DNA Oxidation and Proteinuria Induced by Successive Triathlons Associated with Long-term Training: A Follow-up Case Study

長期トレーニングに付随したトライアスロン競技後のDNA損傷量と尿タンパク量：ケーススタディ

YASUDA, Nobuo
Department of Physical Education
International Pacific University
安田　従生
環太平洋大学体育学部体育学科

CARDOZO-PELAEZ, Fernando
Department of Biomedical and
Pharmaceutical Sciences
The University of Montana
カードゾペレッツ・ファーナンド
米国モンタナ大学薬学部

BOLIN, Celeste
Department of Biomedical and
Pharmaceutical Sciences
The University of Montana
ボリン・セレステ
米国モンタナ大学薬学部

RUBY, Brent
Department of Health and Human Performance
The University of Montana
ルビー・ブレント
米国モンタナ大学健康運動科学部

要旨：本研究の目的は，１人のトライアスリートによる長期間のトレーニングと連続する４大会のトライアスロン競技がDNA損傷に及ぼす影響を検討することであった。被検者は，成人男性トライアスリート（年齢：38 year，身長：185.4 cm，体重：78.8 kg）で，ハーフアイアンマン（1.9 km水泳，90 km自転車，21.1 kmランニング）とフルアイアンマン（3.9 km水泳，180 km自転車，42.2 kmランニング）を含む連続する４競技会を完走した。競技会AとCは，ハーフアイアンマン，競技会BとDは，フルアイアンマンから構成された。競技会B, C, 及びDで，８−ハイドロキシディオキシグアノジン（８−OHdG）をマーカーとしたDNA損傷と修復のバランスを定量化するために，競技会出場前日と出場後１，２，４日目に，24時間尿が採取された。競技会B，C，及びDでは，競技出場後１日目ではDNA損傷と修復が多く見られたが，４日目ではほぼ通常値に戻る傾向を示した。また，連続する競技会で，運動時間および距離が異なるにも関わらず，同様な傾向が見られた。本研究結果により，長期間のトレーニングと連続する競技会出場によって，抗酸化能力が高まることが明らかとなった。

Abstract：The purpose of this study was to determine alterations in whole body DNA oxidation and proteinuria induced by successive triathlons in addition to long-term training by the triathlete. A trained male triathlete (age: 38; height 185.4 cm; body weight: 78.8 kg) completed four successive triathlons (in order, races A, B, C, and D). The races A and C consisted of half-Ironman distances (1.9 km swim, 90 km bike, 21.1 km run) and races B and D full-Ironman distances (3.9 km swim, 180 km bike, 42.2 km run). For all races, 24-hour urine output was collected on the day before (Pre) and after the race (Day 1) to evaluate exercise-induced proteinuria. For races B, C, and D, 24-hour urine output was collected on the day before the race and days 1, 2, and 4 post-race to determine exercise-induced DNA damage accounted for by urinary 8-hydroxy-2'-deoxygunosine (8-OHdG). One-way Analysis of Variance (ANOVA) revealed significant increases in urinary 8-OHdG at Day 1 compared with Day 4. This demonstrated that 8-OHdG excretion tended to be higher during Day 1 post-race gradually returning to the baseline around Day 4. Similar patterns of urinary 8-OHdG

concentrations were observed among three races (races B, C and D) regardless of distance. There were also similar patterns of proteinuria (total protein, albumin, N-acetyl-β-D-glucosaminidase, creatinine) at Pre and Day 1 in all four races (races A, B, C, and D). These observations indicate limited accumulative effects of whole body DNA oxidation based on the transient changes in urinary 8-OHdG excretion after successive races. This may in part be related to an enhanced antioxidant capacity in a trained individual.

キーワード：oxidative stress, 8-hydroxy-2'-deoxyguanosine, renal function, hydration, strenuous exercise

Introduction

Exercise appears to augment the generation of reactive oxygen (ROS) and nitrogen species (RNS) *in vivo* as a function of exercise-induced oxidative stress (Pilger et al., 1997; Radak et al., 2000). The electron transport chain in the mitochondria is considered to be a major ntracellular site associated with ROS and RNS generation during exercise (Okamura et al., 1997; Radak et al., 1999). Exercise-induced ROS and RNS can increase DNA strand breaks and modifications to the bases (Van Remmen et al., 2003). A commonly used oxidative DNA damage marker is 8-hydroxy-2'-deoxyguanosine (8-OHdG)(Pilger et al., 1997; Radak et al., 2000). Specifically, urinary 8-OHdG excretion is regarded to reflect the integrated rate of oxidative DNA damage and repair in the whole body (Pilger et al., 1997; Radak et al., 2000). Previous studies regarding strenuous exercise have demonstrated increased urinary 8-OHdG concentrations after road cycling (Almar et al., 2002), marathon (Tsai et al., 2001), super-marathon running (Radak et al., 2000), 8-d running training camp (Okamura et al., 1997), and 30-d physical training (Poulsen et al., 1996). In contrast, others report no alterations following habitual long-distance running (Pilger et al., 1997), after swimming and running (Inoue et al., 1993), and progressive resistance training (Rall et al., 2000).

With regard to the exercise-induced adaptive responses, repeated exercise and training are considered to enhance antioxidant defense and repair mechanisms *in vivo* (Niess et al., 1996; Hartmann et al., 1994). According to Radak et al. (2003), regular training appears to augment repairing systems as evidenced by the up-regulation of DNA repair enzymes including human 8-oxoguanine DNA glycosylase1 (hOGG1) and oxidized purine-nucleoside triphosphatase (hMTH1) to protect against exercise-induced DNA damage. This observation can result in an exercise-induced adaptation process (Radak et al., 2003).

Previous studies have shown exercise-induced DNA oxidation in a variety of endurance exercise such as ultra-marathon (Radak et al., 2000) and short-course triathlon (Hartmann et al., 1998). Triathlon is a unique endurance exercise event, composed of swimming, cycling and running, which originated as the Hawaii Ironman Triathlon in 1978 (van Rensburg et al., 1986). Hartmann et al. (1998) demonstrated no increase in urinary 8-OHdG excretion up to Day 5 post-exercise after a short-course triathlon. In addition, Palazzetti et al. (2003) observed no change in leukocyte DNA damage in triathletes after a 4-week period of training. Although it remains unclear how repeated exercise and training may influence oxidative DNA damage, to our knowledge, there are no data associated with the long-term training and competition on oxidatively damaged DNA with regard to the half- and full-ironman distance triathlons.

Exercise is associated with an increase in urinary protein (Poortmans, 1985). It has been reported that exercise-induced proteinuria depends on the intensity of exercise, rather than the duration of exercise based on the evidence of strong correlation between lactate and proteinuria (Poortmans, 1985). Urinary total protein excretion is considered to be a combination of glomerular permeability, tubular leakage, tubular secretion, and normal urinary protein excretion by the kidneys (Newman et al., 2000). In addition, urinary albumin (a high-

molecular-weight protein) excretion mainly reflects glomerular permeability (Newman et al., 2000). β_2-microglobulin (a low-molecular-weight protein) is freely filtered at the glomerulus and reabsorbed by the renal proximal tubule (Miyai and Ogata, 1990; Yaguchi et al., 1998). N-acetyl-β-D-glucosaminidase (NAG), a lysosomal enzyme derived from proximal tubular cells, is a sensitive marker of renal tubular disturbance (Miyai and Ogata, 1990; Yaguchi et al., 1998). Thus, increased urinary excretion of β_2-microglobulin and NAG is referred to as common markers of tubular dysfunction (Miyai and Ogata, 1990; Yaguchi et al., 1998).

In terms of the observation using aforementioned proteinuria markers, there are previous reports regarding the effects of strenuous exercise on proteinuria. For example, several studies indicate that strenuous exercise appears to acutely induce renal dysfunction but does not cause chronic to glomerular and tubular function (Miyai and Ogata, 1990) as urinary protein excretion usually returns to the baseline within 24-48 hours (Poortmans, 1985). With respect to triathlon events, some studies have shown transient effects on renal function (Edes et al., 1990; Farber et al., 1987). Moreover, Yaguchi et al. (1998) reports the effect of short-course triathlon on urinary enzyme and protein excretion. These results demonstrated that glomerular damage appears to persist in some individuals who perform strenuous exercise. However, whether there are cumulative effects after repeated triathlon events and training has not been addressed.

Therefore, the purpose of this study was to determine alterations in whole body DNA oxidation and proteinuria induced by successive triathlons which was accompanied by long-term training in a triathlete. It was hypothesized that urinary 8-OHdG and protein concentrations would be increased after each race, while the excretion pattern of urinary 8-OHdG and protein concentrations would be similar over four consecutive half and full Ironman races regardless of race duration. Furthermore, we hypothesized that there would be no cumulative effects on urinary 8-OHdG and protein concentrations over successive triathlon events.

Methods

Subject

A trained male triathlete served as the subject for four successive triathlons over a two-year training cycle. The experimental triathlon events consisted of the race A (half-Ironman), race B (full-Ironman), race C (half-Ironman) and race D (full-Ironman). Table 1 depicts physical and physiological characteristics of the subject before race A (initial race) and after race D (last race). Table 2 describes the race information and experimental conditions. As numerous studies have shown dramatically decreased body weight after triathlon events (Farber et al., 1987, 1991), the measurement of body weight in the races B and C was not considered. However, the evaluation

Table 1. Physical and physiological characteristics of the subject

Characteristic	Before race A	After race D
Age (yr)	38	39
Height (cm)	185.4	185.4
Body weight (kg)	78.8	76.4
$\dot{V}O_2$ peak ($l \cdot min^{-1}$)		
Bicycle	4.83	4.92
Treadmill	4.92	5.40
Tvent ($l \cdot min^{-1}$)		
Bicycle	3.01	3.84
Treadmill	3.42	4.20

$\dot{V}O_2$ peak= peak oxygen uptake, Tvent= ventilatory threshold

Table 2. Conditions and finishing time for succesive races

Record	Race A	Race B	Race C	Race D
Date	9/17/2005	6/25/2006	9/16/2006	10/21/2006
Type	Half-Ironman	Full-Ironman	Half-Ironman	Full-Ironman
Altitude (m)	400 m	Sea level	400 m	Sea level
Ambient temperature (°C)	14-23	12-31	18-29	28-32
Humidity (%)	28-97	21-82	21-47	67-79
Swim	0:33:33	1:08:13	0:33:23	1:15:51
Bicycle	2:43:11	5:12:06	2:34:45	5:19:40
Run	1:28:40	3:33:09	1:27:51	3:55:29
Total*	4:48:53	9:58:43	4:38:45	10:40:16

Half-Ironman= Swim 1.9 km, Bicycle 90 km, and Run 21 km; Full-Ironman= Swim 3.8 km, Bicycle 180 km, and Run 42 km. *Total time includes transition time.

of body weight before and after the race A and D were taken into account in order to determine cumulative effects on physiological conditions. The physical characteristics of the subject in the race A has been reported in a different collaborative study related to muscle glycogen depletion and resynthesis (Gillum et al., 2006). All protocols conformed to the standards set forth by *the Declaration of Helsinki* and approved by the Institutional Review Board of The University of Montana.

Experimental design

As the present study was carried out to demonstrate more closely what occurs during actual competition, the dietary intake before, during, and after each race was based on *ad libitum* food or fluid intake (Farber et al., 1991).

Training Volume

With regard to training volume, the subject recorded time spent in each of the three sections such as swimming, cycling and running involved in the triathlon as described by Margaritis et al. (1997). Training volume was recorded in min per week for each section (Figure 1). The first training session (9 months training period) was from January 1st, 2005 to the race A and the second training session (6 months training period) was after January 1st, 2006 to the race B. Furthermore, the third training session (4 months training period) continued by the end of the race D. However, subject did not have any intense training up to four days after each race was completed.

Exercise Testing

Exercise testing was carried out within two weeks before the race A and after the race D. Ventilatory threshold (Tvent) and peak oxygen uptake ($\dot{V}O_2$peak) were determined with an electronically braked cycle ergometer (Velotron, Seattle, WA) and motorized treadmill (Quinton treadmill, Seattle, WA) to evaluate the subject's fitness level at the beginning and the end of successive races (Table 1). During the exercise test, expired gases were collected and analyzed at 15-second intervals using a calibrated metabolic cart (Parvomedics, Inc., Salt Lake City, UT) which was calibrated with a 3L calibration syringe and medical gases of known concentrations (15.2%O_2, 5.17%CO_2, N_2-balance).

Urinary Sample Collection and Biomarkers

For all races (races A, B, C, and D), 24-hour urine output was collected on the day before (Pre) and after the race (Day 1) to evaluate exercise-induced proteinuria. In the present study, the markers of proteinuria included total protein, albumin, β_2-microglobulin, N-acetyl-β-D-glucosaminidase, and creatinine. Urinary samples for the markers of proteinuria were kept frozen at –80℃ until subsequent analysis.

For races B, C, and D, 24-hour urine output was collected on the day before the race and days 1, 2, and 4 post-race to determine exercise-induced whole body DNA oxidation from measured values of urinary 8-OHdG excretion. As the urinary samples in the race A was not sufficiently stored to analyze 24-h urinary 8-OHdG excretion, only races B, C,

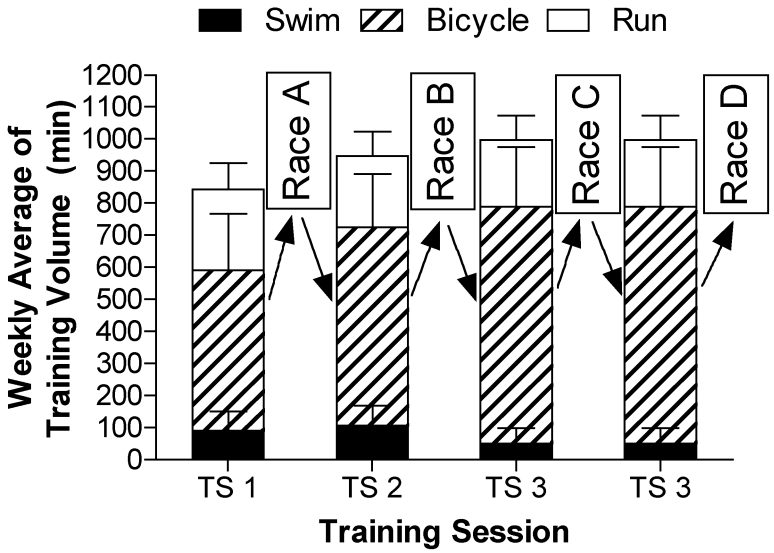

Figure 1. Average of training volume described as minutes per week. TS stands for training session. The first (TS 1), second (TS 2), and third (TS 3) training sessions consisted of 9, 6, and 4 months training period, respectively. The race C was held during the third training session (TS 3). All data are described as mean ± SD.

and D were chosen to determine urinary 8-OHdG excretion. Urine specimens for urinary 8-OHdG analysis were adjusted to the pH of 4-5 by the addition of 2 M HCl and 15 ml aliquots of urine were stored frozen at –20℃ (Germadnik et al., 1997).

Determination of Urinary 8-OHdG Levels

The determination of urinary 8-OHdG levels was based on a modification of Germadnik et al. (1997) using high-performance liquid chromatography with electrochemical detection. Each urinary sample had at least one freeze-thaw step and was centrifuged at 1500 g for 5 min before precipitates were removed. The supernatant underwent a cleanup procedure by solid-phase extraction. The Bond Elut solid-phase extraction columns C18/OH (C18/OH, 500 mg, 3 ml; Varian Inc., Lake Forest, CA) were preconditioned with 10 ml of methanol, 5 ml of water and 10 ml of 50 mM KH_2PO_4, pH 7.5 (buffer A). A 2-ml volume of urine premixed with 1 μl of 8-OHdG standard (1 mg·ml^{-1}) was applied to the first column. The column was washed with 3 ml of buffer A and 3 ml of 5% methanol in buffer A. 8-OHdG was eluted with 3 ml of 15% methanol in buffer A. The eluate then was diluted with 5 ml of water, mixed and applied to another conditioned C18/OH column. The column was dried under vacuum and the absorbed material was eluted with 1.5 ml of 20% methanol in buffer A. To remove methanol, the samples were evaporated for 1.5 h in a SpeedVac® Concentrator SVC200H (Savant, Holbrook, NY) and subsequently filled up with buffer A to give a final volume of 1.5 ml.

As described by Bolin et al. (2004), urinary 8-OHdG was subsequently analyzed by HPLC with a reverse phase YMCbasic column (4.6 mm × 150 mm; particle size 3 μm)(YMC Inc., Wilmington, NC) and quantified using a CoulArray electrochemical detection system (ESA Inc., Chelmsford, MA). An isocratic mobile phase containing 100 mM sodium acetate, pH 5.2, 4% methanol (HPLC Grade) diluted in water passed through C18 Sep-Pak cartridges (Waters Corp., Milford, MA) was prepared to elute the nucleosides from the column. Prior to running the HPLC, the mobile phase was filtered using 0.2 μm nylon filters. Potentials of the four coulometric analytical cells of the CoulArray system placed in series were as follows: 50, 125, 175, 200, 250, 380, 500, 700, 785, 850, 890, and 900 mV. Based on the peak area of the 8-OHdG standard eluting at 9.0

min in the 250 mV channel, the calibration curve for 8-OHdG was demonstrated. In order to obtain highest sensitivity, the electrochemical cells were activated by equilibrating and waking up standards (three times the highest concentration of calibration standards) and were injected to the HPLC. The amount of 8-OHdG calculated using the peak areas from the 10-20 μl injection of the prepared elute. Data were eventually analyzed using CoulArray software for Windows (ESA Inc., Chelmsford, MA).

Determination of Urinary Proteinuria and Specific Gravity

Total protein was based on an improved coomassie blue G method and spectrophotmecally determined (BioAssay Systems, Hayward, CA). Albumin (Alpha Diagnostic International, San Antonio, TX) and the β_2-microglobulin (ALPCO Diagnostics, San Antonio, TX) was tested on the enzyme-linked immunosorbent assay, respectively. The N-acetyl-β-D-glucosaminidase (Roche Applied Science, Indianapolis, IN) and creatinine (Stanbio Laboratory, Boerne, TX) was determined using the colorimetric assay, respectively. To evaluate the subject's hydration status before and after each race, urinary specific gravity was determined with a refractometer (ATAGO, Bellevue, WA).

Statistical Procedure

A Student's dependent t-test was used to determine any significant differences in all proteinuria markers (pre vs. post race). One-way Analysis of Variances (ANOVA) with repeated measures was performed to detect any significant differences for markers associated with whole body DNA oxidation by using a computerized statistical package (Statistica V5.1, Statsoft, Tulsa, OK). Pearson's product moment correlation was used to describe the relationship between urinary measures of 8-OHdG and creatinine. The overall alpha levels were set at $p \leq 0.05$. All data are shown as mean ± SD.

Results

Body weight was decreased in response to race A (78.8 and 74.9 kg for pre and post race, respectively). Body weight returned to 76.3 kg at 4h post-race and reached 77.7 kg at 18 h post-race. In the race D, subject's body weight demonstrated a more substantial decrease (78.6 and 72.7 kg for pre and post race, respectively). Body weight returned to 75.1 kg at 13 h post race.

One-way Analysis of Variances (ANOVA) with repeated measures revealed a significant difference at Day 1 compared to Day 4 in urinary 8-OHdG excretion ($ng \cdot ml^{-1}$)(main effect for time, $p \leq 0.05$; Figure 2A), which demonstrated that 8-OHdG excretion tended to be higher during day 1 post-race and gradually returned to the baseline around day 4. However, no statistical differences were found in 8-OHdG excretion when expressed relative to creatinine concentration ($ng \cdot mg\ creatinine^{-1}$) over successive triathlon events (Figure 2C). However, there was a strong correlation between 8-OHdG and creatinine excretion ($r^2=0.748$, $p<0.05$; Figure 2F). These similar patterns of urinary 8-OHdG excretion were observed among three races regardless of distance (Figure 2).

With or without a creatinine-adjustment, significant increases in all markers of proteinuria markers except β_2-microglobulin were noted at Day 1 ($p<0.05$; Figure 3). Except for β_2-microglobulin, similar patterns of proteinuria (total protein, albumin, N-acetyl-β-D-glucosaminidase) between Pre and Day 1 were observed in all four races regardless of duration (Figure 3). Increased urinary creatinine excretion and specific gravity were found at Day 1 compared with Pre ($P<0.05$, Figure 4A and 4B). Furthermore, urinary creatinine excretion was highly correlated with urinary specific gravity ($r^2=0.921$, $p<0.001$, Figure 4C).

Discussion

Exercise-induced DNA Damage

The main finding in the present study was that a trained male triathlete demonstrated acute changes

in urinary markers of whole body DNA oxidation after each triathlon race. However, there were no patterns that demonstrated cumulative effects over the two-year period of training/competition. Moreover, the patterns of exercise-induced proteinuria were also similar regardless of either half- or full- Ironman triathlon.

With regard to exercise-induced DNA damage, there are conflicting observations using *in vivo* models. It has been shown that DNA oxidation and changes in urinary 8-OHdG excretion are induced by long-distance strenuous sports events such as ultra-marathon running (Radak et al., 2000) and road cycling (Almar et al., 2002). In contrast, some studies

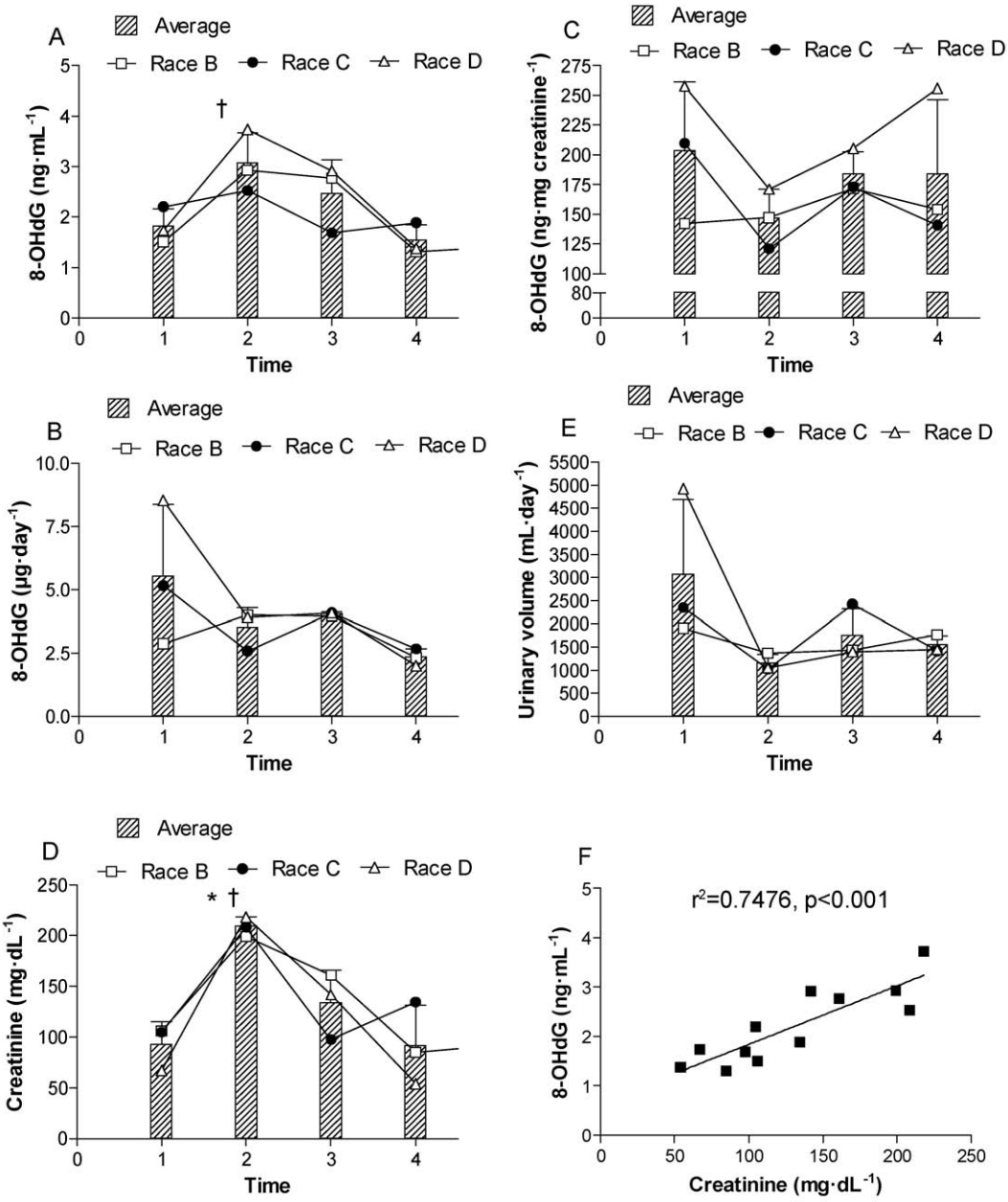

Figure 2. Exercise-induced DNA oxidation. (A) Urinary 8-OHdG concentration ($ng \cdot ml^{-1}$) and (B) ($\mu g \cdot day^{-1}$); (C) urinary creatinine concentration ($mg \cdot dl^{-1}$); (D) urinary 8-OHdG with the creatinine-adjustment; (E) urinary volume ($ml \cdot day^{-1}$); (F) relationship between urinary creatinine ($mg \cdot dl^{-1}$) and 8-OHdG ($ng \cdot ml^{-1}$) before and after each race up to Day 4.
*Significantly different from Pre (one-way ANOVA, main effect for time; $p<0.05$) and
† Significantly different from Day 4 (one-way ANOVA, main effect for time; $p<0.05$). All data are expressed as mean ± SD.

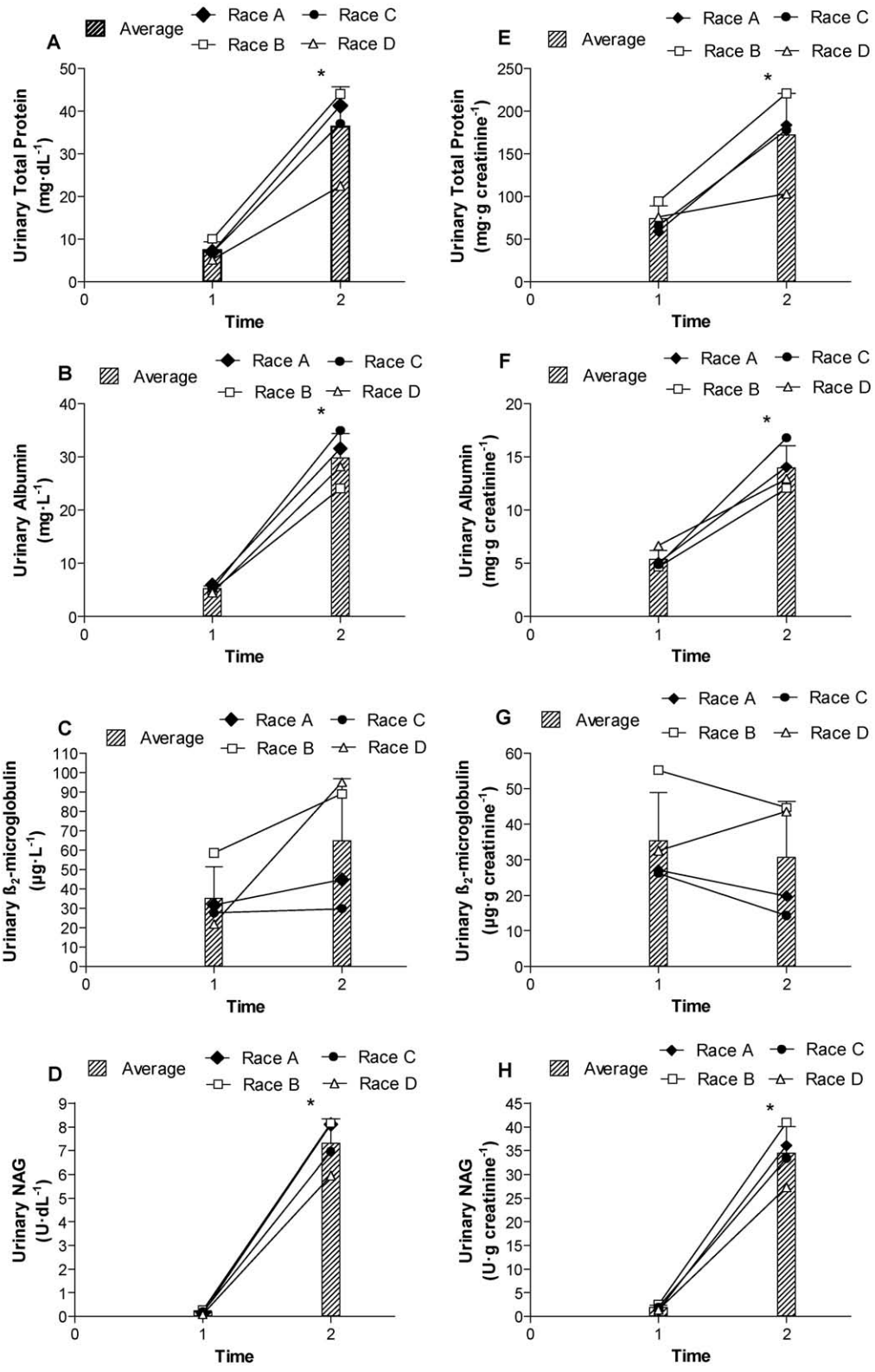

Figure 3. Exercise-induced proteinuria for each triathlon event. (A) urinary total protein concentration (mg·dl^{-1}); (B) urinary albumin concentration (mg·l^{-1}); (C) urinary β_2-microglobulin (μg·l^{-1}); (D) urinary N-acetyl-β-D-glucosaminidase (NAG, U·dl^{-1}); (E) urinary total protein concentration (mg·g creatinine^{-1}); (F) urinary albumin concentration (mg·g creatinine^{-1}); (G) urinary β_2-microglobulin (μg·g creatinine^{-1}); (H) urinary N-acetyl-β-D-glucosaminidase (NAG, U·g creatinine^{-1}). *Significantly different from Pre ($p<0.05$). All data are shown as mean ± SD.

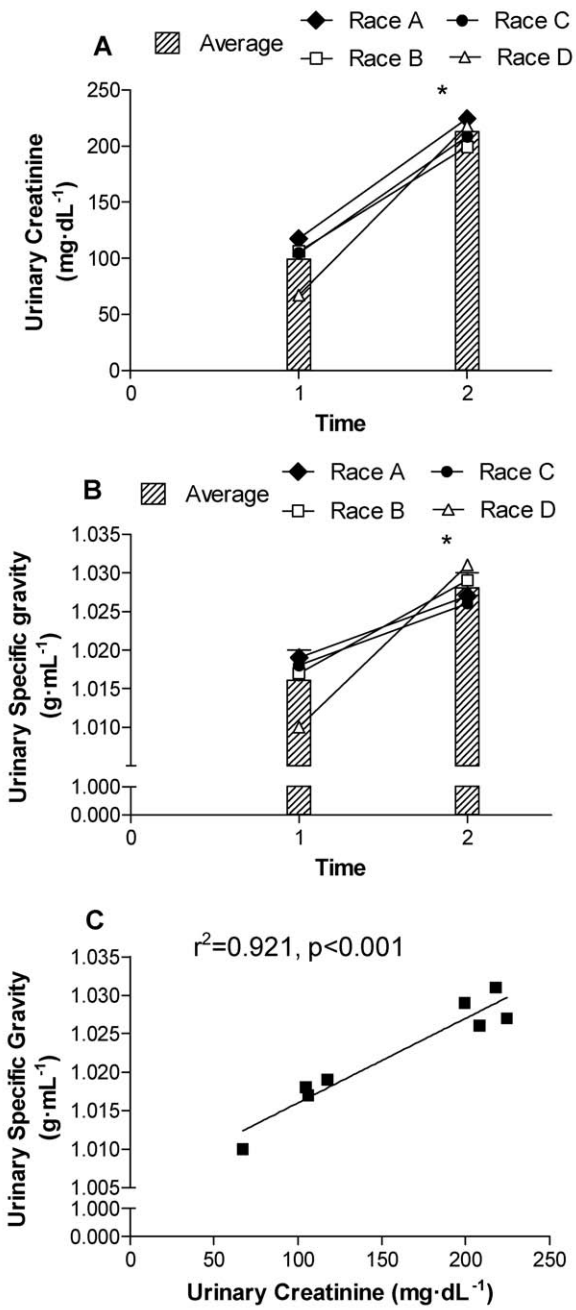

Figure 4. (A) Urinary creatinine ($mg \cdot dl^{-1}$); (B) urinary specific gravity ($g \cdot ml^{-1}$). *Significantly different from Pre ($p<0.05$). All data are shown as mean ± SD. (C) The relation between urinary creatinine ($mg \cdot dl^{-1}$) and urinary specific gravity ($g \cdot ml^{-1}$).

observed no significant changes in urinary 8-OHdG excretion after swimming and running (Inoue et al., 1993) or habitual long-distance run training (Pilger et al., 1997). Hartmann et al. (1998) examined the effects of a short-course triathlon on oxidative DNA damage. The authors reported no alterations of urinary 8-OHdG before and after exercise (up to 4 days post). In the present study, urinary 8-OHdG excretion ($ng \cdot ml^{-1}$) tended to increase on Day 1 post-race and return to the baseline by Day 4 for all three races despite the differences in race duration.

With respect to cumulated effects, the pattern of alterations in urinary 8-OHdG for all three successive races agrees with previously observed changes during an 8-d training camp (Okamura et al., 1997) and during 30-d period of physical training (Poulsen et al., 1996). In those studies, there were no accumulated effects on oxidative DNA damage, while an increase of urinary 8-OHdG was found as long as the exercise was repeated. One possible reason why cumulative effects were not observed may in part be due to adaptive responses induced by long-term regular training, which enhances endogenous antioxidant defense and DNA repairing systems to prevent against exercise-induced DNA damage (Hartmann et al., 1998; Inoue et al., 1993).

Data demonstrated by previous studies do not show a consistent increase in oxidative DNA damage induced by long-term strenuous exercise. Discrepancies could in part stem from the method performed to describe urinary 8-OHdG excretion. The urinary 8-OHdG is commonly standardized with creatinine excreted in the urine in both spot and 24-h urine collection at the steady-state level (Loft and Poulsen, 2000) and before and after prolonged exercise (Hartmann et al., 1998). In the present study, amount of urinary creatinine excretion based on 24-h urine collection was increased at Day 1 post-exercise. An increase in the creatinine excretion into the urine is commonly caused by an increase in the breakdown of skeletal muscle, other tissue breakdown or an enhanced muscle metabolism (Okamura et al., 1997). Some studies have shown the urinary 8-OHdG relative to body weight loss (Almar et al., 2002; Okamura et al., 1997). In contrast, Radak et al. (2000) have demonstrated urinary 8-OHdG excretion ($ng \cdot ml^{-1}$) based on 12-18h urine collection. In the present study, the body weight of the subject dramatically changed after each triathlon event and it has been suggested that this change results from dehydration, energy metabolism turnover, and potentially muscle tissue degradation (Almar et al.,

2002; Okamura et al., 1997). As a result, changes in post-race urinary creatinine excretion may result from several factors. This evidence is strongly supported by previous triathlon studies (Farber et al., 1987; Margaritis et al., 1997). Taken together, it appears that the correction procedure with either creatinine or body weight for 24-h urinary 8-OHdG can only be used when urinary creatinine excretion or body weight remains relatively constant (Almar et al., 2002; Okamura et al., 1997). Therefore, our findings in the present study demonstrate an acute increase in urinary 8-OHdG excretion ($ng \cdot ml^{-1}$) following successive half- and full-triathlon events.

Exercise-induced Proteinuria

The post-exercise proteinuria highly correlates to the intensity of exercise, rather than the duration of exercise, as evidenced by the strong correlation between lactate and proteinuria (Poortmans et al., 1985). According to Poortmans (1985), there are three main variables associated with protein excretion, which are glomerular permeability, tubular reabsorption, and disposal of the absorbed proteins. In terms of exercise, the renal vascular system is constricted (vasoconstriction), which results in an increase in the glomerular permeability and filtration fraction (Poortmans, 1985). These in turn increase plasma protein infiltration, and decrease reabsorption of proteins in the tubular cells, resulting in an elevated urinary excretion of proteins (Miyai and Ogata, 1990). The augmented glomerular permeability and/or impaired tubular reabsorption appeared to cause exercise-induced proteinuria as a result of largely excreted high-molecular-weight proteins (e.g., albumin) in the urine (Khoury et al., 1983; Poortmans, 1985). In contrast, larger amount of low-molecular-weight (e.g., β_2-microglobulin) proteins can be recognized in the urine when tubular function is disturbed (Khoury et al., 1983; Poortmans, 1985). In line with those observations, it has been reported that urinary protein excretion returns to normal within 24-48 hours (Poortmans, 1985). In addition, exercise-induced proteinuria has been considered to be transient and reversible phenomenon, which do not lead to renal disease (Campanacci et al., 1981).

Although numerous studies examined the effects of strenuous exercise on proteinuria, our research interest was to clarify whether or not there are cumulative effects before and after successive triathlon events associated with a long-term training. Our results in the present study confirms previous observations that long-term strenuous exercise is accompanied by a transient impairment of renal function (Farber et al., 1987; Yaguchi et al., 1998), which appears to be similar regardless of triathlon race duration. In the current study, urinary total protein was selected to determine a combination of glomerular and tubular function in the kidney (Newman et al., 2000). Furthermore, albumin, the most abundant protein in the urine, was also chosen as a marker of glomerular permeability (Newman et al., 2000). β_2-microglobulin and NAG were used as common markers of tubular dysfunction (Miyai and Ogata, 1990; Yaguchi et al., 1998). β_2-microglobulin is smoothly filtered at the glomerulus and almost completely reabsorbed by the renal proximal tubule (Miyai and Ogata, 1990; Yaguchi et al., 1998). When the filtered amounts of low-molecular-weight protein surpasses the ability of reabsorption in the tubules, the protein such as β_2-microglobulin increases (Miyai and Ogata, 1990; Yaguchi et al., 1998). NAG is a lysosomal enzyme derived from proximal tubular cells and it does not pass through the intact glomerulus (Miyai and Ogata, 1990; Yaguchi et al., 1998). In the present study, the amount and pattern of proteinuria accounted for by most aforementioned markers were similar over four successive triathlon events. Although no significant increase or similar pattern was observed in β_2-microglobulin for all races, there was a trend for it to be increased after the full-triathlon compared to half-triathlon. Taken together, the augmented values of the proteinuria markers may describe acute functional disturbance of the kidney, but not be a chronic glomerular and tubular damage (Miyai and Ogata, 1990; Yaguchi et al., 1998).

In conclusion, our findings in the present study indicate that no cumulative effects of oxidative DNA damage accounting for urinary 8-OHdG after successive races and training may be due to

sufficient antioxidant buffering capacity in a trained individual, associated with the up-regulation of DNA repair systems to prevent against oxidative DNA lesions. Moreover, the amount and pattern of proteinuria excretion appears to be similar regardless of a half- or full-ironman race. However, further research will be required to clarify the 'threshold' (e.g., exercise duration, intensity and frequency) to induce oxidative damage to DNA and renal dysfunction derived from long-term heavy exercise and training.

Practical implications

Successive triathlon events associated with long-term regular training may enhance endogenous antioxidant defense and DNA reparing systems.

Long-term strenuous exercise is accompanied by a transient impairment of renal function, but may not induce renal disease.

Exercise-induced DNA damage and proteinuria can be used as a marker for monitoring training.

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