Exercise-induced oxidatively damaged DNA in humans: evaluation in plasma or urine?

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Abstract

Physical exercise can induce oxidative damage in humans. 8-Hydroxy-2’-deoxyguanosine (8-OHdG) is a widely known biomarker of DNA oxidation, which can be determined in blood and urine. The aim of the present study was to compare these two biological fluids in terms of which is more suitable for the estimation of the oxidative damage of DNA by measuring the concentration of 8-OHdG one hour after maximal exercise by enzyme immunoassay. The concentration of 8-OHdG increased with exercise only in plasma (p < 0.001), and values differed between exercise tests in both plasma and urine (p < 0.05). In conclusion, plasma appears to be more sensitive to exercise-induced 8-OHdG changes than urine and, hence, a more appropriate medium for assessing oxidative damage of DNA, although the poor repeatability of the measurement needs to be addressed in future studies.

History

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Acute physical exercise can create an imbalance between oxidant and antioxidant levels in humans, a state known as oxidative stress, which can damage enzymes, protein receptors, lipid membranes and DNA (Leewenburgh & Heinecke, 2001). However, regular physical activity can enhance the antioxidant system and suppress the oxidative stress induced by a single bout of exercise (Bloomer, 2008). DNA oxidation is deemed critical for redox-signaling mechanisms (Cobley et al., 2015). Nevertheless, oxidatively damaged DNA may affect mitochondrial function and disturb energy supply (Niess & Simon, 2007), while it also associated with overtraining (Pereira et al., 2013). Therefore, it seems important that the levels of exercise-induced oxidatively damaged DNA are regularly and reliably monitored.

The most frequently used biomarker of DNA oxidation is 8-OHdG (Valavanidis et al., 2009). This compound is mainly formed by the reaction of hydroxyl radical with guanine on both nuclear and mitochondrial DNA, followed by excision from the DNA strands as part of a constantly operating repair mechanism (Valavanidis et al., 2009). 8-OHdG can be determined in both plasma and urine (Cooke et al., 2005; Finaud et al., 2006; Valavanidis et al., 2009), and studies have shown this compound to be an accurate biomarker of DNA oxidation in many diseases associated with oxidative stress, such as diabetes, cancer and Huntington’s disease (Ayala-Pena, 2013; Suzuki et al., 1999; Valavanidis et al., 2009), as well as other pro-oxidant conditions such as air pollution, smoking and acute exercise (Diaz-Castro et al., 2012; Huang et al., 2012; Lu et al., 2014).

Hu et al. (2010, 2015) have raised the possibility of assessing 8-OHdG in different body fluids, that is, plasma, saliva and urine. To our knowledge, the literature concerning the effects of exercise on the accumulation of 8-OHdG is controversial, and it is not clear which biological fluid is the most appropriate for 8-OHdG determination. In relevant studies, measurements have been performed in urine, serum, plasma and leukocytes, with equivocal results regarding the 8-OHdG response to exercise (Alessio, 1993; Bloomer et al., 2006; Clarkson, 1995; Diaz-Castro et al., 2012; Hamurcu et al., 2010; Inoue et al., 1993; Sato et al., 2003; Tsakiris et al., 2006). These equivocal results could be attributed, among other factors, to different exercise types, participants’ characteristics and, obviously, matrices used for analysis.

Given this uncertainty the aim of the present study was to assess oxidatively damaged DNA after maximal exercise in human plasma and urine by using an exercise model that has been recently found to elevate plasma 8-OHdG (Kabasakalis et al., 2014) and a measurement method that is practical and suitable for routine analysis. We have chosen plasma over leukocytes, where DNA is more abundant, in order to make our findings more applicable (since plasma is easier to prepare than leukocytes) and because we considered that plasma 8-OHdG may partly reflect oxidative DNA damage in muscle, which is of more interest than oxidative DNA damage in leukocytes in exercise science. The novelty of this study lies in the side-by-side comparison of plasma and urine for the determination of exercise-induced DNA oxidation.

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Fourteen male national-level swimmers, aged 15.3 ± 1.0 (mean ± SD throughout), participated voluntarily in the study. They had been training for 4.6 ± 1.1 years, at least six times per week. Both the participants and their parents were informed orally and in writing about the details of the experimental procedure, and they provided written informed consent prior to the study. Procedures were in accordance with the Helsinki declaration and were approved by the Institutional Review Board.

The swimmers participated in two identical trials (in a test-retest fashion) in order to check the repeatability of the measurements. Each trial consisted of six 50-m maximal freestyle swimming bouts, one every 5 min, with passive recovery in between. The swimmers started each bout with a push-off from the wall inside the pool. This test has been established as a training tool for improving anaerobic energy production and maximizing the blood lactate concentration (Maglischo, 2003). Time of each bout was recorded with an electronic stopwatch (Selecta W10710, Waterfly, Lelystad, NL). The two trials were performed one week apart. One week before the first trial, each participant's record at 50 m freestyle swimming was determined in order to evaluate exercise intensity during the trials as a percentage of maximal intensity.

Weight and height were measured one week before the first trial in order to calculate body mass index (BMI). The athletes were subjected to blood sampling from an antecubital vein (4 mL), 15 min before and one hour after each trial. The choice of the particular post-exercise sampling time was based on recent findings that plasma 8-OHdG peaked one hour after the end of the same exercise protocol (Kabasakalis et al., 2014). Blood samples were dispensed into EDTA-containing tubes and centrifuged at 1500g and 4 °C for 2 min. The supernatant plasma was collected and stored at −80 °C until analyzed. Urine was collected at the same time points as blood in plastic containers that were stored at −20 °C until analyzed. Hydration of the participants was standardized through the intake of 500 mL of water 90 min before and immediately after each trial.

Plasma and urine samples were used for the determination of 8-OHdG through enzyme immunoassay (Cayman, Ann Arbor, MI). The assay has a measurement range of 10.3–3000 pg/mL and a sensitivity (80% B/B₀) of approximately 30 pg/mL. The coefficient of variation (CV) of the assay was 9.3%. Prior to the main measurements, pilot tests were carried out in order to optimize the necessary dilution of the samples and check the reliability (repeatability) of the assay by performing duplicate measurements and calculating the intra-class correlation coefficient (ICC). As a result of the pilot tests, dilution factors were set at 100 for plasma and 500 for urine.

In addition to 8-OHdG, creatinine was determined in the urine samples (CREA plus, Roche Diagnostics, Mannheim, Germany), and the urine 8-OHdG concentration was normalized to the creatinine concentration (a widely used practice in clinical chemistry to account for differences in renal function).

The distribution of the data set was checked with the Shapiro–Wilk test. Three-way ANOVA (matrix × trial × time) with repeated measures on all three factors was applied to the plasma 8-OHdG concentration and the urine 8-OHdG/creatinine molar ratio, followed by the Bonferroni post-hoc analysis where appropriate. The reliability of these measures and of exercise performance was examined using the ICC. The level of statistical significance was set at α = 0.05.

Weight of the participants was 70.2 ± 6.9 kg, height was 1.79 ± 0.05 m, and BMI was 21.9 ± 2.0 kg/m². Fifty-meter swim time was 28.99 ± 1.14 s during test and 28.77 ± 1.16 s during retest, presenting high repeatability, with ICC of 0.910 and 95% confidence interval (CI) of 0.752–0.970. These times corresponded to 95.8 ± 2.0% and 96.5 ± 2.2% of maximal intensity, respectively. Equally high was the repeatability of the 8-OHdG assay (ICC = 0.988, 95% CI = 0.928–0.998). Both these findings provided a sound basis for examining the biological repeatability of the 8-OHdG levels in plasma and urine.

Plasma and urine 8-OHdG data are presented in Table 1. ANOVA showed significant main effects of matrix (p < 0.001), trial (p = 0.013) and time (p < 0.001), as well as fluid × trial and fluid × time interactions (p < 0.001 for both). Bonferroni post-hoc analysis revealed significantly different plasma 8-OHdG levels between test and retest, as well as between pre- and post-exercise (p = 0.002 and p < 0.001, respectively), the latter result suggesting a significant increase of plasma 8-OHdG with exercise. In accordance with the significantly different values of 8-OHdG between test and retest, there was no significant repeatability in either plasma or urine, as the ICCs before and after exercise were not significantly different from 0.

Formation of RONS commences with the consumption of molecular oxygen in cells and occurs constantly in living systems (Finaud et al., 2006). Acute exercise, especially exercise of long duration and/or high intensity, is included in the stress factors that can cause overproduction of RONS and shift the redox balance toward the state of oxidative stress (Fisher-Wellman & Bloomer, 2009). The exercise protocol

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<th>Plasma&lt;sup&gt;a,b&lt;/sup&gt;</th>
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<td>Before</td>
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<tr>
<td>Test</td>
<td>10.38 ± 2.47</td>
<td>13.59 ± 3.58</td>
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<td>Retest</td>
<td>8.21 ± 1.50</td>
<td>10.60 ± 1.30</td>
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Data are mean ± so of 14 swimmers.

<sup>a</sup>Main effect of trial (p = 0.002).

<sup>b</sup>Main effect of time (p < 0.001).

### References

that was used in the present experimental procedure was of very high intensity and was recently shown to increase the plasma 8-OHdG concentration (Kabasakalis et al., 2014). Therefore, it is reasonable to assume that this exercise protocol caused sufficient RONS production to elicit DNA oxidation, at least transiently.

The existing knowledge concerning the comparison of different body fluids or cells in terms of 8-OHdG accumulation after exercise is limited. Post-exercise values of 8-OHdG were measured in both lymphocytes and urine after moderate exercise (running and swimming) in the study of Inoue et al. (1993). The authors found a significant decrease of 8-OHdG in lymphocytes and no change in urine after intermittent swimming, whereas there was no change in either the lymphocyte or urine values of 8-OHdG after long-distance running. Recently, Yasuda et al. (2015) investigated the effects of repeated bouts of long-duration endurance exercise, performed by moderately trained cyclists, on muscle and urinary levels of 8-OHdG, using high-performance liquid chromatography with electrochemical detection. The results showed no changes in urinary or muscle 8-OHdG values after the repeated exercise bouts. The lack of evidence for DNA oxidation in both of these studies may be explained by the rather moderate intensities of the exercise protocols employed in contrast to a high-intensity exercise session.

In a non-exercising context, Hu et al. (2010, 2015) measured 8-OHdG in different body fluids using on-line solid-phase extraction liquid chromatography–mass spectrometry and found good correlations between the levels in urine, plasma and saliva. Therefore, it is unclear which human biological matrix is most appropriate for the estimation of DNA oxidation through the measurement of 8-OHdG, especially in exercise settings. It seems possible that the specific conditions in each case (e.g. exercise, rest) could indicate the appropriate matrix. It is this rather vague area of the literature that the present study aimed to clarify using a convenient and reliable method of measurement (enzyme immunoassay). Our findings lean on the side of plasma, where we detected a consistent increase in the 8-OHdG concentration with exercise, although with poor reproducibility, apparently due to large biological variability (Pilger et al., 2001), since test procedures were strictly replicated at retest, thus minimizing analytical variability. Attesting to this explanation is the fact that, although the retest values were lower than the pretest ones in terms of means and statistical outcome, five out of the 28 values (18%) at retest were higher than the corresponding values at test, suggesting a random rather than systematic effect. The urine values of 8-OHdG might have been affected by sampling time, since most relevant studies use longer ones, although with longer and continuous-type exercises (Wagner et al., 2011). Our reason for waiting only one hour was to make the procedure convenient for the participants.

In conclusion, a maximal swimming protocol was able to cause an increase in the 8-OHdG concentration in blood plasma. Urine seems to be less suitable for detecting exercise-induced DNA oxidation, at least when samples are collected 1h post-exercise, since neither significant changes nor repeatability of the 8-OHdG concentration were detected.


