm), and a significant difference was also

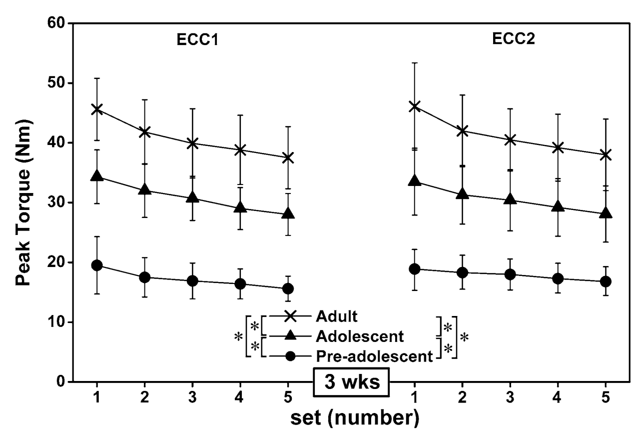

Fig. 1 Changes in peak torque (average of 6 eccentric contractions) over 5 sets for the first (ECC1) and second (ECC2) eccentric exercise bouts of the pre-adolescent, adolescent and adult groups. *Significant ($P < 0.05$) interaction (group × set) effect based on a pairwise comparison between groups

evident between adolescent and pre-adolescent groups. The total work was also significantly greater for adult group (ECC1: 1,536 ± 202 J, ECC2: 1,503 ± 186 J) than adolescent (ECC1 1,082 ± 151 J, ECC2: 1,060 ± 155 J) and pre-adolescent (ECC1: 433 ± 68 J, ECC2: 442 ± 59 J) groups, and significant difference was also found between adolescent and pre-adolescent groups for both bouts.

First exercise bout (ECC1)

All variables except MVC–EE torque changed significantly ($P < 0.05$) following ECC1, and their changes were significantly smaller for pre-adolescent and adolescent groups compared with adult group (Figs. 2, 3, 4). When comparing pre-adolescent and adolescent groups, the changes in all variables except upper arm circumference (Fig. 3a) were significantly smaller for pre-adolescent than adolescent group. When comparing the relative changes in plasma CK activity and Mb concentration from baseline, the results were basically the same as those based on the absolute values (Fig. 4).

Second exercise bout (ECC2)

After the second bout, significant changes in all variables except MVC–EE torque and plasma CK activity and myoglobin concentration were evident (Figs. 2, 3, 4). The changes were significantly smaller for pre-adolescent and

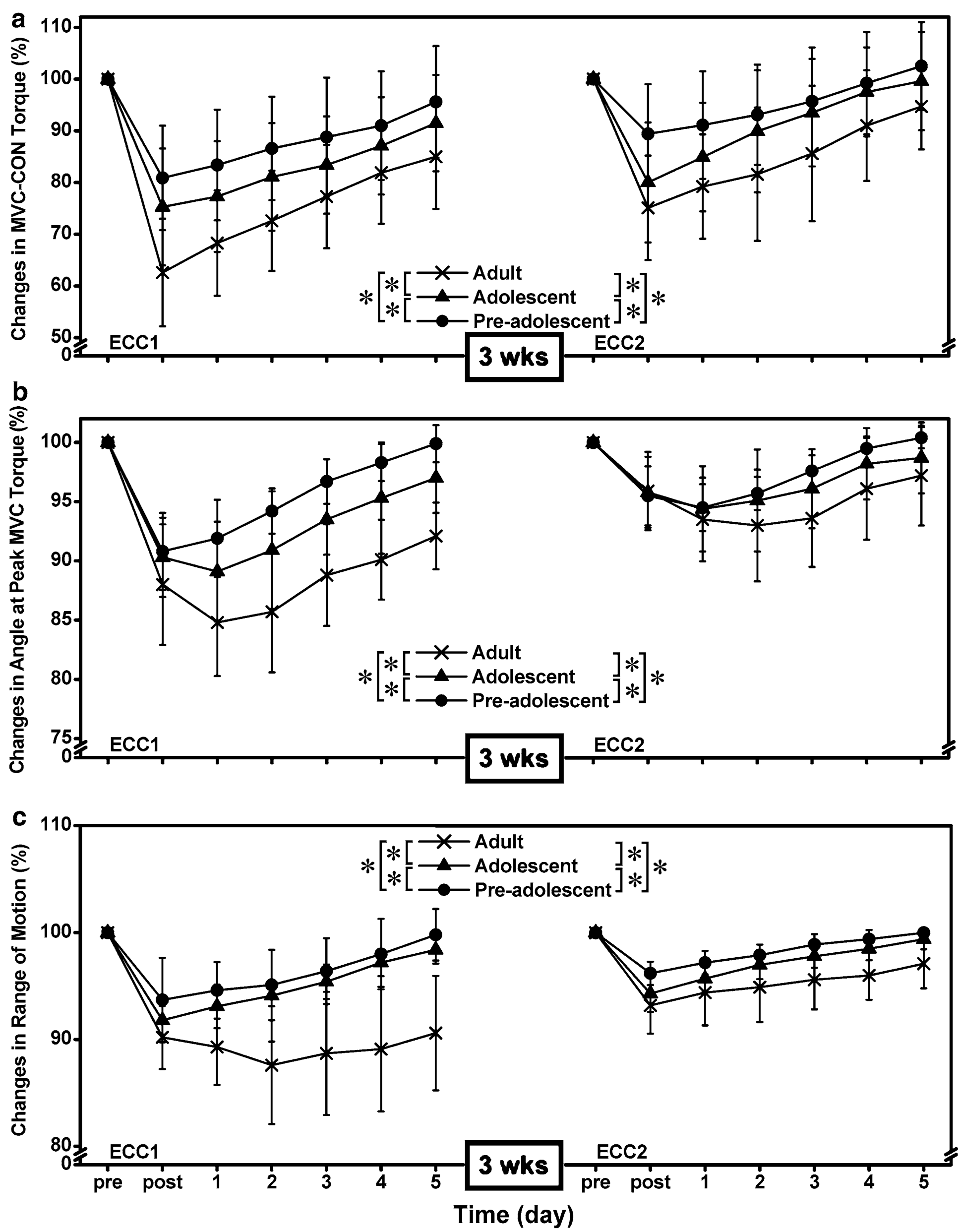

Fig. 2 Normalised changes from baseline (pre, 100 %) in maximal voluntary concentric contraction (MVC–CON) torque (**a**), elbow joint angle at peak MVC torque (**b**) and range of motion of the elbow joint (**c**) immediately (post) and 1–5 days after the first (ECC1) and second (ECC2) eccentric exercise bouts for the pre-adolescent, adolescent and adult groups. *Significant ($P < 0.05$) interaction (group × time) effect based on a pairwise comparison between groups

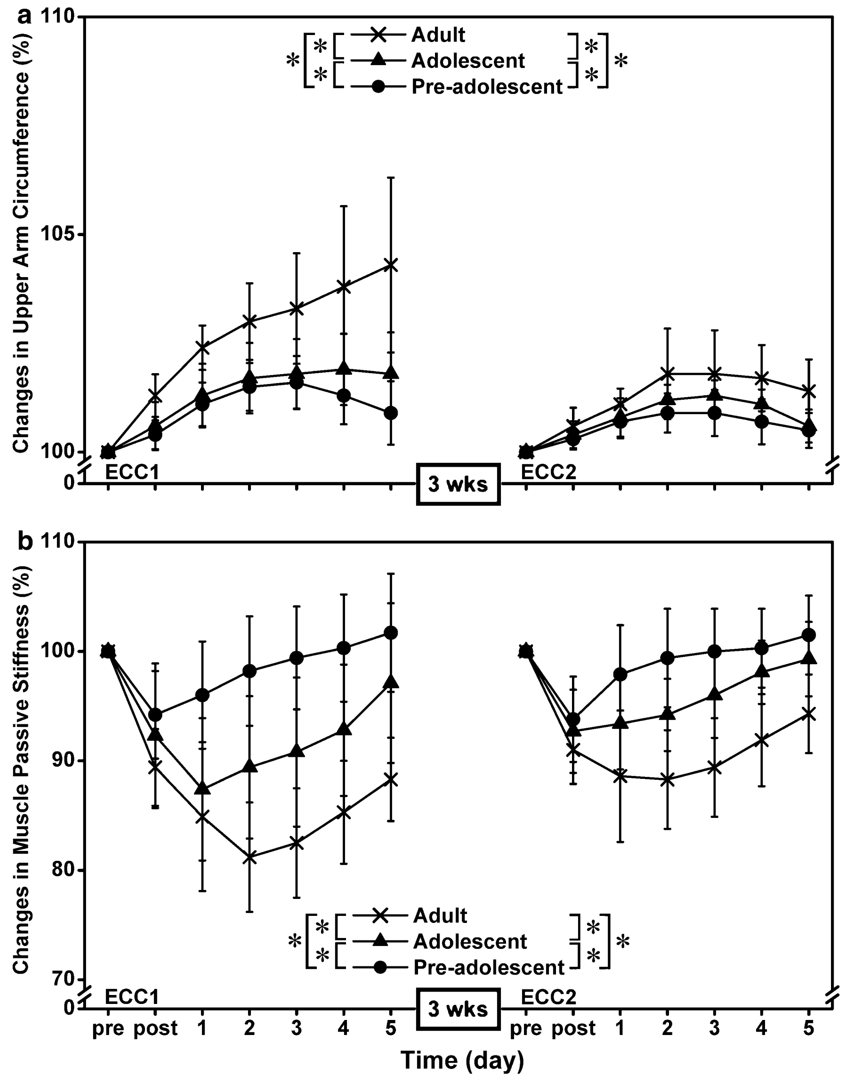

Fig. 3 Normalised changes from baseline (pre, 100 %) in upper arm circumference (**a**) and muscle passive stiffness (**b**) immediately after (post) and 1–5 days following the first (ECC1) and second (ECC2) eccentric exercise bout for pre-adolescent, adolescent, and adult groups. *Significant ($P < 0.05$) interaction (group × time) effect based on a pairwise comparison between groups

adolescent groups compared with adult group for all variables that showed significant changes, and the changes were also significantly smaller for pre-adolescent group than adolescent group for MVC–EF torque, peak torque angle, ROM, circumference, muscle passive stiffness and muscle soreness.

Comparison between bouts

When comparing between ECC1 and ECC2, changes in all dependent variables were significantly smaller following ECC2 than ECC1 for all groups (Figs. 2, 3, 4). The magnitude of protection indicated by the protection index for each dependent variable was not significantly different amongst the groups for all variables except plasma CK activity and Mb concentration (Fig. 5). For plasma CK activity and Mb concentration, the protective effect was significantly greater for adult than other groups, but no significant difference was evident between pre-adolescent and adolescent groups.

Discussion

The present study tested the hypotheses that (1) the magnitude of muscle damage would be smaller for pre-adolescent and adolescent than adult, and for pre-adolescent than adolescent; and (2) the magnitude of protective effect would be also smaller for pre-adolescent and adolescent than adult, and for pre-adolescent than adolescent. The results support the first hypothesis but not the second one.

The changes in the dependent variables following ECC1 and ECC2 for adult group were comparable to those reported after a similar eccentric exercise of the elbow flexors that was performed by young "untrained" adults in our previous studies (e.g. Chen et al. 2007, 2010). Thus, the changes in the dependent variables shown by adult group in the present study are considered to be typical for young adults who do not perform resistance training. The prolonged decrease in MVC–EF torque and ROM, swelling of muscle indicated by increase in upper arm circumference,

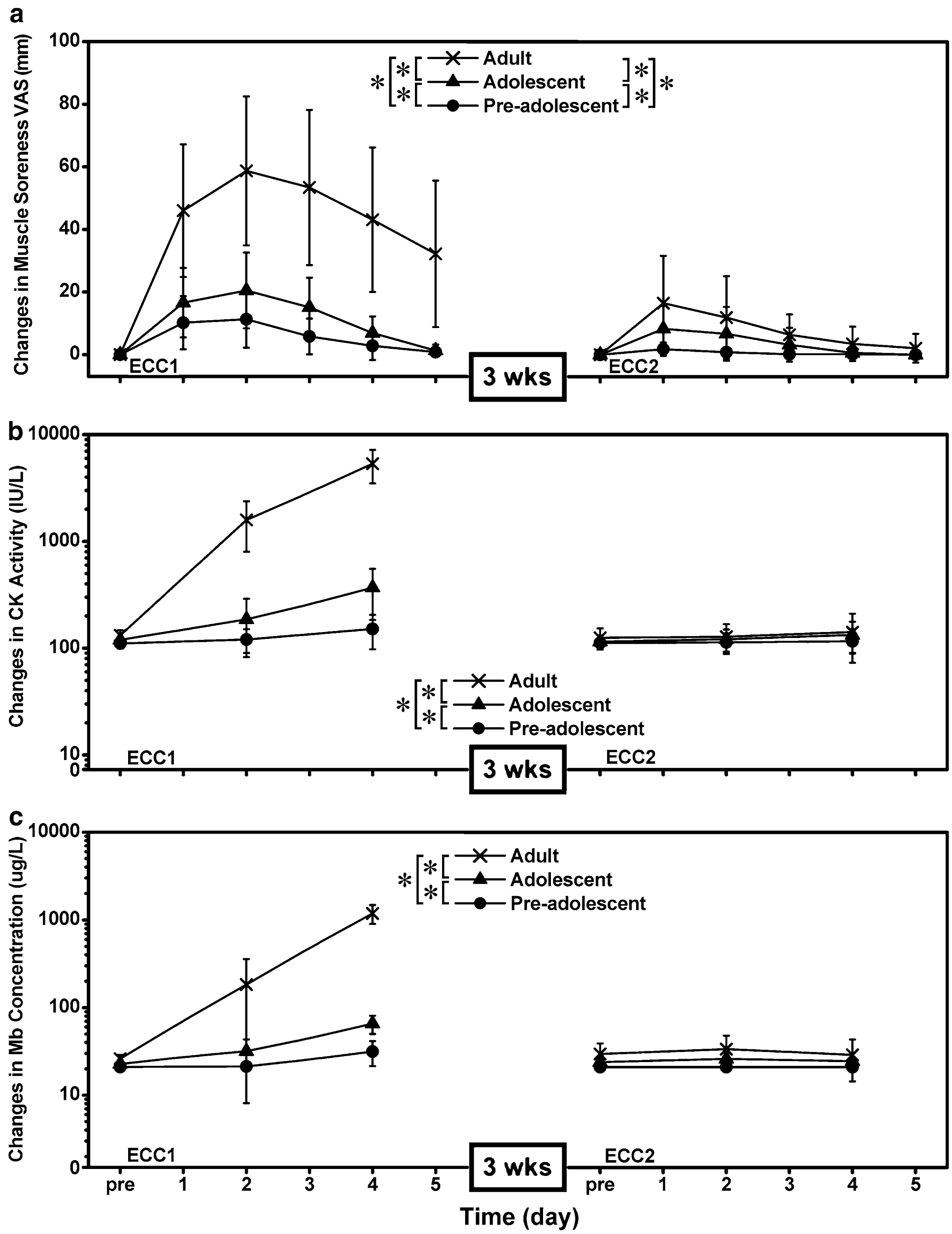

Fig. 4 Changes in muscle soreness assessed by a visual analogue scale (**a**), plasma creatine kinase (CK) activity (**b**) and plasma myoglobin (Mb) concentration (**c**) before (pre) and for 5 days after the first (ECC1) and second (ECC2) eccentric exercise bouts for the pre-adolescent, adolescent and adult groups. *Significant ($P < 0.05$) interaction (group × time) effect based on a pairwise comparison between groups

and large increase in muscle soreness and plasma CK activity as well as Mb concentration collectively indicate that severe muscle damage was induced to the exercised elbow flexors after ECC1 for adult group. The smaller changes in the dependent variables after ECC2 than ECC1 show typical repeated bout effect responses (Chen et al. 2007, 2010). Based on the Tanner's stage (Tanner 1962), all subjects in pre-adolescent group were in the stage I, considered to be pre-puberty, and all subjects in adolescent group were in the stage III–IV, puberty to late puberty. A significant difference in the resting plasma testosterone concentration was also evident between pre-adolescent and adolescent groups, as well as adolescent and adult groups with no overlaps (Table 1). Thus, it seems reasonable to assume that the differences in the changes in the dependent variables after the eccentric exercise among groups were associated with the differences in the maturity. Although the present study used only indirect markers of muscle damage, the differences in the changes in the dependent variables likely represent the differences in the susceptibility to eccentric exercise-induced muscle damage.

As shown in Fig. 1, eccentric contraction torque generated during the exercise was significantly smaller for pre-adolescent and adolescent groups than adult group, and for pre-adolescent than adolescent group. The total work performed during the eccentric exercise was approximately

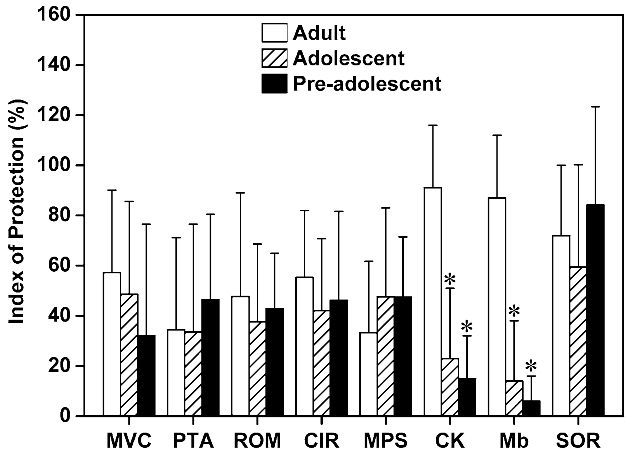

Fig. 5 Comparison in protective index [(ECC1 − ECC2)/ECC1 × 100 %] amongst the pre-adolescent, adolescent and adult groups for maximal voluntary concentric contraction torque of the elbow flexors (MVC), peak torque angle (PTA), range of motion (ROM), upper arm circumference (CIR), muscle passive stiffness (MPS), plasma CK activity (CK), plasma myoglobin concentration (Mb) and muscle soreness (SOR). *Significantly ($P < 0.05$) different from the adult group

28 % for pre-adolescent and 70 % for adolescent group when compared with that of adult group (100 %). The pre-exercise MVC–EF torque of pre-adolescent and adolescent groups was also approximately 25 and 71 %, respectively, of that of adult group (100 %). All subjects reached the target torque that was set at 10 % greater than their maximal voluntary isometric contraction torques, and from the observation, all subjects performed the two eccentric exercise bouts with their maximal effort. Thus, although the torque and total work were smaller for pre-adolescent and adolescent groups when compared with adult group, it seems likely that the relative intensity of the eccentric exercise was similar amongst groups.

The present study, using an arm eccentric exercise model, confirmed that decrease in muscle function was significantly smaller, and DOMS was less developed for boys than young adult men, which were shown in the previous studies (Gorianovas et al. 2013; Marginson et al. 2005) in which leg eccentric exercises were used. The present study was the first to show that the changes in other markers of muscle damage (i.e. ROM, upper arm circumference, plasma CK activity, plasma Mb concentration, muscle passive stiffness) were also smaller for boys than young adults. When comparing the magnitude of changes in the dependent variables after ECC1 among groups, it varied amongst the variables, but importantly, the differences between pre-adolescent/adolescent groups and adult group were greater than those between pre-adolescent and adolescent groups. This suggests that more distinct differences in the susceptibility to eccentric exercise-induced muscle damage exist between children and adults than between pre-adolescent and adolescent boys.

One might argue that the greater magnitude of muscle damage in adult group than other groups, and in adolescent group than pre-adolescent group was simply due to greater mechanical strain induced by greater force generation. However, as discussed above, the relative force production level during eccentric contractions appeared to be similar among groups. It is possible that greater muscle damage of adults was due to their greater lean body mass or greater volume of the elbow flexors. It has been shown that CK activity in the blood is affected by muscle mass such that the greater the skeletal muscle volume, the greater the resting CK activity (Brancaccio et al. 2007). The present study found significantly smaller baseline plasma CK activity and Mb concentration for pre-adolescent than adult group (Table 1), however, the difference was small. Thus, it is unlikely that the difference in the muscle volume could explain the difference in the susceptibility to eccentric exercise-induced muscle damage. It does not appear that simply bigger, stronger muscles were associated with greater damage in the adult group.

Marginson et al. (2005) explained that less muscle damage in pre-adolescent boys than young adult men after plyometric jumps could be related to greater flexibility, fewer fast-twitch muscle fibres, and greater habitual physical activities for boys than adult men. They also reported that the angle at peak torque during isometric contractions of knee extensors for pre-adolescent boys (90° of knee extension, full knee extension = 0°) was greater than that of adult men (80° of knee extension); the angle shifts to a long muscle length. Several studies have shown that a shift of "optimum angle" to a long muscle length makes muscle less susceptible to muscle damage (e.g. Brockett et al. 2001; McHugh and Tetro 2003; McHugh and Pasiskos 2004). The angle at peak torque during eccentric contractions of the elbow flexors was greatest for pre-adolescent group followed by adolescent group, and adult group. It is also noted that the angle at peak torque of pre-exercise MVC–EF torque was smaller for pre-adolescent group than other groups (Table 1), indicating that the optimum angle was at a longer muscle length for pre-adolescent group. This might have contributed to the less muscle damage for pre-adolescent than adult group. It is possible that the flexibility of the shoulder joint was also attributed to the difference in the muscle damage among groups. It has been shown that children are more flexible in their shoulders than adult men (Boone and Azen 1979), and the range of shoulder extension decreases with increasing age in boys between 6 and 17 years old (Koley and Singh 2008). The shoulder joint was set at 45° flexion during the eccentric exercise for all subjects in the present study. It might be that muscle length of bicep brachii was shorter for pre-adolescent and adolescent boys than adult men, inducing less extension of the muscles for the boys than adult men. The present study did not control and account for possible differences in the shoulder joint flexibility among individuals and age groups, since it was thought that the comparison between the groups could be better made by the same exercise protocol. It is interesting to investigate further if the differences in markers of muscle damage across the age groups still exist when the shoulder joint angle is adjusted to account for the difference in the shoulder flexibility among the groups.

As shown in Table 1, pre-exercise muscle passive stiffness indicated by the displacement of the upper arm tissue in response the probe pressure was significantly greater for pre-adolescent and adolescent groups than the adult group, and significantly greater for pre-adolescent than adolescent group. This could indicate that muscles are more compliant for pre-adolescent and adolescent boys than adult men, although it should be noted that the method that the muscle passive stiffness assessed in the present study might not represent the muscle's longitudinal stiffness. Our previous study showed that an 8 weeks knee flexor flexibility training increased hip joint ROM, shifted the optimum angle of the knee flexors to a longer muscle length, and attenuated muscle damage induced by 60 maximal knee flexor eccentric contractions (Chen et al. 2011). McHugh et al. (1999) also suggested that stiffer muscles would result in greater overstretching of the sarcomeres during exercise and hence resulting in more damage. Thus, it is possible that more compliant muscles of children make them less susceptible to eccentric contraction-induced muscle damage. ROM of the elbow joint was also significantly greater for pre-adolescent group than adolescent and adult groups (Table 1). We noticed that all pre-adolescent boys were able to overextend the elbow joint. This suggests that the elbow flexors were not fully extended at the end of each eccentric contraction. These might also have contributed to the less muscle damage in pre-adolescent boys in the present study.

The less magnitude of muscle damage for the pre-adolescent and adolescent groups than the adult group may be also associated with a difference in tendon compliance. Kubo et al. (2006) showed that more compliant tendon structures partially accounted for variations in torque–angle curves of the knee extensors among young men, and stated that greater compliance of the tendon structures might require a longer muscle length (greater joint angle) to attain sarcomere lengths that could elicit peak torque. In a separate study, Kubo et al. (2001) reported that the tendon of the knee extensors was more compliant in pre-adolescent boys (10.8 years, $4.1 \pm 0.9 \times 10^{-2}$ mm/N) than adolescent boys (14.8 years, $2.9 \pm 1.1 \times 10^{-2}$ mm/N) and adult men (24.7 years, $1.8 \pm 0.3 \times 10^{-2}$ mm/N), showing that the compliance of tendon structures decreases with increasing age. If this is also the case for the elbow flexors, possible differences in the tendon properties between children and adults could have attributed to the difference in muscle damage between them. Investigation of muscle–tendon behaviour during eccentric contractions using ultrasonography or other techniques may shed light on the mechanisms underpinning the less muscle damage of children than young adults.

It has been reported that adult muscles have greater proportion of fast-twitch fibres compared with pre-mature muscles (Lexell et al. 1992). Since muscle damage has been reported to occur predominantly to fast-twitch fibres (Friden et al. 1983; Jones et al. 1986; Friden and Lieber 1992), it is possible that the smaller proportion of fast-twitch fibres in pre-adolescent and adolescent boys attributed to less muscle damage when compared with adult men. However, it is not known how much difference in the muscle fibre type existed for the elbow flexor muscles among groups in the present study. It is assumed that the levels of muscle activation during eccentric contractions are different such that the activation was less for children than adults. Further studies are necessary to compare motor unit recruitment between adults and children.

Previous studies (Rowland 1996; Pratt et al. 1999) reported different daily activity levels between pre-adolescents and young adults such that children generally had greater levels of habitual physical activity than adults. It has been shown that the magnitude of muscle damage induced by eccentric contractions is attenuated by prior submaximal eccentric (Chen et al. 2007) or maximal isometric (Chen et al. 2013) contractions. It is possible that children had obtained protective adaptation to eccentric exercise-induced muscle damage through daily activities, but young adults had not, because of reduced daily physical activities. Unfortunately, the activity levels of the participants were not assessed in the present study, thus it is not known whether the greater muscle damage in adult group was due to less daily activities when compared with pre-adolescent and adolescent boys. The differences in the magnitude of muscle damage among groups might be associated with the usage of the elbow flexors in daily activities. Further studies are necessary to investigate these possibilities to elucidate why the magnitude of eccentric exercise-induced muscle damage increases from pre-adolescent, adolescent to post-adolescent.

As shown in Fig. 5, the magnitude of the protective effect conferred by the ECC1 on the second bout was similar amongst groups for all dependent variables except plasma CK activity and Mb concentration. Gorianovas et al. (2013) reported that the protective effect conferred by the first bout against the muscle damage in the second bout was less for boys than adult men, and Marginson et al. (2005) also reported less protective effect for boys than adults; however, this was not found in the present study. The discrepancy between the studies is likely explained by the differences in muscle (leg muscles vs. elbow flexors) and/or exercise protocol (drop jumps or plyometric jumps vs. maximal eccentric exercise of the elbow flexors) used in the studies. Since it has been shown that the greater the magnitude of muscle damage in the initial bout, the greater the magnitude of muscle damage (Nosaka et al. 2001; Chen et al. 2007), we hypothesised that the magnitude of protective effect would be less for pre-adolescent and adolescent boys compared with adult men, and for pre-adolescent than adolescent boys. However, this was not supported by the results. Regarding plasma CK activity and Mb concentration, however, the magnitude of the protective effect was greater for adult followed by adolescent and pre-adolescent group, supporting the hypothesis and the findings of the study by Gorianovas et al. (2013). The greater protective effect was most likely due to the greater increase in plasma CK activity and Mb concentration after ECC1 for adult group than pre-adolescent group. Overall, it appears that muscles adapt to eccentric exercise similarly among pre-adolescent, adolescent and young adult men.

In conclusion, pre-adolescent boys experience less severe symptoms of muscle damage than adolescent boys and adults after eccentric exercise, and adolescent boys have significantly smaller extent of eccentric exercise-induced muscle damage compared with adults. However, no significant difference in the magnitude of the protective effect was evident amongst groups. These results indicate that the magnitude of muscle damage is increased from pre-adolescent, adolescent to post-adolescent, but the adaptability to eccentric exercise is similar among them. It appears that changes in muscle–tendon complex characteristics with maturation make muscles more susceptible to eccentric exercise-induced muscle damage, but further studies using ultrasonography or other techniques are warranted to examine this. It seems likely that children are less susceptible to eccentric exercise-induced muscle damage than adults. Thus, theoretically eccentric exercise can be prescribed to children, but it should be investigated whether children can benefit from eccentric training.

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Conflict of interest The authors declare that we have no any potential conflict of interest.

Ethical standards The authors declare that the experiments comply with the current laws of the country in which we were performed.

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