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Assessment of whole-body DNA oxidation following prolonged exercise in adolescent males and females matched for aerobic capacity

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Abstract

OBJECTIVE: The purpose of this study was to investigate the effects of moderately extended cycling exercise on oxidative DNA damage (accounted for by urinary 8-hydroxy-2´-deoxyguanosine) in adolescent males and females matched for aerobic capacity.

MATERIALS AND METHODS: Twenty-nine aerobically active adolescent males and females matched for peak oxygen uptake (VO_2peak) relative to fat free mass (ml/kg FFM/min) participated in this study. Two-hour urinary samples were taken at three time points before (-2-0h), immediately (0-2h) after and 24-26 h after 60 min of cycling exercise at 65%VO_2peak, followed by the analysis of urinary 8-OHdG (a potential marker of whole-body DNA damage and repair) determined with high performance liquid chromatography with electrochemical detection.

RESULTS: The two-way (time x sex) analysis of variance demonstrated no significant main effects for time, sex or interaction regarding urinary 8-hydroxy-2´-deoxyguanosine level following moderate-intensity endurance exercise.

CONCLUSIONS: These results of the present study suggest that no detrimental DNA damage can be observed after moderately prolonged exercise in aerobically fit males and females, potentially because of the enhanced antioxidant defense responses. Furthermore, the endurance-trained adolescent males and females appear to have similar DNA oxidation responses at the whole-body level when normalized to peak oxygen uptake relative to fat free mass.

INTRODUCTION

Since exercise increases oxygen consumption, it appears to induce an imbalance between reactive oxygen species and antioxidants, causing oxidative stress (Graille *et al.* 2020). In this regard, it has been represented that eccentric exercise and/or high-intensity endurance, inclusive of unaccustomed exercise, may lead to the alterations of the antioxidant defense systems followed by oxidative DNA damage (Radak *et al.* 2013). Contrastingly, there may be protective effects

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of regular endurance exercise training (enhancement of aerobic capacity) in conjunction with the up-regulation of endogenous antioxidant defense and repair systems (improvement of antioxidant capacity), which has been demonstrated by previous studies that trained athletes have less oxidative DNA damage compared with untrained individuals (Pittaluga *et al.* 2006; Yasuda *et al.* 2015).

Most of the oxidatively damaged DNA is basically repaired by the base excision repair pathway, and the oxidized products are excreted in the urine (Graille *et al.* 2020). One of the most widely studied oxidized metabolites is 8-hydroxy-2'-deoxyguanosine (8-OHdG), regarded as a biomarker for the dynamics of oxidative DNA damage and repair from all cells *in vivo* (whole-body level)(Loft *et al.* 1992). Such a sensitive biomarker of DNA damage induced by the hydroxyl radical attack at the C8 position of the nucleobase guanine or its nucleoside guanosine was originally represented in 1984 by Kasai and Nishimura (Kasai & Nishimura, 1984).

Regarding urinary 8-OHdG levels following endurance exercise, previous investigations on healthy adults have represented augmented urinary 8-OHdG excretion after 60 min of cycling exercise at 70%VO_2peak (Orhan *et al.* 2004), after the end of a running marathon (Tsai *et al.* 2001) and a 2-day ultramarathon race (Miyata *et al.* 2008). In contrast to those data, other studies have described no significant change of the urinary 8-OHdG excretion after a single bout of intense exercise (treadmill running, cycling and a 20-km run) (Sumida *et al.* 1997), a 5-h cycling and 40 km time trial (Yasuda *et al.* 2015), a 4-day and 3-week stage road cycling race (Almar *et al.* 2002) and a 894-km relay trail run (Rowlands *et al.* 2012). With reference to some investigations on healthy adolescents, although little has been done on urinary 8-OHdG levels, recent study has represented significantly elevated plasma 8-OHdG levels after exercise in adolescent swimmers (Kabasakalis *et al.* 2019). In contrast, another exercise study has no significant alterations in adolescent male wrestlers (Hamurcu *et al.* 2010).

In the matter of sex comparative phenomena, some researchers have depicted no sex-specific differences in healthy adults at serum 8-OHdG levels, taking into consideration exercise training status and dietary intake (Bloomer & Fisher-Wellman, 2008). Kabasakalis *et al.* (2014) have shown no sex-specific discrepancies in plasma 8-OHdG levels following endurance and high-intensity exercise in adolescent swimmers, albeit there is little data as to exercise-induced DNA oxidation in adolescent males and females matched for aerobic capacity.

It has been suggested that sex steroid hormones may have an influence on the magnitude of oxidative DNA damage (Bloomer *et al.* 2009). Particularly, past studies have indicated estrogen (i.e., 17β-estradiol, one of the sex steroid hormones) can have a potential role of endogenous antioxidant to cell (Kapiszewska *et al.* 2005). In this respect, some investigations suggest that women have higher 17β-estradiol levels and therefore have less oxidative DNA damage than men (Borras *et al.* 2003), whereas others have shown no direct relationship between 17β-estradiol levels and exercise-induced DNA damage (Yasuda & Yano, 2018). Collectively, it remains to be clarified whether there are potentially protective effects of 17β-estradiol concentration on exercise-induced whole-body DNA oxidation in adolescent males and females during the growth period.

When examining sex-specific differences in relation to endurance exercise, an important consideration is to normalize aerobic capacity with a training background in men and women (Lambert *et al.* 2013). Accordingly, matching men and women for aerobic capacity regarded as peak oxygen uptake (VO_2peak) expressed relative to fat free mass (VO_2peak ml/kg FFM/min) helps to identify aerobic capacity between sexes since men commonly have higher VO_2peak and greater fat free mass (Armstrong & Welsman, 2001).

Taken collectively, no studies have evaluated how training status influence exercise-induced whole-body DNA oxidation in adolescent males and females matched for aerobic capacity, albeit many studies have represented the relation between exercise and oxidative stress (Pepe *et al.* 2009). Given the determination of effects of aerobic capacity, including 17β-estradiol levels, on exercise-induced whole-body DNA oxidation in adolescents over the growth and maturation, it could provide critical information to comprehensively improve the exercise training of each young athlete at the optimal level. Consequently, the objective of this study was to clarify the effects of endurance exercise on oxidative DNA damage (accounted for by urinary 8-OHdG excretion) in adolescent males and females matched for aerobic capacity. It was hypothesized that aerobically active adolescent males and females matched for aerobic capacity could have similar exercise-induced DNA oxidation responses at the systemic level.

MATERIAL AND METHODS

Experimental approach to the problem

The participants reported to the laboratory for a proficiency session in order to get used to the experimental protocol at least one week before the experimental trial. Upon their first arrival in the lab, all participants underwent anthropometric measurements, ventilatory threshold (Tvent) and VO_2peak. Consequently, VO_2peak (an index of aerobic capacity) was divided by fat free mass to equally match the physical fitness levels of the participants (ml/kg FFM/min) (Haapala *et al.* 2018), and then each dependent variable was analyzed based on the values. All participants carried out a one-hour cycling exercise corresponding to a constant

Tab. 1. Descriptive characteristics of the participants

Variable	Males (n=15)	Females (n=14)
Age (yrs)	15.1±1.7	15.6±1.0
Height (cm)	165.8±6.9	156.3±3.1*
Body mass (kg)	58.4±12.1	52.1±4.3*
Body mass index (kg/m^2)	21.1±3.2	21.3±1.6
Body surface area (m^2)	1.642±0.182	1.501±0.065*
Body fat (%)	13.1±6.0	23.7±5.1
Fat free mass (kg)	50.3±7.8	39.6±3.0*
VO_2peak (1/min)	3.9±0.6	2.9±0.4*
VO_2peak (ml/kg/min)	68.3±10.2	55.5±7.0*
VO_2peak (ml/kg FFM/min)	78.4±9.5	72.5±5.7
Tvent (1/min)	1.6±0.2	1.3±0.2*
Tvent (ml/kg/min)	27.8±4.0	24.9±2.8*
Tvent (ml/kg FFM/min)	31.8±3.2	32.6±2.9

VO_2peak=peak oxygen uptake, Tvent=ventilatory threshold, FFM=fat free mass, All data are shown as mean±SD. *Significantly different from males (p<0.05).

output of 65%VO_2peak on a stationary cycle ergometer on another occasion (within two weeks after completing the maximal exercise test). All tests were performed in our exercise physiology laboratory.

Participants

Thirty-two physically active adolescent males and females (age: 15-18) were recruited as participants who engaged in athletic club activities (intermediate- to long-distance runners, cross-country skiers, and alpine skiers) from junior high school to high school. After the participants made the first visit to the laboratory, a researcher conducted a screening test using a questionnaire to confirm the medical history and exercise habits that have been performed so far.

All participants have maintained a daily regime of athletic club activities (2-2.5 h/day and 5-6 days/week) for ≥ 6 months. Participants were excluded if they have been taking alcohol, caffeine and nutritional supplements containing antioxidants or medications that could affect oxidative stress or anti-inflammatory conditions. Specifically, female participants reported normal menstrual function for no less than the 6 months without use of any form of oral contraceptives taken prior to participating in this study. After the screening test, a total of 29 individuals (15 males and 14 females) finally participated in this experiment. The female participants executed the submaximal exercise protocol in the earlier follicular phase of the menstrual cycle (within 5-9 days after the start of the menses) to standardize hormonal effects having an impact on metabolic responses to exercise (Tarnopolsky & Ruby, 2001). All experimental procedures were conducted in compliance with the ethical standards of the Helsinki Declaration, and approval given by the University's human ethics review board. Participants provided written informed consent before participating in the present study.

Preliminary measurements

Each participant undertook a physical examination and then evaluated their body composition during a preliminary visit to the laboratory. After an overnight fast of 12 hours (*ad libitum* water intake was allowed), all males and females arrived at the lab (7:00-7:30), followed by anthropometric measurements (height, body mass and percent body fat). Body mass and percent body fat based on bioelectrical impedance analysis (InBody 270, Fujitex Corporation, Tokyo, Japan) were digitally quantified on a calibrated scale with the participants wearing underwear and barefoot. Fat free mass was computed by subtracting fat mass from body weight (Mondal *et al.* 2017). Next, all individuals carried out an incremental exercise test until volitional exhaustion to assess Tvent and VO_2peak implemented on an electromagnetically braked cycle ergometer (Excalibur 600™, Lode B.V., Groningen, Netherlands). Each participant fulfilled a 5 min warm up prior to the testing. All participants accomplished a cycling exercise at an initial power output of 0 W for 3 min, which was increased by 25 W every 1 min until volitional exhaustion. Pedaling frequency was 60 rpm during submaximal exercise. Expired gas samples were analyzed with an automated breath by breath gas analysis system (Oxycon Pro™, Erich Jaeger GmbH, Hoechberg, Germany). Their heart rate was also continuously recorded using an electrocardiograph (BSM-2401 ECG monitor, Nihon Kohden, Tokyo, Japan). The measurements for VO_2peak were based on acquiring at least two of the following three criteria: a plateau in VO_2, respiratory exchange ratio

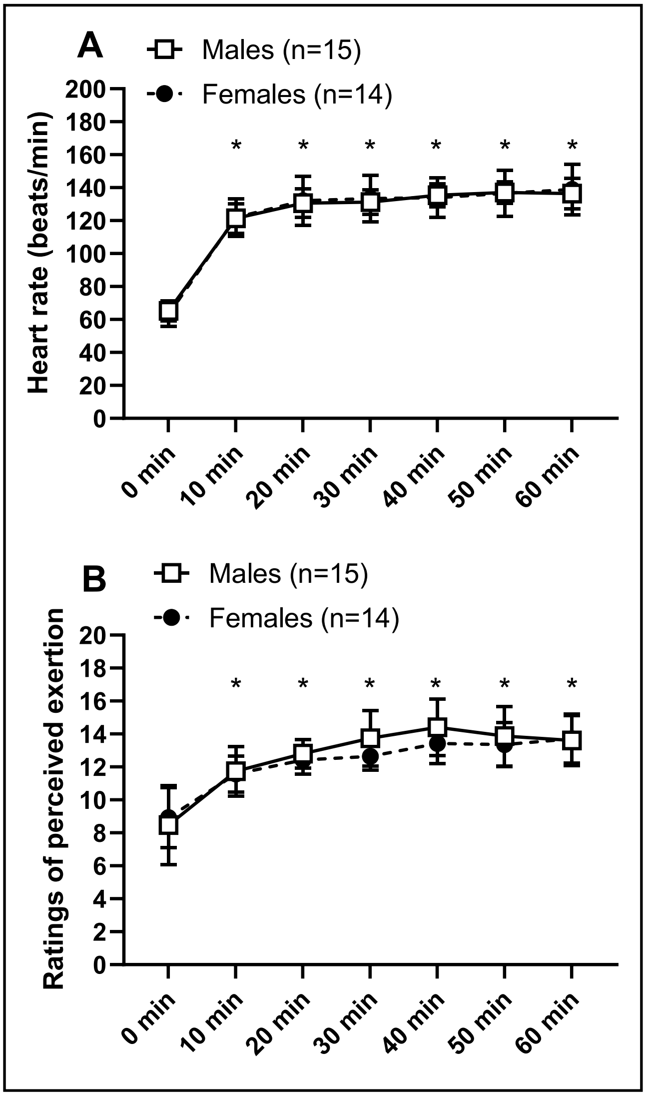

Fig. 1. (A) Heart rate (beats/min) and (B) ratings of perceived exertion during 1-h of prolonged exercise at 65%VO_2peak in adolescent males and females. *Significantly different from baseline (main effect for time, *p*<0.001).

≥ 1.1 and volitional exhaustion (ACSM guideline, 2009).

Assessment of ventilatory threshold (Tvent)

In the present study, Tvent was regarded as an index of anaerobic threshold as shown previously (Yasuda *et al.* 2008). At the same time, three methods were used to quantify Tvent: (i) ventilatory equivalent method (VEQ method) (Shimizu *et al.* 1991), (ii) excess carbon dioxide ($ExCO_2$ method) (Anderson & Rhodes, 1989) and (iii) modified V-slope method using 15-s averaged data (Beaver *et al.* 1986). Two investigators separately assessed each testing record concurrently with the three detection methods per procedure by Gaskill *et al.* (2001).

Experimental procedures

After an overnight fast of 12 hours, all participants came to the lab in the morning (7:00-7:30) on the day of the submaximal exercise trial. Each participant was instructed to abstain from exhaustive exercise for 2 days prior to the trial. Moreover, the participants were requested to maintain and record their normal diet in energy balance with a macronutrient composition of 60% carbohydrate, 25% fat and 15% protein for 5 days preceding the submaximal exercise trial (Yasuda *et al.* 2021).

Each participant carried out 1-h cycling exercise corresponding to a constant power output at 65%VO_2peak, wearing clothing made of an evaporative polyester fabric (Gavin *et al.* 2001), on a stationary cycle ergometer. Heart rates and ratings of perceived exertion were monitored to keep exercise intensity constant, recorded every 10 min over submaximal exercise. To be on the safe side, from the perspective of preventing dehydration during exercise and maintaining the body's energy supply, the participants drank a 6.2% commercially available carbohydrate solution (Pocari Sweat®, Otsuka Pharmaceutical Co., Ltd.; energy: 104.6 kJ/100 ml, carbohydrate: 6.2%, Na^+: 21 mEq/l, K^+: 5 mEq/l, Cl^-: 17 mEq/l, osmolality: 340 mOsm/l) immediately before the start (0 min) of cycling exercise (3 ml/kg body mass) and every 20 mins up to 40 min during 1-h exercise (2 ml kg/body mass) as demonstrated by Nassis *et al.* (1998). At 60 min (the completion of the exercise), carbohydrate solution was not consumed. The laboratory temperature and relative humidity were constant during all submaximal trials (21.0-21.6°C and 41-45%, respectively).

Urinary sample collection

Two-hour urinary specimens were taken at three time points [before (-2 to 0 h), immediately after (0 to 2 h) and 24-26 h after 1-h exercise] since it was difficult for participants to collect 24-hour urine over a long period of time from a practical point of view (Waller *et al.* 1971a, 1971b). Each participant collected their urinary samples with a sterile urine collection cup, transferring it to a 15 ml tube. All urinary samples were stored at -80°C for the subsequent analysis of the urinary 8-OHdG.

Quantification of urinary 8-OHdG level

Urine 8-OHdG levels were determined using high performance liquid chromatography (HPLC) with electrochemical detection (ECD) on the basis of the protocol demonstrated by Nakano *et al.* (2003). Succinctly, the detection of 8-OHdG in urine was carried out using a 2-column switching HPLC method. The HPLC system consisted of two pumps, an automatic injection device, two analytical reverse phase columns, a column oven, a valve unit, an electrochemical detector, and an integrator. The electrochemical detector operated at +370 mV with respect to the Ag-AgCl reference electrode. The main mobile phase was treated with 1000 ml 30 mM phosphate buffer (pH 6.9) consisting of ethylenediamine tetraacetic acid, disodium salt (200 mg/l), and 20.8 ml tetrahydrofuran. During the process of biochemical assays, 400 μl of the HPLC mobile phase was added to 100 μl of urine, centrifuged at 10,000 rpm for 5 minutes, and then 35 μl of the mixture was infused into the HPLC system. In order to normalize the urinary 8-OHdG concentration, it was consequently expressed as nanogram per milligram creatinine.

Determination of steroid hormone (17β-estradiol and testosterone) levels

Urinary 17β-estradiol concentration was analyzed by radioimmunoassay as demonstrated previously (Tominaga *et al.* 1975). A 0.5 ml aliquot of the urine sample was hydrolyzed with 0.075 ml HCl by heating at 100 ° C for 60 minutes. After extracting 17β-estradiol with 4 ml of ether, the extract was washed with 0.5 ml of distilled water and evaporated to dryness under nitrogen. The dried extract was dissolved in 0.1 ml of a benzene-methanol mixture and applied to a Sephadex LH-20 microcolumn. The column was eluted with the same solvent. Urinary testosterone concentration was analyzed based on a commercially available ELISA kit (Abcam, Cambridge, UK). Finally, urinary 17β-estradiol and testosterone concentrations were described as nanogram per milliliter.

Statistical Analyses

A two-way analysis of variance (ANOVA) for repeated measures on two factors (sex x sampling time) proceeded with a statistical package (Statistica V5.1 for Windows, Statsoft, Tulsa, OK, USA), after confirming the normal distribution of the dependent variables with the D'Agostino-Pearson omnibus test. When significance was recognized, the location of the difference was clarified using a Tukey's post-hoc test. Effect sizes for t-test and ANOVA were calculated with Cohen's d and eta squared (η^2), respectively (Kirk, 2007). The degree of effect sizes based on Cohen's d and η^2 is as follows: $0.2 < d < 0.5$ = small effect, $0.5 < d < 0.8$ = medium effect, and $d > 0.8$ = large effect; $0.01 \leq \eta^2 < 0.06$ = small effect, $0.06 \leq \eta^2 < 0.14$ = moderate effect, $\eta^2 \geq 0.14$ = large effect, respectively (Kirk, 2007). Pearson's correlation analysis was performed to clarify the relation of percent change in urinary 8-OHdG concentration with indexes of aerobic fitness (Tvent and VO_2peak) and sex steroid hormone concentration (17β-estradiol and testosterone). Statistical significance was set at the 0.05 level. All values are shown as mean±SD.

RESULTS

Descriptive characteristics and physiological responses

Descriptive characteristics of the participants are described in Table 1. There was similar aerobic capacity in Tvent (ml/kg FFM/min, p=0.512, Cohen's d=0.3) and VO_2peak (ml/kg FFM/min, p=0.064, Cohen's d=0.7) between males and females when normalized relative to fat free mass (after matching-process).

Significant differences were observed in heart rate (beats/min, main effect for time, p<0.001, η^2=0.85), but for sex (p=0.814, η^2=0.01) or interaction (p=0.970, η^2=0.01) over 1-h moderately extended exercise (Figure 1A). Furthermore, there were significant alterations in ratings of perceived exertion (main effect for time, p<0.001, η^2=0.58), whereas no significant changes were noted for sex (p=0.089, η^2=0.02) or interaction (p=0.428, η^2=0.03, Figure 1B). As shown in the data above, both males and females completed prolonged exercise while maintaining 65% of exercise intensity as constant as possible (Figure 1A and 1B).

Additionally, significant discrepancies were found with respect to loss of body mass (kg, main effect for sex, p<0.05, η^2=0.10), but not for time (p=0.933, η^2=0.01) or interaction (p=0.959, η^2=0.01) immediately after endurance exercise. Moreover, significant differences were identified regarding percent change of body mass (kg) between adolescent males (-0.55±0.4%) and females (-0.16±0.2%; mean±SD) (p<0.01, Cohen's d=0.8) before and shortly after moderate-intensity aerobic exercise.

Urinary biomarkers

In terms of urinary 8-OHdG concentration (ng/ml), no significant changes were found for time (p=0.421, η^2=0.02), sex (p=0.771, η^2=0.01) or interaction (p=0.894, η^2=0.01) before and after 1-h of long-lasting exercise (Figure 2A). Furthermore, there were no significant discrepancies in urinary 8-OHdG concentration relative to creatinine (ng/mg creatinine) for time (p=0.432, η^2=0.02), sex (p=0.610, η^2=0.01) or

Tab. 2. Association of the percent change in urinary 8-OHdG concentration (ng/mg creatinine) before (-2-0h) and after exercise (24-26h) with Tvent and VO_2peak (ml/kg FFM/min) in adolescent males and females

	Tvent			VO_2peak		
	r	**p**	**Significance**	**r**	**p**	**Significance**
Males (n=14)						
PC-u-8-0HdG	0.340	0.235	ns	0.637	0.147	ns
Females (n=11)						
PC-u-8-0HdG	0.532	0.092	ns	0.297	0.375	ns

Tvent: ventilatory threshold, VO_2peak: peak oxygen uptake, r: correlation coefficient, p: probability value, ns: no significance, PC-u-8-0HdG: percent change in urinary 8-hydroxy-2'-deoxyguanosine concentration

Tab. 3. Relation of 8-0HdG (ng/mg creatinine) with 17β-estradiol and testosterone concentration (ng/ml) in urine before (-2-0h) and after exercise (24-26h) in adolescent males and females

	17β-estradiol			Testosterone		
	r	p	Significance	r	p	Significance
Males (n=12)						
u-8-0HdG	0.040	0.853	ns	0.213	0.368	ns
Females (n=11)						
u-8-0HdG	0.192	0.391	ns	0.131	0.583	ns

Tvent: ventilatory threshold, VO_2peak: peak oxygen uptake, r: correlation coefficient, p: probability value, ns: no significance, u-8-0HdG: urinary 8-hydroxy-2'-deoxyguanosine concentration

interaction (p=0.928, η^2=0.01, Figure 2B). In addition, no significances were recognized in relation to percent change urinary 8-OHdG (ng/mg creatinine) for time (p=0.654, η^2=0.01), sex (p=0.795, η^2=0.01) or interaction (p=0.270, η^2=0.03, Figure 2C). With regard to urinary creatinine concentration (mg/dl), no significant changes were identified (main effect for time, p=0.784, η^2=0.01), in connection with the lack of significant discrepancies for sex (p=0.154, η^2=0.03) or interaction (p=0.894, η^2=0.01, Figure 2D). As to urinary 17β-estradiol concentration (ng/ml), significant main effects were noted for sex (p<0.05, η^2=0.09), notwithstanding no significances for time (p=0.937, η^2=0.01) or interaction (p=0.450, η^2=0.01). Moreover, no significant differences were observed in urinary testosterone concentration (ng/ml) for time (p=0.268, η^2=0.03), sex (p=0.357, η^2=0.03) or interaction (p=0.834, η^2=0.01, Figure 2F).

Correlation analysis

Pearson's correlation analysis demonstrated no significant association of the percent change in urinary 8-OHdG concentration (ng/mg creatinine) before (-2-0 h) and after exercise (24-26 h) with Tvent (ml/kg FFM/min) and VO_2peak (ml/kg FFM/min) (Table 2). Additionally, there was no relation of 8-OHdG with 17β-estradiol and testosterone concentration in urine before (-2-0 h) and after exercise (24-26 h) in adolescent males and females (Table 3).

DISCUSSION

The main findings of the current study were that aerobically fit adolescent males and females matched for aerobic capacity appear to have similar DNA oxidation responses at the systemic level following 1-h of moderately prolonged exercise. To our knowledge, this was the first study to determine endurance exercise-induced whole-body DNA oxidation in adolescent males and females matched for VO_2peak relative to fat free mass.

In terms of endurance exercise-induced whole-body DNA oxidation accounted for by urinary 8-OHdG levels, previous studies dealing with healthy adults have reported elevated urinary 8-OHdG excretion after 60 min of cycling exercise at 70%VO_2peak (Orhan *et al.* 2004), following the end of a running marathon (Tsai *et al.* 2001) and a 2-day ultramarathon race (Miyata *et al.* 2008). As opposed to those data, other investigations have shown no significant differences of the urinary 8-OHdG excretion after a single bout of intense exercise (treadmill running, cycling and a 20-km run) (Sumida *et al.* 1997), a 5-h cycling and 40 km time trial (Yasuda *et al.* 2015), a 4-day and 3-week stage road cycling race (Almar *et al.* 2002) and a 894-km relay trail run (Rowlands *et al.* 2012).

In line with the aforementioned findings, while much less work has been done on urinary 8-OHdG levels, past research coping with healthy adolescents have exhibited significantly increased plasma 8-OHdG concentration following exercise in adolescent swimmers (Kabasakalis *et al.* 2019). On the contrary, another investigation has depicted no remarkable oxidative DNA damage attributable to plasma 8-OHdG levels after regular wrestling exercise in adolescent male wrestlers (Hamurcu *et al.* 2010), which is in accordance with our findings that no significant whole-body DNA damage explained by urinary 8-OHdG levels was observed after 1-h of moderately prolonged exercise, aside from poor correlation found between aerobic capacity and whole-body DNA oxidation.

A more plausible explanation for the differences in the aforementioned results may be ascribed to the disparity in cardiorespiratory fitness level for each individual. It has been introduced that a higher aerobic fitness results in the lower DNA damage in humans (Soares *et al.* 2015b; Mota *et al.* 2010), which indicates an up-regulation of antioxidant capacity at the tissue (Marciniak *et al.* 2009; Mota *et al.* 2010) and systemic levels (Yasuda *et al.* 2015). In this respect, VO_2peak, together with Tvent, have been regarded as a reference standard for aerobic capacity to use oxygen at peak metabolic demands in connection with cardiovascular, respiratory and metabolic function because it can be augmented by regular endurance exercise (Lyerly *et al.* 2009; Soares *et al.* 2013). In fact, lower aerobic capacity has been associated with several cardiovascular diseases as well as all causes of mortality (Lyerly *et al.* 2009). Thus, it is plausible that individuals with

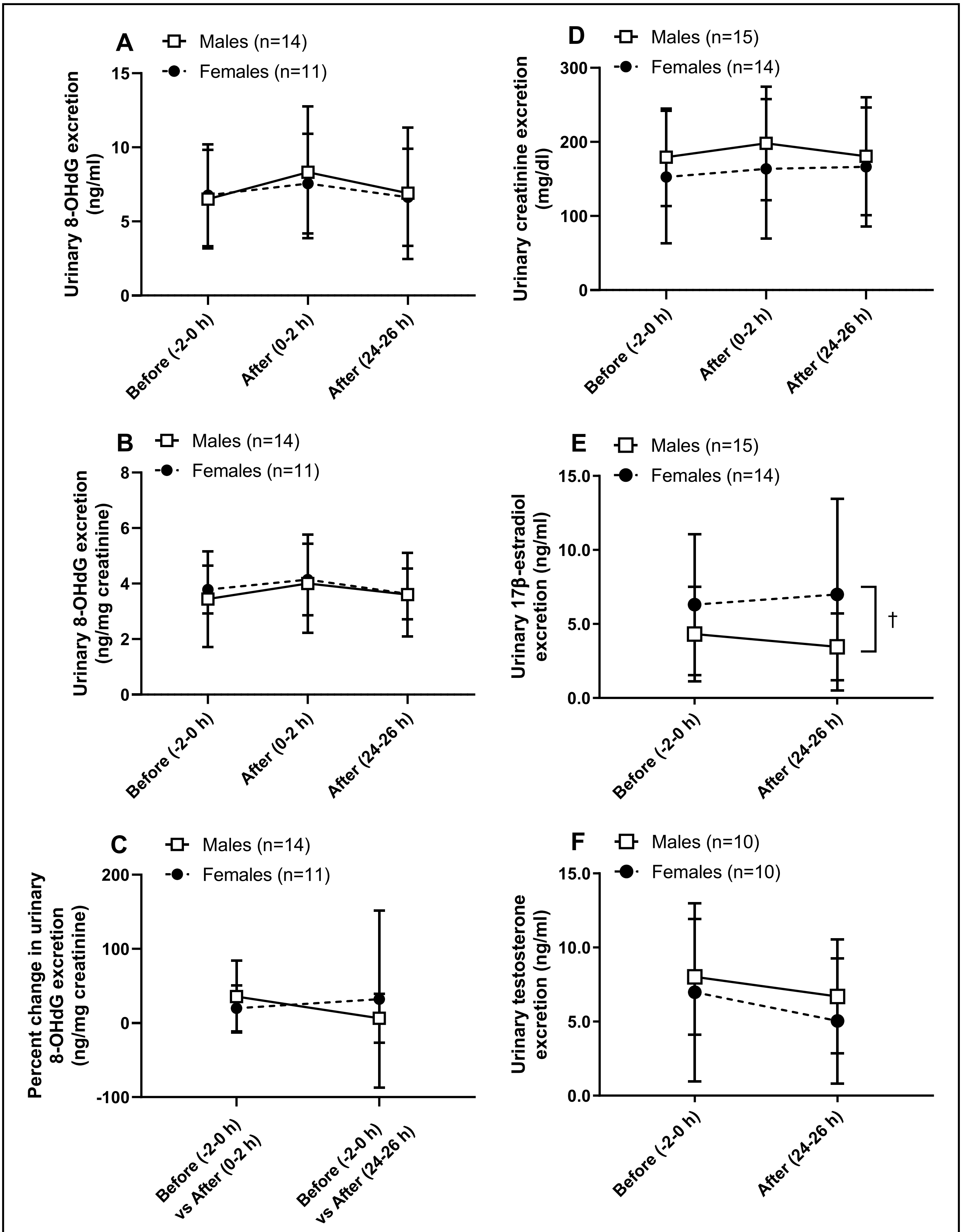

Fig. 2. (A) 8-OHdG (ng/ml), (B) 8-OHdG relative to creatinine (ng/mg creatinine), (C) percent change in 8-OHdG relative to creatinine (ng/mg creatinine), (D) creatinine (mg/dl), (E) 17β-estradiol (ng/ml) and (F) testosterone concentration (ng/ml) in urine before and after 1-h of moderately extended exercise in adolescent males and females. †Main effect for sex (*p*<0.05).

higher levels of aerobic capacity would have lower levels of DNA damage (Soares *et al.* 2013). Collaterally, the production of relatively lower levels of reactive oxygen species induced by regular endurance exercise training at moderate intensity can be beneficial as it may lead to an up-regulation of some antioxidant enzymes such as such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase (Rowlands *et al.* 2012; Soares *et al.* 2015c). On the other hand, prolonged exercise at high-intensity may significantly restrict visceral blood flow, which temporarily deprives oxygen supplies to tissues, causing overproduction of reactive oxygen species (Shephard & Johnson, 2015). In this context, Moreno-Villanueva *et al.* (2019) attempted to clarify lymphocyte DNA damage following single-bout exhaustive exercise in trained (maximal oxygen uptake: $VO_2max > 55$ ml/kg/min) and untrained ($VO_2max < 45$ ml/kg/min) adult male individuals, reporting that only the latter represented DNA damage, whereas the trained individuals speedily repaired oxidatively damaged DNA. Taken collectively, as no conclusions can be drawn about the precise relationship between aerobic capacity and exercise-induced whole-body DNA oxidation, additional work is required to verify whether enhanced aerobic capacity after endurance exercise training plays a causal role in preventing exercise-induced oxidative DNA damage, or whether it is a mere epiphenomenal event associated with potential endogenous antioxidants.

As to the sex-specific comparisons in antioxidant capacity accounted for by 8-OHdG levels, it has been demonstrated that men have different reactions to free-radical production after exercise compared to women (Ginsburg *et al.* 2001; Mastaloudis *et al.* 2004). Contrariwise, some investigators have noted no differences in healthy adult men and women at serum 8-OHdG levels, in view of exercise training status and dietary intake (Bloomer & Fisher-Wellman, 2008). In addition, another study has shown that the adaptation to changes in antioxidant capacity could be different between sexes (Ilhan *et al.* 2004).

Along the lines of those phenomena, recent investigation on adolescent males and females has recounted no sex-specific disparities in urinary 8-OHdG excretion after endurance and high-intensity exercise (Kabasakalis *et al.* 2014). Similarly, our findings of the present study showed no sex-specific divergence in urinary 8-OHdG concentration after 1-h of endurance exercise at moderate intensity. It is reasonable to suppose that training adaptation with the enhanced levels of antioxidant system may have occurred in adolescent males and females, though relatively little work has been directed towards exercise-induced DNA oxidation in adolescent males and females matched for aerobic capacity. On this matter, it is necessary to compare males and females who have similar aerobic capacity in order to examine whether there are differences between males and females in terms of DNA damage and its ability to repair. Accordingly, an essential contemplation is required to normalize aerobic capacity predicated on a training background in men and women when clarifying sex-specific differences in metabolism pertaining to prolonged exercise (Lambert *et al.* 2013; Tarnopolsky & Ruby, 2001). In other words, matching men and women for VO_2peak expressed relative to fat free mass (ml/kg FFM/min) contributes to ascertaining factual aerobic capacity between men and women since there is a tendency for men to commonly have higher VO_2peak and greater fat free mass compared to women (Armstrong & Welsman, 2001). Therefore, the present study was conducted in adolescent males and females matched for VO_2peak expressed relative to fat free mass.

Adolescence refers to the transition between childhood and adulthood whose onset comprises pubertal maturation (Harden *et al.* 2014). In that period, sex steroid hormones such as estrogens (i.e., 17β-estradiol) and androgens (i.e., testosterone) play pivotal roles in developmental and reproductive functions (Courant *et al.* 2010). Resting testosterone secretion in adolescence is known to be higher in males than in females (Harden *et al.* 2014). However, it has been demonstrated that testosterone is highly responsive to training and competition in women (Crewther *et al.* 2011), which indicates some potential to elevate testosterone concentration due to chronic adaptations. In this regard, previous investigations have represented greater exercise-induced testosterone augmentation in adult elite women compared with non-athletes (Cook *et al.* 2012). In the present study, no significant disparities were noted between males and females in urinary testosterone concentration. The reason why there was no discrepancy in testosterone levels between males and females may be related to the degree of training adaptation of females in this study (Courant *et al.* 2010). While it remains unclear whether testosterone has the potential as an endogenous antioxidant in the body, detailed studies should be conducted to validate the characteristics of testosterone following acute and chronic exercise in adolescent males and females, as the results may differ depending on the type of sample handled and the method of analysis (Alexander *et al.* 2021).

It has been known that women may have a higher antioxidant potential compared to men by virtue of the antioxidant effect of sex steroid hormones such as 17β-estradiol (Demirbag *et al.* 2005). Some studies on female rats found a higher antioxidant tissue capacity and lower susceptibility to oxidative stress (Katalinic *et al.* 2005). In human studies, premenopausal women appear to be less susceptible to oxidative stress than postmenopausal women, connoting a relationship between estrogens and antioxidant capacity (Trevisan *et al.* 2001). Although there have been previous studies in which the potential of estrogens as an antioxidant was not recognized, inconsistent findings with regard to the effects of sex hormones on the female redox balance could be attributed to the potential reaction of estrogens as antioxidants and pro-oxidants (Nathan & Chaudhuri, 1998). Supplementally, blood estrogen levels in female athletes can be reduced due to exercise-induced imbalances (Warren & Perlroth, 2001).

According to Bloomer *et al.* (2009), 17β-estradiol can be endogenous antioxidant to cell. Especially, preceding investigation has manifested the protective effect of 17β-estradiol on oxidative DNA damage under resting condition (Kapiszewska *et al.* 2005). In this connection, some studies have signified that women have higher 17β-estradiol levels in association with less oxidative DNA damage compared to men (Borras *et al.* 2003). In contrast, other reports have exhibited no apparent link between 17β-estradiol levels and oxidative DNA damage on the basis of correlation analysis (Yasuda & Yano, 2018). To some degree, the divergence concerning the influence of 17β-estradiol on oxidatively damaged DNA may have partially been owing to inter- and intra-individual variability on endogenous antioxidant status (Yasuda & Yano, 2018). However, there remain unanswered questions regarding whether the potential impact of 17β-estradiol on oxidative DNA damage is causative or an epiphenomenon in adolescent males and females over the growth and maturation period.

When it comes to the effects of carbohydrate supplementation on oxidative DNA damage after exercise training, one study has demonstrated that consumption of a carbohydrate solution resulted in less DNA damage and diminished the acute post-exercise inflammation response, providing better recovery during training (de Sousa *et al.* 2012). Though it was considered that carbohydrate intake during 1-h of moderate endurance exercise was sufficient to keep an euglycemic state constant in this study (Baker & Jeukendrup, 2014; Henriksen, 2002), the question has been raised as to whether carbohydrate consumption is effective in reducing the marked increase in exercise-induced DNA oxidation at the systemic level (Yasuda *et al.* 2015). Accordingly, further investigations are required to shed light on those phenomena.

Limitations of the study

The rationale behind 24-hour collection is that variability of urinary protein loss fluctuates significantly over 24-hours and collection of urine samples shorter than this period may not accurately reflect the actual amount of daily protein loss (Ginsberg *et al.* 1983). However, this method has major disadvantages, which are that the handling of the urinary samples becomes complicated and the compliance of the participants lowers because it takes a long time to collect the samples, inclusive of improper mixing or spillage (Somnathan *et al.* 2003). Thus, 2-hour urine collection was used in this study as a surrogate measure of 24-hour urinary excretion (Somnathan *et al.* 2003). Besides, it is important to mention that urinary creatinine excretion is constant under normal conditions but creatinine excretion is not stable after exhaustive/long-lasting exercise as suggested previously (Almar *et al.* 2002; Orhan *et al.* 2004; Yasuda *et al.* 2015). In this study, creatinine excretion tended to increase after endurance exercise, regardless of no significant difference observed. From the above, caution should be taken when assessing urinary 8-OHdG levels using creatinine-corrected values after prolonged exercise. Additionally, dietary variables were not included in any correlation analysis with either whole-body DNA oxidation or sex steroid hormonal biomarkers. Furthermore, as biological maturation measurement using the Tanner scale was not used for logistical reasons in this study, there may have been some error in assessing maturity. Moreover, concomitant quantification of body mass and percent body fat in the present study was carried out by bioelectrical impedance analysis, which is convenient, ease and low cost for athletes and investigators, yet it is inherently disposed to assessment errors compared with other devices such as dual-energy X-ray absorptiometry (Moon, 2013).

In conclusion, the findings of the current study imply that no detrimental DNA damage can be observed after moderately prolonged exercise in aerobically trained males and females, potentially by virtue of the augmented antioxidant defense responses. Furthermore, the endurance-trained adolescent males and females appear to have similar DNA oxidation responses at the systemic level when matched for peak oxygen uptake relative to fat free mass.

Perspectives

The assessment of endurance exercise-induced whole-body DNA oxidation is needed to answer the question of how much the enhanced antioxidant defense system induced by training adaptations are sufficient to protect adolescent males and females who are engaged in sports activities during a growth period. Bearing in mind this, it is worth mentioning that when investigating the effects of endurance exercise training on whole-body DNA oxidation, it might be important to contemplate the genetic variations of DNA repair systems, namely the hOGG1 Ser326Cys polymorphism (Soares *et al.* 2015a), in conjunction with potential endogenous antioxidants, since it could have an influence on the findings in adolescent males and females.

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DISCLOSURE STATEMENT

All authors declare no conflict of interest in the present study.

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