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# Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a marker of oxidative stress in rheumatoid arthritis and aging: Effect of progressive resistance training

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*Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG), as a measure of oxidative stress, was measured before and after 12 weeks of progressive resistance strength training in 8 healthy elderly (65–80 yr) and eight healthy young (22–30 yr) men and women, and in eight adults (25–65 yr) with rheumatoid arthritis (RA).*

*Training subjects exercised at 80% of their one-repetition maximum and performed eight repetitions per set, three sets per session, on a twice-weekly basis. 8-OHdG was measured at baseline and follow-up (at least 24 hr after the last exercise session) in the RA and elderly subject groups, and at baseline only in young subjects.*

*Baseline 8-OHdG levels were greater among subjects with RA compared to both healthy young ($P < 0.001$) and elderly ($P < 0.05$) subjects. There were no changes in 8-OHdG levels in either RA or elderly subjects as a result of the strength training intervention.*

*These results suggest that subjects with RA have higher levels of oxidative stress than young and elderly healthy individuals. Furthermore, there is no change in oxidative stress, measured by urinary 8-OHdG, in elderly healthy individuals or in subjects with RA after a 12-week strength training intervention.* (J. Nutr. Biochem. 11: 581–584, 2000) 

**Keywords:** rheumatoid arthritis; oxidative stress; exercise; aging; 8-OHdG; inflammation

## Introduction

8-Hydroxy-2′-deoxyguanosine (8-OHdG) has been recognized as a biomarker of oxidative DNA damage by endogenously generated oxygen radicals.[1] The urinary excretion of 8-OHdG is a reflection of the integrated rate of oxidative DNA damage and the whole body repair of DNA.[2] In vivo, oxygen radicals are produced during normal metabolic processes.[3] There are many defense systems within the body that protect cellular macromolecules against oxidation, yet oxidative DNA damage still occurs.

Certain physiologic conditions lead to increased oxidative damage. 8-OHdG levels in the DNA of rat organs have been shown to increase during normal aging.[4–6] Moreover, reactive oxygen species production in patients with rheumatoid arthritis (RA) has been found to be more than five-fold greater than in either healthy control subjects or in patients with nonrheumatic diseases.[7] Furthermore, because exercise increases oxygen consumption, the generation of reactive oxygen species is increased by exercise, which may result in damage to several types of tissues and DNA.[8,9]

Results from recent studies of urinary 8-OHdG excretion

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**Table 1** Subject characteristics

| | Elderly | RA | Young |
|---|---|---|---|
| *n* | 8 | 8 | 8 |
| Age (yr) | 70.3 ± 5.0 | 41.8 ± 12.6 | 25.8 ± 2.5 |
| Gender (F/M) | 5/3 | 5/3 | 5/3 |
| BMI (kg/m$^2$) | 25.1 ± 2.8 | 25.0 ± 4.3 | 22.8 ± 2.4 |
| $VO_{2\ max}$ (mL/kg/min) | | | |
| Baseline | 20.7 ± 5.0 | 22.9 ± 4.2 | 40.2 ± 10.3[a] |
| Follow-up | 21.2 ± 5.5 | 21.2 ± 3.7 | 37.0 ± 7.2 |
| 8-OHdG (nmol/day) | | | |
| Baseline | 24.82 ± 16.35 | 45.43 ± 16.67[b] | 7.14 ± 11.49 |
| Follow-up | 15.50 ± 10.74 | 30.11 ± 31.17 | —[c] |

Data are mean ± SD.
[a]Young > Elderly and RA, $P < 0.001$.
[b]RA > Young, $P < 0.001$ and RA > Elderly, $P < 0.05$.
[c]Not measured.
RA–rheumatoid arthritis. 8-OHdG–8-hydroxy-2′-deoxyguanosine. BMI–body mass index. $VO_{2\ max}$–maximal oxygen uptake.

after exercise have been conflicting, with some demonstrating increased 8-OHdG excretion after repeated heavy exercise,[10] and others showing no change in 8-OHdG excretion.[11–13] Some of this discrepancy may be related to the time-since-exercise that 8-OHdG was measured as well as the type and duration of exercise studied, which has included exercise on a treadmill,[11] bicycle ergometer,[12] a short-distance triathlon,[13] and long-distance running.[10]

To our knowledge, no studies have been done examining the effect of progressive resistance training on oxidative damage. The present study was designed to examine the effect of a 12-week strength training intervention on oxidative DNA damage as measured by urinary 8-OHdG excretion in healthy young and elderly individuals, and subjects with RA.

## Materials and methods

### *Human subjects protocol*

Subjects and study design have been described in detail elsewhere.[14] Briefly, eight subjects with RA (25–65 yr), and eight healthy young (22–30 yr) and eight healthy elderly (65–80 yr) sedentary men and women were studied. Like healthy elderly individuals, patients with RA exhibit reduced physical activity, abnormal immunity, and changes in body composition; however, in RA, these changes seem to occur independent of chronological age. Thus, RA could provide a useful model of these phenomena dissociated from aging, and it is of interest to compare these individuals to both young and elderly healthy subjects. Before acceptance in the study, all subjects passed a complete physical exam and completed a maximal $O_2$ uptake ($VO_{2\ max}$) test on a cycle ergometer. All of the subjects with RA met the American College of Rheumatology criteria for RA,[15] and were considered by their rheumatologists to be under good disease control. Potential subjects had no known medical illnesses other than RA, and were not consuming vitamin or mineral supplements or any medications (other than RA subjects). The research protocol was approved by the New England Medical Center/Tufts University Human Investigation Review Committee.

All subjects were admitted to the metabolic research unit of the Jean Mayer U.S.D.A. Human Nutrition Research Center on Aging at Tufts University (HNRCA; Boston, MA) for 3 days of baseline studies. Thereafter, all subjects visited the HNRCA on a twice-weekly basis for 12 weeks of strength training. Subjects were admitted to the HNRCA a second time for 3 days of follow-up studies at the end of the 12-week period.

Exercise training consisted of a 12-week regimen of progressive resistance strength training of all major muscle groups, on a twice-weekly basis. Subjects trained at 80% of their one-repetition maximum (1-RM; maximal weight that can be lifted once with acceptable form) on five different machines for trunk (abdominal and back extension), upper body (chest press), and lower body (leg press and leg extension) strength. All subjects exercised on Keiser pneumatic resistance equipment (Keiser Sports Health Equipment, Fresno, CA USA). Strength training was performed at baseline and every 2 weeks thereafter, and the exercise load was increased accordingly to maintain a constant training intensity. Subjects performed three sets of eight repetitions on each machine and training sessions lasted approximately 45 min. Each session was preceded by a warm-up period consisting of approximately 15 min of water exercises (calisthenics and water walking).

Maximal oxygen uptake was determined for all subjects before and after the 12-week study period using a continuous incremental protocol on an electronically braked cycle ergometer. The $VO_{2\ max}$ was recorded as the highest $VO_2$ for 1–1 1/2 minutes during the test. Subjects were encouraged to exercise to exhaustion.

### *Urinary 8-OHdG measurement*

Urine samples were collected over 48 hr at baseline and follow-up (at least 24 hr after the last exercise session) in the RA and elderly subject groups, and at baseline only among young subjects. Urinary 8-OHdG was measured by an enzyme-linked immunosorbent assay according to assay kit instructions provided by the manufacturer (Genox Corporation, Baltimore, MD USA).

### *Statistical analysis*

Comparisons between groups at baseline and follow-up were done by analysis of variance (ANOVA) with Tukey's HSD test. Differences pre- versus posttraining sessions at each time point were compared between groups by ANOVA. Statistical significance was taken at $\alpha = 0.05$. All data analysis was performed using SYSTAT software version 7.0.1 (Systat, Inc., Evanston, IL USA).

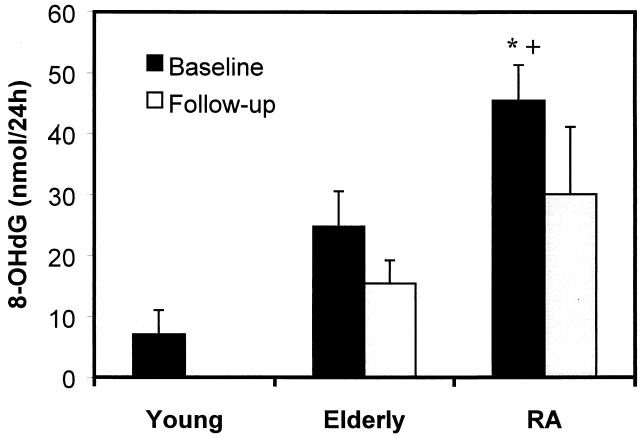


**Figure 1** Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) in young, elderly, and rheumatoid arthritis (RA) subjects at baseline and follow-up. Values are mean ± SE, $n = 6-8$. *Significantly different from young group by Tukey's HSD multiple comparison ($P < 0.001$). +Significantly different from elderly group by Tukey's HSD multiple comparison ($P < 0.05$).

## Results

Subject characteristics are shown in Table 1. Results of the strength training intervention have been discussed in detail elsewhere.[14,16,17] Briefly, there were no differences among the three groups of subjects with regard to an increase in strength, which was calculated as the average of 1-RMs on the five machines: RA subjects 57% increase in strength, young subjects 44%, and elderly subjects 36% (data not shown).

Young subjects had a significantly greater $VO_{2\ max}$ compared to both elderly and RA subjects at baseline and follow-up ($P < 0.001$). Comparisons between groups were performed using both unadjusted data and data adjusted for total body potassium (data not shown). There were no differences among groups in terms of body weight or body mass index, and these parameters did not change as a result of the strength training intervention.

Baseline urinary 8-OHdG levels were greater in subjects with RA compared to both healthy young ($P < 0.001$) and elderly ($P < 0.05$) subjects (Figure 1). There was also a trend toward elderly subjects' having greater baseline levels of 8-OHdG than young subjects ($P = 0.07$). There were no changes in 8-OHdG levels among any subject groups as a result of the strength training intervention.

## Discussion

The results of the present study demonstrate that subjects with chronic inflammation are under greater oxidative stress (measured by urinary 8-OHdG excretion) than young or elderly healthy individuals. Furthermore, these results demonstrate no difference in oxidative DNA damage among any of the subject groups after 12 weeks of high-intensity progressive resistance strength training.

Our finding that subjects with RA had increased oxidative DNA damage compared to healthy young and elderly individuals is consistent with the findings of Miesel et al.,[7] who suggested that elevated mitochondrial oxidative stress may contribute to the pathology of RA. Furthermore, the trend toward elderly subjects having a greater level of urinary 8-OHdG excretion than young subjects is consistent with the greater oxidative stress seen with aging (reviewed in Wei[18]).

Our finding that urinary 8-OHdG excretion is unchanged by a strength training intervention is supported by other studies in which no change in oxidative DNA damage (as determined by urinary 8-OHdG excretion) was found in young healthy subjects after a single session of exhaustive exercise consisting of either incremental exercise to exhaustion on a bicycle ergometer or treadmill, or a short-distance triathlon.[11–13] However, others have demonstrated that prolonged endurance exercise (long-distance running) augments the urinary excretion of 8-OHdG, suggesting that oxidative DNA damage is induced by consecutive heavy exercise.[10]

The apparent discrepancy in these findings may be related to the type, intensity, and duration of exercise performed as well as the training status of subjects studied and the timing (hours since exercise) of urine sample collection. We have demonstrated that 12 weeks of high-intensity progressive resistance strength training does not affect immune function as measured by peripheral blood mononuclear cell subpopulations, cytokine and prostaglandin $E_2$ production, proliferative response, and delayed type hypersensitivity skin response.[14] The present findings regarding oxidative damage are consistent with our earlier results, and lend additional support to the notion that high-intensity progressive resistance strength training has no measurable damaging effects on the immune system or inflammatory response.

In conclusion, we found evidence of increased oxidative damage, determined by urinary excretion of 8-OHdG, in subjects with RA compared to healthy young and elderly subjects. Furthermore, 12 weeks of high-intensity progressive resistance strength training had no effect on urinary excretion of 8-OHdG in any of the subject groups.

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