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*Original article*

# A new method for measuring oxidative stress using blood samples

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## Abstract

***Objective*:** 8-hydroxydeoxyguanosine (8-OHdG), isoprostane, and other oxidative stress metabolites such as hydro-peroxide are known as markers of deoxyribonucleic acid (DNA) oxidative damage, lipid peroxidation, and blood oxidation respectively. We examined the utility of measuring the blood oxidative stress (bOS) and antioxidant power (bAP) sampled during an anti-aging medical checkup.

***Methods*:** bOS and bAP were measured in 29 participants (males: 14; females: 15; mean age ± standard deviation: 43.9 ± 11.5 years old) using the i-Pack Oxystress Test. bOS was assessed based on levels of oxidative derivative generated from chromogen by isolated radical after Fenton reaction via the colorimetric method, while bAP was assessed based on the specimen ability of reducing the complex composed of thiocyanic acid compound and trivalent iron ion to ferrous iron via colorimetric method. Using markers consisted of the Anti-Aging QOL Common Questionnaire (AAQol), general physical examination, functional age and risk factors for pathological aging, evaluated using the Age Management Check System, we analyzed the correlation between bOS or bAP values and these markers. All subjects provided written informed consent.

***Results*:** No correlation was detected between bOS and levels of 8-OHdG or isoprostane. Among all the markers, bOS correlated with 3 items (hours of sleep, r = 0.494; body mass index (BMI), r = 0.415; lifestyle-related habits, r = 0.564) and bAP with 13 (basal metabolic rate, r = 0.383; erythrocyte count, r = 0.431; hemoglobin levels, r = 0.591; hematocrit, r = 0.543; γ-glutamyltranspeptidase γ GTP), r = 0.474; total protein levels, r = 0.437; uric acid levels, r = 0.378; high-density lipoprotein (HDL) , r = −0.500; triglyceride levels, r = 0.458; dehydroepiandrosterone sulfate levels (DHEA-s) , r = 0.372; hormonal age, r = −0.370; mental and physical stress, r = 0.377; glycative stress, r = 0.414). bOS reflected oxidative stress in a different manner from known stress markers 8-OHdG and isoprostane. Considering the correlation between bOS or bAP and inspection items in anti-aging medical checkup, bOS and bAP were determined to be useful markers.

***Conclusion*:** Our study suggested that bOS and bAP may function as markers for oxidative stress.

***KEY WORDS*:** anti-aging medical checkup, blood oxidative stress (bOS), blood antioxidant power (bAP)

## Introduction

Oxidative stress is considered risk factors for pathological aging and promotes glycative stress. While physicians have measured oxidative markers to find the relationship with many diseases, the significance of each marker is not fully understood. Reactive oxygen species (ROS) are known to cause oxidative stress through oxidative deoxyribonucleic acid (DNA) damage, protein degeneration, and lipid peroxidation. While human bodies can tolerate moderate oxidative stress, given our antioxidant network which protects sensitive cells and organelles from such damage, this protection can be overwhelmed under conditions of excessive stress, with cell and tissue damage leading to onset and development of oxidative stress-related diseases [1].

8-hydroxydeoxyguanosine (8-OHdG), isoprostane, and hydro-peroxide (ROOH; lipid-, protein- and nucleic acid-peroxide) are recognized as the major markers for oxidative stress. 8-OHdG is the DNA base deoxyguanosine (dG) hydroxylated at the C-8 position and is regarded as a marker of DNA oxidative damage [2]. Levels of 8-OHdG are also elevated in diabetic patients [3], and it is reported 8-OHdG is a biomarker of macrovascular complication in type 2 diabetes [4]. Isoprostane is an oxidized product of biomembrane degradation which is commonly used as a marker indicating lipid peroxidation; like 8-OHdG, isoprostane levels are also elevated in diabetic patients [5]. In addition, 8-OHdG and isoprostane levels elevated in patients with lifestyle-related diseases such as heart diseases and arteriosclerosis, indicating these two are important markers in preventive medicine [4]. Elevated levels of hydro-peroxide are a marker of blood oxidation and have been found to correlate with a number of diseases.

The anti-aging medical checkup assesses functional age

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and risk factors for pathological aging. These two markers are useful for identifying aging weak points, which can then be shored up to ensure good health and longevity. However, despite the obvious importance of the anti-aging medical checkup in the field of preventive medicine, few studies have examined the relationship between anti-aging medical checkup inspection items and blood oxidation.

Here, we measured blood oxidative stress (bOS) and blood antioxidant power (bAP) in subjects receiving the Doshisha University Anti-aging Medical Checkup and examined the relationship between bOS or bAP values and 44 items. In addition, we compared bOS and known stress markers, 8-OHdG and isoprostane levels.

## Methods

### Subjects

Subjects were 29 examinees who participated in the Doshisha University Anti-aging Medical Checkup (males: 14; females: 15; mean age ± standard deviation: 43.9 ± 11.5 years old). All subjects provided written informed consent.

### Inspection items

The present study focused primarily on bOS and bAP measured using the SPOTCHEM™ i-Pack Oxystress Test (Arkray Inc., Nakagyo-ku, Kyoto, Japan). Pearson correlation analysis was conducted between these two markers and 44 items in the anti-aging medical checkup, anthropometry examination, blood biochemistry examination, urine analysis, and degree of aging using the Age Management System (Ginga Kobo, Naka-ku, Nagoya, Japan)[6-8].

### Anti-aging QOL Common Questionnaire (AAQol)

The AAQol was obtained from the official website of the Japanese Society of Anti-Aging Medicine (http://www.anti-aging.gr.jp/anti/pdf/2011monshin.pdf). Subjective symptoms were divided into 3 categories: "physical symptoms", "mental symptoms" and "lifestyle habits", each assessed on a 5-point Likert scale ranging from 1 to 5 points or asked to fill in the numbers[6-8]. In AAQol, only lifestyle habit-related items were measured in the present study.

### Anthropometry examination

For anthropometry examination, we measured body composition, degree of atherosclerosis, bone density, and degree of glycation. In addition to body weight and height, we measured body mass index (BMI), percentage body fat (%), muscle mass (kg), weight bearing index (WBI), bone mass (kg), and basal metabolic rate (kcal) using a bioelectrical impedance analyzer for muscle mass (Physion MD; Nippon Shooter Ltd., Chiyoda-ku, Tokyo, Japan). Degree of atherosclerosis and bone density were evaluated via acceleration plethysmography (Dyna Pulse SDP-100; Fukuda Denshi, Bunkyo-ku, Tokyo, Japan) and ultrasonography (A-1000 Exp II; GE Healthcare Japan, Hino, Tokyo, Japan), respectively. Degree of glycation was measured at the right upper arm, approximately 10 cm above the elbow, using an AGE reader™ (DiagnOptics, Groningen, the Netherlands). Measured values (AF values),—the integral data of advanced glycation endproducts (AGEs) autofluorescence (AF) —were then scaled for a Japanese population (glycation age = [AF values – 1.112] / 0.0175).

### Blood biochemical examination

Blood biochemical examination was performed at LSI Medience Co. (Chiyoda-ku, Tokyo, Japan). A total of 19 inspection items were evaluated: leukocyte count (/μL), erythrocyte count ($\times 10^4$/μL), hemoglobin (g/dL), hematocrit (%), aspartate transaminase (AST; U/L), alanine transaminase (ALT ; U/L), alkaline phosphatase (ALP ; U/L), γ-glutamyltranspeptidase (γGTP ; U/L), total protein (g/dL), creatinine (mg/dL), uric acid (mg/dL), high-density lipoprotein (HDL; mg/dL), low-density lipoprotein (LDL ; mg/dL), triglyceride (mg/dL), fasting plasma glucose (FPG; mg/dL), hemoglobin A1c (HbA1c ; %), dehydroepiandrosterone sulfate (DHEA-s; μg/dL), cortisol (μg/dL), insulin-like growth factor -1 (IGF-1 ; ng/mL).

### Assessment of degree of aging

Degree of aging was evaluated using the Age Management Check System (Ginga Kobo). The database used with this system allowed us to calculate relative functional age for muscles, blood vessels, nerves, hormones, and bones, as well as risk factors for pathological aging (decreased immunological function [immune stress] and antioxidant capacity [oxidative stress], increased mental and physical stress and glycative stress, and lifestyle-related habits) were calculated.

### i-Pack Oxystress Test

bOS and bAP were measured using the SPOTCHEM™ i-Pack Oxystress Test (Arkray Inc.). bOS was evaluated via the colorimetric method, based on the amount of oxidative derivative generated from chromogen (N,N-bis(2-hydroxyethyl)-1,4-phenylenediamine sulfate) by isolated radicals (alkoxy radical [·OR] or peroxy radical [·OOR]) through metabolism of reactive oxygen after a Fenton reaction. bAP was also evaluated via the colorimetric method, based on the amount of reduced decolorized complex in a specimen formed from red complex with thiocyanic compound and trivalent iron ion.

### Free Radical Analytical System

Derivatives of reactive oxygen metabolite (d-ROM) and biological antioxidant potential (BAP) tests were performed using the FRAS4 (Wismerll Company Limited, Bunkyo-ku, Tokyo, Japan). d-ROM was evaluated via the colorimetric method based on the amount of amaranth radical cation generated from chromogen oxidation by free radicals. BAP was also evaluated via the colorimetric method based on the degree of color change of trivalent iron ion after reduction into divalent ferrous ion by antioxidants in blood.

### Urine analysis

Urine analysis was performed at Nikken Seil Co., Ltd. (Fukuroi, Shizuoka, Japan). Levels of 8-OHdG and isoprostane in urine were measured using an enzyme-linked immunosorbent assay (ELISA). Speed of generation of 8-OHdG and isoprostane (ng/kg/h) in collected urine samples was calculated based on the time elapsed since last micturition, urinary volume, and compound concentration in sample.

### *Statistical analyses*

All analyses were performed using SPSS II software (IBM Japan, Chuo-ku, Tokyo, Japan), and data were expressed as mean ± standard deviation (SD). Independent t-test was used for mean comparison of gender-specific bOS and bAP, while Pearson correlation analysis was used to compare relationships between bOS or bAP values and items in the anti-aging medical checkup. Significance level was set at 5%.

### *Ethical considerations*

This study complied with the ethical principles set forth by the "Helsinki Declaration (including the amaendments made in the Tokyo general meeting in 2004)", "Standards for the Implementation of Clinical Trials on Pharmaceutical Products (Ministry of Health, Labour and Welfare Ordinance No. 28, dated March 27, 1997)", and "Ethic Guidelines for Epidemiology Research (Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare Ordinance, dated November 1, 2007)". The protocol was approved by the Doshisha University Ethical Review (approval number #832).

## *Results*

### *Correlation between i-Pack Oxystress test and FRAS4*

Performance of i-Pack Oxystress test was evaluated using findings from the FRAS4. Using Pearson correlation analysis, we compared bOS and bAP between the two methods (***Fig. 1-a, 1-b***), with findings showing strong correlation for both items (bOS vs. d-ROM, r = 0.973; bAP vs. BAP, r = 0.975). These results suggest that these testing methods displayed similar accuracy in measuring bOS and bAP.

### *Inspection items and measured values in this study*

34 inspection items and measured values for the anti-aging medical checkup are shown in ***Table 1*** (a; inspection items including 8-OHdG and isoprostane, b; bOS and bAP). Data are shown mean ± SD. On stratifying bOS and bAP values by gender, bAP values in women were significantly lower than in men (p = 0.002, ***Fig. 2***).

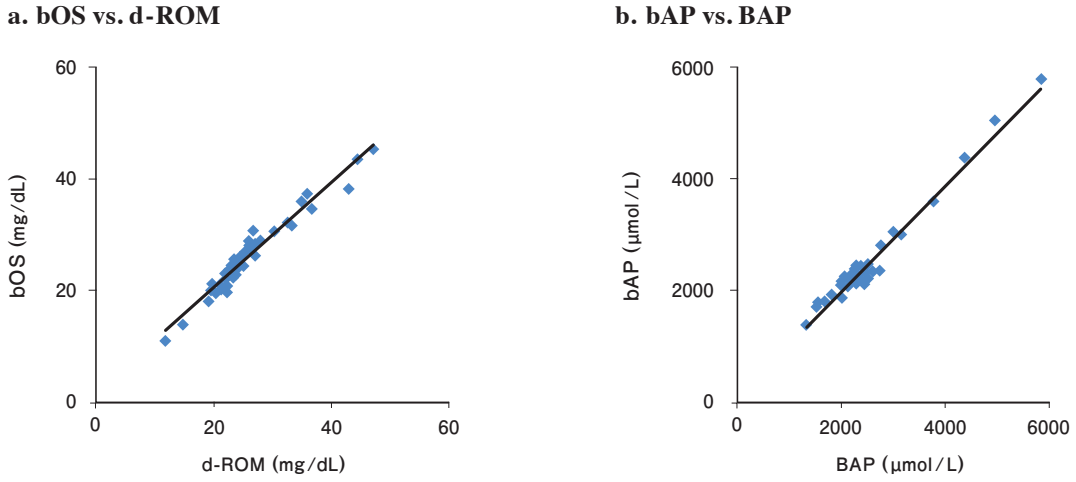


***Fig 1.*** **Correlation of the bOS and bAP between two testing methods (i-Pack Oxystress Test vs. FRAS4).**

Performance of i-Pack Oxystress test was assessed using Pearson correlation analysis to compare bOS and bAP. Strong correlations were shown in both. bOS : y = 0.948x + 1.619, r = 0.973 (a); bAP: y = 0.939x + 119.4, r = 0.975 (b). bOS, blood oxidative stress; bAP, blood antioxidant power; d-ROM, derivatives of reactive oxygen metabolite; BAP, biological antioxidant potential.

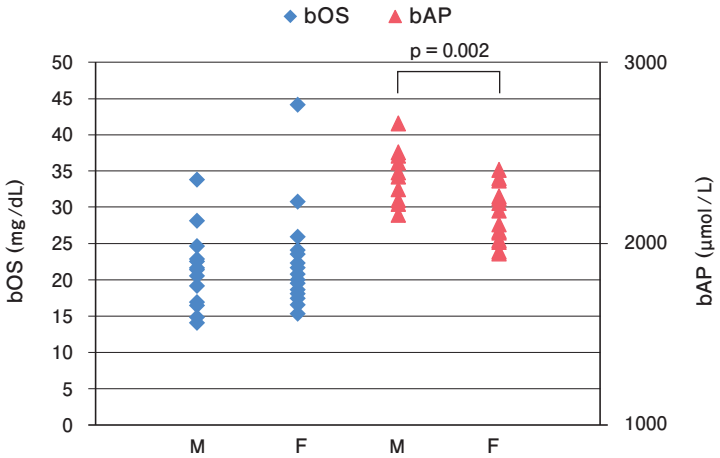


***Fig 2.*** **Gender-specific bOS and bAP values.**

Stratification of bOS and bAP values by gender. bAP values were significantly lower in women than in men (p = 0.002). bOS, blood oxidative stress; bAP, blood antioxidant power; M, male; F, female.

***Table 1-a. Inspection items and measured values (including 8-OHdG and isoprostane)***

| | Mean ± SD |
|---|---|
| Age (years) | 43.9 ± 11.5 |
| Hours of sleep (h/day) | 6.3 ± 0.9 |
| Exercise (times/week) | 1.4 ± 1.8 |
| Alcohol consumption (mL/week) | 1102.1 ± 1646.7 |
| BMI | 21.0 ± 2.4 |
| Percentage body fat (%) | 21.2 ± 5.6 |
| Muscle mass (kg) | 21.8 ± 6.4 |
| WBI | 0.9 ± 0.1 |
| Bone mass (kg) | 9.4 ± 3.0 |
| Basal metabolic rate (kcal) | 1309.3 ± 216.2 |
| Bone (T score) | −0.046 ± 1.3 |
| Vascular age (years) | 43.9 ± 13.6 |
| Glycation (skin AF) | 1.7 ± 0.4 |
| Leukocyte count (/μL) | 5724 ± 1316 |
| Erythrocyte count ($\times 10^4/\mu L$) | 473 ± 31 |
| Hemoglobin (g/dL) | 14.2 ± 1.2 |
| Hematocrit (%) | 43.7 ± 3.1 |
| AST (U/L) | 19.6 ± 4.5 |
| ALT (U/L) | 17.0 ± 5.7 |
| ALP (U/L) | 209.6 ± 73.4 |
| γGTP (U/L) | 28.2 ± 19.5 |
| Total protein (g/dL) | 7.3 ± 0.3 |
| Creatinine (mg/dL) | 0.78 ± 0.15 |
| Uric acid (mg/dL) | 5.0 ± 1.1 |
| HDL (mg/dL) | 72.6 ± 18.8 |
| LDL (mg/dL) | 111.8 ± 31.0 |
| Triglyceride (mg/dL) | 68.7 ± 27.0 |
| FPG (mg/dL) | 83.7 ± 5.8 |
| HbAlc (%) | 5.0 ± 0.3 |
| DHEA-s (μg/dL) | 189.6 ± 111.5 |
| Cortisol (μg/dL) | 9.4 ± 3.1 |
| IGF-1 (ng/mL) | 168.4 ± 45.2 |
| 8-OHdG in urine (ng/kg/h) | 7.56 ± 2.61 |
| Isoprostane in urine (ng/kg/h) | 2.79 ± 21.68 |

SD, standard deviation; 8-OHdG, 8-hydroxydeoxyguanosine; BMI, body mass index; WBI, weight bearing index; AF, autofluorescence; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; γGTP, γ-glutamyltranspeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; DHEA-s, dehydroepiandrosterone sulfate; IGF-1, insulin-like growth factor-1; 8-OHdG, 8-hydroxydeoxyguanosine.

***Table 1-b. Findings for bOS and bAP values***

| | Mean ± SD |
|---|---|
| bOS (mg/dL) | 21.9 ± 6.3 |
| *Male* | 21.2 ± 5.3 |
| *Female* | 22.6 ± 7.2 |
| bAP (μmol/L) | 2238.4 ± 173.8 |
| *Male* | 2334.3 ± 173.8 |
| *Female* | 2148.9 ± 151.3 |

bOS, blood oxidative stress; bAP, blood antioxidant power; SD, standard deviation.

## *Relationship between bOS or bAP values and other inspection items in anti-aging medical checkup*

The relationship between bOS or bAP values and other 34 inspection items in the anti-aging medical checkup was assessed using Pearson correlation analysis (***Table 2***). bOS and bAP/bOS ratio correlated with 2 items each (hours of sleep, r = 0.494 and BMI, r = 0.415 for bOS; and hours of sleep, r = −0.523 and percentage body fat, r = −0.449 for bAP/bOS ratio), and bAP with 10 (basal metabolic rate, r = 0.383; erythrocyte count, r = 0.431; hemoglobin levels, r = 0.591; hematocrit, r = 0.543; γGTP, r = 0.474; total protein levels, r = 0.437; uric acid levels, r = 0.378; HDL, r = −0.500; triglyceride levels, r = 0.458;, and DHEA-s, r = 0.372). 8-OHdG and isoprostane correlated with 2 items (vascular age, r = 0.450 and leukocyte count, r = 0.420) and 4 items (percentage body fat, r = −0.533; vascular age, r = 0.392; hemoglobin levels, r = 0.386; hematocrit; r = 0.411), respectively, with no items overlapping with those correlated with bOS. Notably, no correlation was observed between bOS and either 8-OHdG (r = −0.135) or isoprostane (r = −0.277).

## *Relationship between bOS, bAP markers and functional age or risk factors for pathological aging*

The relationship between bOS, bAP, or bAP/bOS ratio and functional age or risk factors for pathological aging (calculated using the Age Management Check system) was assessed using Pearson correlation analysis. bOS, bAP, bAP/bOS ratio, and isoprostane correlated with 1 item (lifestyle-related habits, r = 0.564), 3 items (hormonal age, r = −0.370; mental and physical stress, r = 0.377; glycative stress, r = 0.414), 1 item (lifestyle-related habits, r = −0.553), and 1 item (antioxidant capacity [oxidative stress], r = −0.392), respectively. No correlation was observed between 8-OHdG and functional age or risk factors for pathological aging (***Table 3***).

***Table 2. Relationship between bOS, bAP markers and other inspection items in anti-aging medical checkup.***

| | bOS | | bAP | | bAP/bOS | | 8-OHdG | | Isoprostane | |
|---|---|---|---|---|---|---|---|---|---|---|
| | r | p value | r | p value | r | p value | r | p value | r | p value |
| Age (years) | 0.162 | 0.401 | -0.042 | 0.828 | -0.331 | 0.080 | 0.004 | 0.985 | -0.055 | 0.780 |
| Hours of sleep (h/day) | 0.494 | 0.008** | -0.096 | 0.625 | -0.523 | 0.004** | -0.209 | 0.295 | -0.280 | 0.157 |
| Exercise (times/week) | 0.228 | 0.243 | -0.176 | 0.370 | -0.324 | 0.093 | -0.218 | 0.276 | -0.139 | 0.490 |
| Alcohol consumption (mL/week) | 0.028 | 0.888 | 0.130 | 0.510 | -0.016 | 0.935 | -0.112 | 0.578 | 0.018 | 0.928 |
| BMI | 0.415 | 0.025* | 0.121 | 0.533 | -0.347 | 0.065 | -0.281 | 0.148 | -0.317 | 0.100 |
| Percentage body fat (%) | 0.336 | 0.075 | -0.329 | 0.082 | -0.449 | 0.014* | -0.338 | 0.079 | -0.533 | 0.004** |
| Musscle mass (kg) | 0.082 | 0.673 | 0.348 | 0.064 | 0.058 | 0.766 | 0.010 | 0.959 | 0.114 | 0.564 |
| WBI | 0.058 | 0.763 | 0.322 | 0,088 | 0.098 | 0.613 | 0.195 | 0.319 | 0.251 | 0.197 |
| Bone mass (kg) | 0.102 | 0.597 | 0.354 | 0.060 | 0.029 | 0.880 | 0.010 | 0.960 | 0.107 | 0.589 |
| Basal metabolic rate (kcal) | 0.091 | 0.639 | 0.383 | 0.041** | 0.067 | 0.730 | -0.074 | 0.708 | 0.046 | 0.817 |
| Bone (T score) | 0.261 | 0.172 | 0.048 | 0.805 | -0.146 | 0.449 | -0.047 | 0.814 | -0.161 | 0.414 |
| Vascular age (years) | 0.051 | 0.792 | -0.147 | 0.447 | -0.158 | 0.414 | 0.450 | 0.016* | 0.392 | 0.039* |
| Glycation (Skin AF) | 0.048 | 0.803 | -0.038 | 0.844 | -0.106 | 0.584 | 0.286 | 0.140 | 0.083 | 0.674 |
| Leukocyte count (/$\mu$L) | 0.237 | 0.216 | -0.051 | 0.793 | -0.149 | 0.440 | 0.420 | 0.026* | 0.357 | 0.062 |
| Erythrocyte count (/$\mu$L) | -0.241 | 0.208 | 0.431 | 0.020* | 0.305 | 0.108 | 0.036 | 0.855 | 0.303 | 0.117 |
| Hemoglobin (g/dL) | -0.095 | 0.623 | 0.591 | 0.001** | 0.255 | 0.182 | 0.191 | 0.330 | 0.386 | 0.043* |
| Hematocrit (%) | -0.145 | 0.452 | 0.543 | 0.002** | 0.242 | 0.205 | 0.173 | 0.380 | 0.411 | 0.030* |
| AST (U/L) | -0.066 | 0.733 | -0.020 | 0.916 | -0.018 | 0.924 | 0.191 | 0.331 | 0.188 | 0.338 |
| ALT (U/L) | 0.047 | 0.810 | 0.245 | 0.201 | 0.032 | 0.870 | 0.225 | 0.250 | 0.176 | 0.370 |
| ALP (U/L) | 0.206 | 0.284 | -0.074 | 0.703 | -0.308 | 0.104 | -0.159 | 0.419 | -0.161 | 0.412 |
| $\gamma$GTP (U/L) | 0.096 | 0.621 | 0.474 | 0.009** | 0.081 | 0.678 | 0.034 | 0.865 | 0.195 | 0.319 |
| Total protein (g/dL) | -0.050 | 0.797 | 0.437 | 0.018* | 0.251 | 0.189 | -0.048 | 0.807 | 0.041 | 0.834 |
| Creatinine (mg/dL) | -0.018 | 0.927 | 0.208 | 0.289 | 0.128 | 0.517 | 0.125 | 0.533 | 0.232 | 0.244 |
| Uric acid (mg/dL) | 0.148 | 0.452 | 0.378 | 0.047* | -0.024 | 0.903 | 0.143 | 0.477 | 0.228 | 0.254 |
| HDL (mg/dL) | 0.088 | 0.648 | -0.500 | 0.006** | -0.322 | 0.089 | -0.341 | 0.076 | -0.306 | 0.113 |
| LDL (mg/dL) | 0.274 | 0.151 | 0.075 | 0.698 | -0.358 | 0.057 | -0.154 | 0.433 | -0.192 | 0.329 |
| Triglyceride (mg/dL) | 0.333 | 0.078 | 0.458 | 0.012* | -0.124 | 0.521 | 0.015 | 0.940 | 0.069 | 0.726 |
| FPG (mg/dL) | 0.201 | 0.295 | 0.186 | 0.333 | -0.182 | 0.346 | -0.223 | 0.254 | -0.162 | 0.409 |
| HbA1c (%) | -0.024 | 0.903 | -0.205 | 0.287 | -0.113 | 0.560 | -0.035 | 0.860 | -0.145 | 0.463 |
| DHEA-s ($\mu$g/dL) | 0.146 | 0.450 | 0.372 | 0.047* | 0.051 | 0.792 | -0.170 | 0.388 | -0.080 | 0.684 |
| Cortisol ($\mu$g/dL) | -0.117 | 0.545 | 0.165 | 0.392 | 0.113 | 0.561 | 0.006 | 0.976 | 0.043 | 0.828 |
| IGF-1 (ng/mL) | 0.007 | 0.971 | 0.251 | 0.189 | 0.270 | 0.156 | -0.136 | 0.490 | -0.130 | 0.511 |
| 8-OHdG in urine (ng/kg/h) | -0.135 | 0.494 | 0.088 | 0.656 | 0.218 | 0.265 | — | — | — | — |
| Isoprostane in urine (ng/kg/h) | -0.277 | 0.154 | 0.191 | 0.331 | 0.328 | 0.088 | 0.799 | <0.001** | — | — |

*p<0.05, **p<0.01. bOS, bAP, and bAP/bOS ratio correlated with 2 items, 10 items, and 2 items, respectively. No correlation was observed between bOS and either 8-OHdG or isoprostane. bOS, blood oxidative stress; bAP, blood antioxidant power; 8-OHdG, 8-hydroxydeoxyguanosine; BMI, body mass index; WBI, weight bearing index; AF, autofluorescence; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; $\gamma$GTP, $\gamma$-glutamyltranspeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; DHEA-s, dehydroepiandrosterone sulfate; IGF-1, insulin-like growth factor-1.

***Table 3. Relationship between bOS, bAP and functional age or risk factors for pathological aging.***

| | bOS | | bAP | | bAP/bOS | | 8-OHdG | | isoprostane | |
|---|---|---|---|---|---|---|---|---|---|---|
| | r | p value | r | p value | r | p value | r | p value | r | p value |
| Functional age | | | | | | | | | | |
| Muscle age (years) | 0.120 | 0.534 | -0.206 | 0.283 | -0.328 | -0.083 | -0.043 | 0.827 | -0.188 | 0.339 |
| Vascular age (years) | 0.165 | 0.394 | 0.018 | 0.925 | -0.241 | 0.208 | 0.000 | 1.000 | -0.046 | 0.815 |
| Nervous age (years) | 0.171 | 0.375 | -0.103 | 0.596 | -0.275 | 0.148 | 0.042 | 0.834 | -0.041 | 0.834 |
| Hormonal age (years) | -0.058 | 0.764 | -0.370 | 0.048* | -0.190 | 0.323 | 0.218 | 0.265 | 0.182 | 0.353 |
| Bone age (years) | -0.128 | 0.507 | -0.229 | 0.233 | 0.006 | 0.976 | -0.021 | 0.915 | -0.014 | 0.944 |
| Risk factors for pathological aging | | | | | | | | | | |
| Immune stress (points) | 0.098 | 0.614 | 0.351 | 0.062 | 0.107 | 0.580 | -0.204 | 0.298 | -0.223 | 0.254 |
| Antioxidant capacity [oxidative stress ](points) | 0.267 | 0.161 | 0.194 | 0.313 | -0.204 | 0.289 | -0.237 | 0.225 | -0.392 | 0.039* |
| Mental and physical stress (points) | 0.108 | 0.576 | 0.377 | 0.044* | 0.156 | 0.418 | -0.142 | 0.471 | -0.127 | 0.519 |
| Lifestyle related habits (points) | 0.564 | 0.001** | -0.248 | 0.195 | -0.553 | 0.002** | -0.209 | 0.287 | -0.266 | 0.171 |
| Glycative stress (points) | 0.065 | 0.736 | 0.414 | 0.025* | 0.105 | 0.587 | -0.217 | 0.268 | -0.232 | 0.236 |

*p<0.05, **p<0.01. Pearson correlation showed that bOS and bAP/bOS ratio were correlated with lifestyle-related habits and bAP with hormonal age, mental and physical stress, and glycative stress. bOS, blood oxidative stress; bAP, blood antioxidant power; 8-OHdG, 8-hydroxydeoxyguanosine.

# *Discussion*

## *Utility of measuring bOS*

While a number of oxidative stress markers have been reported, few evaluation methods have sufficiently established [9]. 8-OHdG and isoprostane are generally regarded as the major oxidative stress markers, as their metabolic pathways have been clarified in detail. The relative chemical stability of 8-OHdG renders it resistant to secondary metabolism, and the substance is generally excreted into urine [10], where it can be detected with high sensitivity [11]. 8-OHdG levels peak after vigorous exercise, and time to restoration of normal levels seems to depend on exercise intensity [12]. Degree of blood oxidation is another major oxidative stress marker, reflecting the balance between bOS and bAP. However, given the relatively large number of substances involved in this complex system of checks and balances, the mechanism behind blood oxidation has not been clarified as those of 8-OHdG and isoprostane.

In the present study, no correlation was observed between bOS and 8-OHdG or isoprostane (Table 2), demonstrating that bOS reflected oxidative stress in a different manner from 8-OHdG and isoprostane and thereby highlighting the utility of measuring bOS. However, unlike 8-OHdG or isoprostane, we were unable to identify the specific substances that reflect bOS or bAP. As with previous research [13], we also used the bAP/bOS ratio as an item in the present study. Of note, more Anti-aging inspection items correlated with bAP (13 items) than bAP/bOS ratio (2 items), suggesting that using bOS and bAP by themselves is more efficient than using ratios such as bAP/bOS when assessing blood oxidation using anti-aging medical checkup inspection items.

## *Significance of measuring bOS and bAP in anti-aging medical checkup*

The ease of measuring d-ROM and BAP using FRAS4 has facilitated examination of the relationship between bOS and a number of diseases. Blood oxidation is known to be involved in obesity, cancer, autoimmune diseases, atherosclerosis, and a range of other conditions [14-16]. Given that bOS and bAP have been shown here to be correlated with some of the inspection items in anti-aging medical checkup, blood oxidation may also function as an oxidative stress marker in anti-aging medical checkup. Previous studies have reported that BMI, carbohydrate/energy ratio, and blood vitamin levels influence variation in levels of 8-OHdG and isoprostane in urine, and that oxidative stress markers and BMI have a hairpin curve relationship [17].While we did not detect the same results in the present study, factors influencing variation in degree of bOS will be analyzed in detail in future studies by increasing the continuous study.

Risk factors for pathological aging were calculated in our study using the Age Management Check. However, only isoprostane—not bOS or bAP—was found to be correlated with “antioxidant capacity [oxidative stress]” (Table 3). Given that the Age Management Check is patented by Ginga Kobo, details regarding the calculation method are unclear. Precision of assessment can likely be improved by feed backing the results in this measurement system and evaluating with bOS- and bAP- reflected data.

### *Relationship between bOS and sleeping*

We focused particular attention to the relationship between bOS and "hours of sleep" among inspection items in the anti-aging medical checkup, as "hours of sleep" was the only item correlated with bOS both men and women after gender-specific stratification of bOS values (data not shown) and the correlation was positive (***Table 2***). "Hours of sleep" is a basal item for lifestyle-related habits and seems to reflect quality of sleep. Given the positive correlation between these two items, we can conclude not that "long duration of sleep results in high bOS" but instead that "those with low stress tolerance need more hours of sleep". This relationship is supported by findings that blood oxidation is associated with fatigue-related diseases, like chronic fatigue syndrome [18].

## *Conclusion*

Taken together, these present findings suggest that bOS and bAP as evaluated during an anti-aging medical checkup are useful oxidative stress markers.


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## *Conflicts of interest*

The authors have no conflicts of interest in this study.

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