Uric acid, ferritin and γ-glutamyltransferase can be informative in prediction of the oxidative stress

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The anti-oxidant system is affected not only by aging but also many lifestyle factors. We aimed to clarify the determinants of medical check-up items affecting the anti-oxidant system. We studied 959 Japanese individuals who underwent anti-aging health check-ups (mean age: 61.1 years) at Tokai University from 2006 to 2016. As parameters of oxidative stress, we measured serum total anti-oxidant status, 8-hydroxy-2′-deoxyguanosine, and isoprostane. Anti-aging health check-up data and lifestyle information were collected from participants in this study. Stepwise multiple regression analyses were conducted to identify determinants that influence serum total anti-oxidant status, 8-hydroxy-2′-deoxyguanosine, and isoprostane, respectively. Serum total anti-oxidant status was significantly correlated with uric acid, vitamin A, folate, and valine. 8-hydroxy-2′-deoxyguanosine was significantly correlated with age, ferritin, drinking habit, and vitamin E. Isoprostane was significantly correlated with vitamin E, γ-glutamyltransferase, ferritin, and smoking habit. The strong antioxidant powers of uric acid and vitamins were confirmed. It was suggested that branched-chain amino acids themselves such as valine or peptides containing them may possess antioxidant ability because of its strong correlation. Uric acid, ferritin, and γ-glutamyltransferase, which are common items measured in medical checkups, can be informative in predicting the oxidative stress situation in a general medical examination.

Key Words: anti-aging health check-ups, oxidative stress-related marker, uric acid, ferritin, γ-glutamyltransferase

Humans consume oxygen to obtain the energy needed for sustaining life, but the process of metabolizing oxygen produces reactive oxygen species (ROS), which causes bodily harm. Antioxidant systems to remove ROS from our bodies do exist, but these systems fluctuate not only with age but also under the impact of many lifestyle habits. If too much ROS accumulates in the body and causes oxidative stress, DNA, lipids, proteins, enzymes and other biopolymers in the body are subjected to oxidative damage. This can lead to cancer and a variety of other disorders such as lifestyle diseases and neurodegenerative diseases, and promotes aging.(1)

According to the 2017 Annual Report on the Aging Society released by the Cabinet Office (Japan), more than 27% of Japan’s population today are aged 65 or over, and by 2065 the percentage will be closer to 40%.² In order to stay healthy for longer as we approach this “aged society,” how we control oxidative stress is an important issue. Accurately evaluating oxidative stress in the body and taking steps to reduce it are expected to be useful in preventing disease and controlling aging.

For more than ten years now, the Health Screening Center of Tokai University, Tokai University School of Medicine has been focusing attention on the oxidative risk caused by aging and lifestyle habits. We have been accumulating data on this issue in our “anti-aging health check-ups,” in operation since 2006.(3) We have also been researching how health is impacted and whether a healthy life span can be extended by improving lifestyle habits without depending on supplements, and thereby avoiding oxidation risks.

Serum total antioxidant status (STAS), a water-soluble oxidant substance in blood, has been measured as an indicator for understanding antioxidant capacity against oxidative stress.(4) 8-hydroxy-2′-deoxyguanosine (8-OHdG) is known as an indicator of DNA damage caused by oxidative stress,(5) while 8-isoprostaglandin F2α (isoprostane), a product arising when phospholipids that serve an important function in the body are subjected to oxidative stress, is known as a marker of lipid system oxidative damage.(6) In this research, particular attention was focussed on three of the oxidative stress-related markers measured in anti-aging health check-ups—STAS, 8-OHdG and isoprostane—in order to study factors that impact the oxidant-antioxidant balance. We studied the correlation between these and various items measured in anti-aging health check-ups, as well as information given by medical questionnaire.

Materials and Methods

Study participants. The participants were 959 patients (526 males, 433 females, ages 27–89, mean age 61.1 years) who had undergone anti-aging health check-ups between June 2006 and March 2016 at the Health Screening Center of the Tokai University School of Medicine. Those who were being treated for hypertension, diabetes or dyslipidemia were excluded from the study (Table 1).

Table 1. Study participants

|                          | Male       | Female     |
|--------------------------|------------|------------|
| Health check-up period   | June 2006–March 2016 |           |
| Participants             | 526        | 433        |
| Age at time of check-up  | Mean age 59.8 ± 11.1 years (27–84) | Mean age 62.2 ± 11.8 years (27–89) |

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Statistical analysis. First, each of STAS, 8-OHdG and isoprostane, and items measured in general health check-ups at the hospital, were subjected to correlation analysis. Here, factors recognized as having a significant correlation (p<0.005), vitamins previously reported as antioxidants (vitamin A, vitamin C, vitamin E, β-carotene, vitamin B12 and folic acid), amino acids (valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine), age, gender, smoking habit, drinking habit and exercise habits were used as candidates for explanatory variables. Multiple regression analysis (step-wise method) was then carried out using STAS, 8-OHdG and isoprostane as the target variables, respectively. The category explanatory variables, a strong multiple collinearity was observed. As a result of studying multiple collinearity between the explanatory variables gender, smoking habit, drinking habit and exercise regime were analyzed using dummy variables for each, namely Male: 1, Female: 0, Smokes: 1, Does not smoke: 0, Drinks: 0, Drinks a little: 1, Drinks at least 360ml each time and on at least five days a week: 2, and Exercises: 1, Does not exercise: 0. As a result of studying multiple collinearity between the branched-chain amino acids (BCAA) valine, leucine and isoleucine and between erythrocyte count, hemoglobin and hematocrit. Therefore, correlation analysis was conducted on at least five days a week: 2, and Exercises: 1, Does not exercise: 0. Of BCAA, valine was used for STAS and isoleucine and between erythrocyte count, hemoglobin and hematocrit. Therefore, correlation analysis was conducted on at least five days a week: 2, and Exercises: 1, Does not exercise: 0.

Results

Table 2 shows the results of correlation analysis between oxidative stress-related markers and general health check-up items

|                | STAS      | p   | 8-OHdG    | p   | Isoprostane | p   |
|----------------|-----------|-----|-----------|-----|-------------|-----|
| Height         | 0.160*    | 0.005 | -0.094    | 0.053 | 0.056       | 0.327 |
| Weight         | 0.102     | 0.073 | -0.059    | 0.226 | 0.144       | 0.011 |
| BMI            | 0.010     | 0.866 | 0.002     | 0.974 | 0.151       | 0.007 |
| Girth          | 0.197*    | <0.0001 | 0.003    | 0.942 | 0.065       | 0.125 |
| Body fat       | -0.111    | 0.052 | 0.072     | 0.136 | 0.040       | 0.480 |
| Systolic blood  | 0.055     | 0.198 | 0.022     | 0.536 | 0.000       | 0.996 |
| Diastolic blood | 0.103     | 0.015 | -0.028    | 0.441 | -0.002      | 0.963 |
| LDL cholesterol | -0.035   | 0.406 | -0.059    | 0.096 | -0.183*     | <0.0001 |
| HDL cholesterol | -0.100   | 0.017 | 0.037     | 0.301 | -0.019      | 0.654 |
| Neutral fat     | 0.055     | 0.193 | -0.068    | 0.057 | -0.056      | 0.184 |
| High sensitivity | -0.009   | 0.829 | -0.005    | 0.888 | 0.062       | 0.141 |
| Leukocyte count | 0.056     | 0.181 | 0.032     | 0.371 | -0.015      | 0.729 |
| Erythrocyte count | —       | —      | -0.018    | 0.613 | -0.128*     | 0.002 |
| Hemoglobin      | 0.250*    | <0.0001 | 0.000    | 0.998 | —           | —    |
| Hematocrit      | —         | —      | -0.007    | 0.851 | —           | —    |
| Platelets       | -0.052    | 0.215 | -0.088    | 0.013 | -0.075      | 0.073 |
| Serum iron      | 0.070     | 0.095 | -0.025    | 0.488 | 0.070       | 0.094 |
| Ferritin        | 0.116     | 0.006 | 0.131*    | <0.0001 | 0.137*     | 0.001 |
| Total protein   | 0.267*    | <0.0001 | 0.050    | 0.231 | -0.158*     | 0.003 |
| Albumin         | 0.331*    | 0.002 | 0.039     | 0.494 | 0.257       | 0.015 |
| AST             | 0.109     | 0.009 | 0.054     | 0.128 | 0.070       | 0.094 |
| ALT             | 0.116     | 0.006 | 0.035     | 0.320 | 0.048       | 0.255 |
| γ-GT            | 0.022     | 0.608 | -0.054    | 0.132 | 0.138*      | 0.000 |
| LDH             | 0.012     | 0.784 | 0.083     | 0.020 | 0.067       | 0.108 |
| ALP             | 0.001     | 0.991 | 0.131*    | 0.002 | -0.163*     | 0.002 |
| CHE             | 0.157     | 0.154 | -0.143    | 0.012 | -0.128      | 0.237 |
| Serum amylase   | 0.084     | 0.118 | 0.103     | 0.014 | -0.087      | 0.105 |
| Fasting blood glucose | 0.093 | 0.026 | -0.066    | 0.063 | 0.080       | 0.056 |
| HbA1c           | 0.399*    | <0.0001 | -0.101    | 0.006 | -0.053      | 0.221 |
| Uric acid       | 0.441*    | <0.0001 | -0.039    | 0.276 | -0.033      | 0.425 |
| Urea nitrogen   | 0.012     | 0.827 | 0.005     | 0.909 | 0.053       | 0.326 |
| Creatinin       | 0.286*    | <0.0001 | —         | —    | —           | —    |

*p<0.005
and healthy life. It is therefore important to know the levels of these individual oxidative stresses by aging but also by various lifestyle habits. It is therefore important to find markers of oxidative stress that sensitively reflect the impact of ROS on the body. 8-OHdG is an oxidized derivative of deoxyguanosine (dG), and a marker of DNA oxidative damage. As this dG has low oxidation reduction potential and is easily susceptible to oxidation by ROS, 8-OHdG is widely used today as a marker of oxidative stress that sensitively reflects the impact of ROS on the body. Because 8-OHdG shows the degree of damage to DNA, the higher the value is, the worse oxidation damage is. In this research, it showed a positive correlation with age and ferritin and a negative correlation with drinking habit and vitamin Eα (standard β = –0.173 and –0.136, respectively). Adjusted R-squared was 0.100 (p<0.0001). Because 8-OHdG shows a strong antioxidant capacity seems to have been reconfirmed this time. 8-OHdG is an oxidized derivative of deoxyguanosine (dG), and a marker of DNA oxidative damage. As this dG has low oxidation reduction potential and is easily susceptible to oxidation by ROS, 8-OHdG is widely used today as a marker of oxidative stress that sensitively reflects the impact of ROS on the body. Because 8-OHdG shows the degree of damage to DNA, the higher the value is, the worse oxidation damage is. In this research, it showed a positive correlation with age and ferritin and a negative correlation with drinking habit and vitamin Eα (standard β = –0.173 and –0.136, respectively). Adjusted R-squared was 0.100 (p<0.0001). Because 8-OHdG shows a strong antioxidant capacity seems to have been reconfirmed this time. 8-OHdG is an oxidized derivative of deoxyguanosine (dG), and a marker of DNA oxidative damage. As this dG has low oxidation reduction potential and is easily susceptible to oxidation by ROS, 8-OHdG is widely used today as a marker of oxidative stress that sensitively reflects the impact of ROS on the body. Because 8-OHdG shows the degree of damage to DNA, the higher the value is, the worse oxidation damage is. In this research, it showed a positive correlation with age and ferritin and a negative correlation with drinking habit and vitamin Eα (standard β = –0.173 and –0.136, respectively). Adjusted R-squared was 0.100 (p<0.0001). Because 8-OHdG shows a strong antioxidant capacity seems to have been reconfirmed this time.

### Table 3. Results of multiple regression analysis on oxidative stress-related markers

| Target variable | Explanatory variable | Parameter estimates | Model conformity |
|-----------------|----------------------|---------------------|-----------------|
|                 |                      | Estimate | SE    | t value | p      | Standard β | Adjusted R² | p      |
| STAS            | Uric acid            | 42.849   | 3.642 | 11.766  | <0.0001| 0.541     | 0.463       | <0.0001|
|                 | Folic acid           | 1.646    | 0.470 | 3.504   | 0.001  | 0.138     | 0.100       | <0.0001|
|                 | Vitamin A            | 0.890    | 0.276 | 3.218   | 0.001  | 0.138     | 0.128       | <0.0001|
|                 | Valine               | 0.299    | 0.120 | 2.040   | 0.013  | 0.112     |             |        |
| 8-OHdG          | Age                  | 0.052    | 0.009 | 5.644   | <0.0001| 0.202     | 0.100       | <0.0001|
|                 | Ferritin             | 0.005    | 0.001 | 4.789   | <0.0001| 0.173     | 0.100       | <0.0001|
|                 | Drinking             | –0.762   | 0.160 | –4.770  | <0.0001| –0.137    |             |        |
|                 | Vitamin Eα           | –0.090   | 0.023 | –3.857  | <0.0001| –0.136    |             |        |
| Isoprostane     | Vitamin Eα           | –0.062   | 0.013 | –4.722  | <0.0001| –0.217    |             |        |
|                 | γ-GT                 | 0.004    | 0.001 | 2.703   | 0.007  | 0.128     |             |        |
|                 | Ferritin             | 0.002    | 0.001 | 2.573   | 0.010  | 0.119     |             |        |
|                 | Smoking              | 0.266    | 0.119 | 2.239   | 0.026  | 0.100     |             |        |

Discussion

Our bodies are constantly exposed to a wide variety of oxidative stresses. The degree of these stresses is greatly impacted not only by aging but also by various lifestyle habits. It is therefore important to find markers of oxidative stress that sensitively reflect the impact of ROS on the body.
reported to be related to hepatitis and other inflammatory diseases, malignant tumors and myocardial infarction. A feature common to all of these is cell disintegration, and it is thought that ferritin existing inside cells flows into the blood, causing serum ferritin levels to rise. During inflammation, moreover, ferritin synthesis is also promoted by TNFα and other inflammatory cytokines, and this is also thought to cause a rise in ferritin. Ferritin, used as an inflammatory marker, is also reported to be actively involved in the production of ROS in the locus of inflammation. Ferritin causes increased production of superoxides by neutrophils, iron ions transformed from trivalent to divalent act as catalysts of a Harber-Weiss reaction, and hydroxyl radicals are produced. Hydroxyl radicals are strong ROS that cause DNA damage and various other types of oxidative stress. In this research, strong correlations between ferritin and two oxidative stress markers (8-OhD and isoprostone) have been recognized. This is thought to corroborate the reaction leading to increased ROS production by ferritin itself.

Isoprostane is a substance formed through oxidation of phospholipids existing in cell membranes and lipoproteins by free radicals, and links to smoking, diabetes and arteriosclerosis have been reported. The higher the isoprostane value, the higher the degree of oxidation is thought to be. In this research, a positive correlation with smoking habit and γ-GT was recognized. It has been reported in many studies that smoking is a cause of ROS generation, and the positive correlation with isoprostone could be seen to corroborate previous reports. We wonder why the correlation with other markers would not be seen. Probably the low smoking rate (14%) of this study may influence on the result. Isoprostone is known as the sharp oxidation damage index that is easier to be oxidized than a nucleic acid or a protein. In the subjects of this study that had such low smoking rate, there is a possibility that only the sharper index “isoprostone” might show a correlation with smoking. γ-GT is known as a sensitive marker of liver disorders. Its function is an enzyme that hydrolyzes a γ-glutamyl compound or transfers the γ-glutamyl group to a peptide or an amino acid. Glutathione, known as a strong antioxidant, is a type of γ-glutamyl peptide; when hydrolyzed by γ-GT, it is broken down into glutamic acid and cysteinyl glycine. Paolicchi reported that γ-GT is involved in the production of oxidant LDL cholesterol. Elevation of serum γ-GT activity is thought to be related to an increase in oxidative stress. The fact that isoprostane and γ-GT showed positive correlations in this research suggests that γ-GT is useful as an oxidative stress marker.

Vitamins have previously been reported to have a strong antioxidant capacity. We found that folic acid and vitamin A showed a significant correlation with STAS, and vitamin Eα with 8-OhD and isoprostone, each moving toward antioxidation. It was suggested that ingesting these vitamins daily in food or via supplements could be effective in raising their own antioxidant capacity and reducing oxidative stress inside the body.

Also this time, the branched-chain amino acid valine was shown to have a significant positive correlation with STAS. Since strong multiple collinearity was recognized between three branched-chain amino acids, we used valine to represent branched-chain amino acids as a candidate explanatory variable, based on the result of a single correlation this time. However, leucine and isoleucine also show significant positive correlation with STAS in correlation analysis, respectively, and all branched-chain amino acids are thought to show positive correlation with STAS. It has been suggested that branched-chain amino acids act as antioxidants. In our research, the standard β of valine was 0.112, which is lower than that of uric acid and vitamins, but since the p value was sufficiently small and significant, the possibility of branched-chain amino acids acting as antioxidants was suggested. The amino acids used in the analysis this time were limited to nine in number—valine, methionine, leucine, isoleucine, tyrosine, phenylalanine, lysine, histidine and arginine—which could be measured reliably when the anti-aging health check-ups were started in 2006. Besides these, however, other amino acids have been reported to have strong antioxidant capacity. We have at present measured 40 different amino acids, and we would like to increase the number of amino acids to be analyzed by our studies in future. The three models created this time all had sufficiently small p values, but the coefficients of determination for 8-OhD and isoprostane, in particular, were small. Many variables that impact the target variables are still thought to exist. In future, we would like to increase our data and conduct further study.

In this research, uric acid and vitamins were confirmed to have strong antioxidant capacity. Moreover, a positive correlation was recognized between STAS and valine as a representative of branched-chain amino acids, and the possibility was suggested that branched-chain amino acids themselves or the peptides they contain could have antioxidant capacity. Uric acid, γ-GT and ferritin have even been measured in general health check-ups, and this finding could be informative when predicting the state of oxidative stress in patients undergoing general check-ups.

Author Contributions
KO designed the study, analyzed data, and wrote the manuscript with contributions and suggestions from all authors; EK, EK, CY, CO, NU, NK, AK, Nİ and YN collected data. All authors contributed to the discussion and interpretation of the results.

Conflict of Interest
No potential conflicts of interest were disclosed.

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