SOD2 mRNA as a potential biomarker for exercise: interventional and cross-sectional research in healthy subjects

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The health-promoting effects of exercise are explained by the biological adaptation to oxidative stress via maintenance of mitochondrial function especially in muscles. Although the induction of antioxidant enzymes in muscle is a useful indicator of exercise, it is not widely used due to the invasiveness of muscle biopsies. To explore more suitable biomarkers for exercise, we examined mRNA levels of antioxidant enzymes in peripheral blood mononuclear cells of 14 volunteers in an exercise intervention study. These results were validated in a cross-sectional study of 392 healthy individuals, and we investigated the association between exercise habits, smoking, alcohol consumption, mitochondrial DNA, malondialdehyde, and various clinical features. The 2-week exercise increased superoxide dismutase 1 at the end of exercise and superoxide dismutase 2 from week 4 onwards. In the cross-sectional study, superoxide dismutase 2 correlated positively with exercise habits and number of mitochondrial DNA, and negatively with malondialdehyde levels. Multivariate binomial regression analysis showed that superoxide dismutase 2 was positively associated with exercise habits in nonsmoking individuals. These results suggest that mRNA levels of superoxide dismutase 2 in blood might be a potentially useful biomarker for exercise in healthy individuals. This study was registered with University Hospital Medical Information Network (No: 000038034).

Key Words: oxidative stress, exercise, mitochondria, biomarker, lifestyles

Increasing physical activity, such as through exercise, can prevent various diseases and extend life expectancy.1-4 It is thought that exercise training improves health by maintaining mitochondrial function, especially in muscles.5-7 Exercise training involves repeated exposure of skeletal muscle to an acute increase in various stresses, including oxidative stress.8 During exercise, oxygen is utilized in the muscle mitochondria for substrate metabolism and ATP production, generating reactive oxygen species (ROS).9,10 Relatively low concentrations of ROS are produced in muscle cells during exercise, which stimulate the expression of various antioxidant enzymes, such as catalase (CAT), glutathione peroxidase 1 (GPX1), and superoxide dismutase (SOD) via its related transcription factors. These adaptive responses are involved in increasing mitochondrial biogenesis and anti-oxidative capacity.11-16

Although establishing an exercise routine is a preventative measure for many lifestyle diseases, many people fail to participate in it.17 To motivate people to start and continue their exercise habits, we searched for biomarkers that confirm the effects of short-term exercise, compared to existing long-term markers, such as HbA1c and blood pressure. We found previously that mRNA levels of several antioxidant enzymes were significantly increased in peripheral blood mononuclear cells (PBMC) after relatively intense aerobic exercise for 1 h per day over 4 weeks.16 Furthermore, it has been reported that exercise-induced changes in muscle mitochondria also occur in PBMCs.11,18 However, there is little information on blood biomarkers for the effects of mild and short exercise training. In this study, we evaluated antioxidant enzymes in PBMCs after exercise by comparing mRNA levels before and after 2-week daily 30-min runs (5 days a week). In addition, we examined the relationship between exercise habits among different lifestyles using blood tests and relative mRNA levels of antioxidant enzymes in the peripheral blood of 392 workers who underwent health examinations in a cross-sectional study.

Materials and Methods

Study design. The study consisted of two arms, an intervention study on 14 participants and a cross-sectional validation study of 392 subjects. In the intervention study, exercise training was conducted at Kochi University, Japan. The study period was from November 2019 to January 2020. Exercise training continued at 5 times per week for 2 weeks. Blood samples were withdrawn for analysis before and after the intervention. The Human Ethics Committee of Kochi University approved the study (#31-120), and all subjects provided written informed consent. This study was registered with UMIN (UMIN 000038034).

A cross-sectional study was conducted to explore the relationship between mRNA levels of antioxidant enzymes in the blood and oxidative stress-related parameters with regard to exercise training habits. The participants were healthy workers (n = 392) who underwent comprehensive medical examination at Juntan University Health Maintenance Center in Okayama Prefecture from December 2019 to March 2020 without comorbidities nor medical treatments. This study was approved by the Human Ethics Committee of Okayama University (#1903-027) and Kochi University (#2020-49) after the authors moved their research base university.

Exercise training. Exercise training for the intervention study consisted of jogging for 30 min 5 days a week for 2 weeks,
at the university campus (Fig 1). For the cross-sectional study, the information about the extent of exercise training per week was obtained from the data of self-reported questionnaires performed by medical staffs in Junpukai Health Maintenance Center.

**Blood tests and medical measurements.** For intervention study, a blood sample (10 ml) was collected at three different times (First, before the start of exercise; Second, after the day of the final exercise; Third, after 2 weeks of the final exercise session. Fig 1). Isolation of the peripheral blood mononuclear cells from whole blood was conducted using Lymphoprep™ (STEMCELL Technologies, Vancouver, Canada), according to manufacturer’s protocol. The supernatant was also collected for analysis of malondialdehyde (MDA).

For the cross-sectional study, venous blood samples were obtained from the participants with overnight fasting state by medical staffs in Junpukai Health Maintenance Center. The whole blood with the anticoagulant was used for measurement of HbA1c, relative expression of anti-oxidant enzymes mRNA and mitochondria DNA. Plasma or serum isolated from the blood was used for the measurement of alanine aminotransferase (ALT), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-c), uric acid, and fasting plasma glucose (FPG), using the automated XE-2100 (Sysmex, Kobe, Japan) and H7700 (Hitachi High-Technologies, Tokyo, Japan) analyzers.

For assessment of oxidative stress, we measured the plasma or serum level of MDA using the method described previously. Briefly, 10 μl of plasma, diluted with 90 μl of PBS (−), was added to 200 μl of 1% phosphoric acid and reacted with 0.67% 2-thiobarbituric acid (TBA) stock solution. The samples were boiled at 95°C for 30 min, centrifuged at 800 × g for 5 min, and the supernatant was measured for the fluorescence detector set at 535 nm excitation and 585 nm emission.

Body mass index (BMI) and conventional blood pressure were measured by trained medical staffs. Information of age, sex, and lifestyle factors were obtained using self-reported questionnaires, including cigarette smoking on scales of dichotomized [no smoking (no smoking + past smoking) or present smoking] or dichotomized (no smoking, past smoking, or present smoking), and alcohol consumption (dichotomized). Exercise habits were collected as described above.

**mRNA levels of antioxidant enzymes.** Total RNA was purified from PBMC or whole blood using ISOGEN (NIPPON GENE, Tokyo, Japan) in combination with High-Salt Precipitation Solution (NIPPON GENE). RNA concentration was measured using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Complementary DNA (cDNA) was synthesized from 1 μg of total RNA in a 20 μl reaction volume using the ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan) according to the manufacturer’s protocol. An aliquot of cDNA was used as a template for quantitative PCR using Thunderbird SYBR qPCR Mix (Toyobo, Osaka, Japan) on the StepOne Plus Real-time PCR System (Applied Biosystems, Foster City, CA) operated in the relative gene expression mode using the ROX dye as a passive reference (see Supplemental Table I* for the primers). Primers were chosen from PrimerBank or q Primer Depot, such that each amplicon spanned at least one intron. The relative expression levels were calculated using the ΔΔCt method with GAPDH as the endogenous control. For example, relative SOD1 gene expression analysis was performed using the eq. 2−ΔΔCt [ΔΔCt = ΔCt (SOD1) − ΔCt (GAPDH)]. For the intervention study, the mean relative expression level of post-intervention to pre-intervention is presented.

**Measurements of mitochondrial DNA.** Total DNA was extracted as described in detail previously. Briefly, 200 μl of a whole blood was diluted in alkaline lysis reagent (100 μl of 25 mM NaOH and 100 μl of 0.2 mM disodium EDTA; pH was adjusted to 12). Samples were heated at 95°C for 10 min and were added neutralizing reagent (200 μl of 40 mM Tris-HCl at pH of 5), then centrifuged at 1,500 rpm for 5 min. In the next step, 1 μl of supernatant was used for measurement of relative mitochondrial DNA (mtDNA) copy number normalized to the expression level of nuclear β-globin. The calculation method using RT-qPCR and the primers were according to the protocol previously described. Forward and reverse primers for the β-globin gene were 5'-GAAGAGCCAAAGGAGGTAC-3' and 5'-CACTTTATCCAGTCTTCC-3', respectively. Forward and reverse primers for the mitochondrial ND1 gene were 5'-AACATACCATGGCCCAACTT-3' and 5'-GCAGGAGGTTGTAGTGAGCCC-3', respectively. Briefly, after denaturation at 95°C for 5 min, the samples were subjected to 40 cycles of incubation at 95°C for 0.1 s, 58°C for 5 s, and 72°C for 18 s. The mtDNA copy number was calculated using the following equation: relative copy number = 2−ΔΔCt [ΔΔCt = ΔCt (β-globin) − ΔCt (ND1)].

**Statistical analysis.** Data are expressed as mean ± SEM. Differences in mean values of several clinical parameters were analyzed according to age, sex, exercise habits, alcohol consumption, and smoking habits using the Mann–Whitney U test or unpaired t test, and ANOVA or Kruskal–Wallis test. We performed logistic regression analysis to investigate the association of antioxidant enzymes mRNA with clinical parameters and lifestyles. The covariates for adjustment were age, systolic blood pressure (SBP), diastolic blood pressure (DBP), LDL-c, uric acid, log-transformed values of BMI, WBC count, ALT, fasting plasma glucose (FPG), HbA1c, mRNA levels antioxidant enzymes (SOD1, SOD2, GPX, and CAT), mtDNA, and three-part scale variables of smoking (ANOVA), dichotomized or dichotomized variables of smoking, and dichotomized scale variables of exercise habit and alcohol consumption. All probability

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values for $p<0.05$ were regarded as statistically significant. All statistical analyses were performed using GraphPad Prism 5.0 for Mac (GraphPad Software, Inc., San Diego, CA) and PASW Statistics 18 for Mac.

### Results

#### Characteristics of subjects of the two studies. The characteristics of the intervention study are shown in Table 1. This population consisted of healthy individuals who were non-smokers and non-regular alcohol drinkers. About the half of the participants had an exercise routine.

The characteristics of the cross-sectional study are shown in Table 2 and 3. The values of BMI, blood pressures, ALT, triglyceride, uric acid, FPG, MDA, mRNA levels of SOD2, GPX1, and mtDNA copy numbers were higher in males than in females. Age had no significant effect on the mRNA levels of antioxidant enzymes. When the degree of smoking was dichotomized (no smoking, past smoking, and present smoking), the values of mtDNA and WBC increased in a stepwise manner according to non-smoking, past smoking and present smoking groups. When the degree of exercise was dichotomized (no exercise and exercise training habits groups), the values of SOD2 mRNA and mtDNA were significantly higher in the exercise group (Table 3). Finally, SOD2 and GPX1 mRNA levels were significantly higher in the regular alcohol consumption group (Table 3).

#### mRNA levels of antioxidant enzymes in the intervention study. Compared to the mRNA levels before exercise, those of SOD1 increased significantly at the end of two weeks of intervention, and the mRNA levels of SOD1 and SOD2 were significantly upregulated two weeks after the end of the intervention period. MDA decreased slightly immediately after the exercise and increased significantly two weeks after the exercise (Fig. 2). The increase in SOD2 was independent of prior exercise habits (Fig. 3A and B). Before the exercise intervention at baseline, SOD2 was significantly higher in individuals with exercise habits compared to those without (Fig. 3C).

#### Antioxidant enzymes, clinical parameters and lifestyle in the cross-sectional study. Among the antioxidant enzymes (SOD1, SOD2, CAT, and GPX1), only SOD2 correlated significantly and positively with exercise habits (Table 4). SOD2 also had positive correlations with GPX1, CAT, mtDNA, and alcohol consumption, and a negative correlation with SOD1 expression. Copy numbers of mtDNA correlated negatively with WBC count and positively with SOD1 and SOD2 levels. MDA correlated negatively with SOD2, GPX1, and CAT, and positively with mtDNA (Table 4). Stratification according to sex and smoking habits showed a significant correlation between SOD2 mRNA levels and exercise habits in non-smoking group (Table 5).

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**Table 1.** Characteristics of participants in the intervention study

| Variables          | Age (years) | % |
|--------------------|-------------|---|
| Age                | 23.4 ± 0.6  |   |
| Body mass index (kg/m²) | 22.4 ± 0.4 |   |
| Total              | 14          |   |
| Sex                | Male        | 11 |   |
|                    | Female      | 3  |   |
| Smoking            | 0           |   |
| Alcohol consumption | No          | 9  |   |
| (units/week)       | <10         | 5  |   |
| Exercise           | No          | 7  |   |
| (days/week)        | 1–5         | 4  |   |
| (days/week)        | >6          | 3  |   |

**Table 2.** Characteristics of the participants of the validation study stratified by sex and age

| Variables          | Total (n = 392) | Male (n = 221) | Female (n = 171) | Age <46 (n = 205) | Age ≥47 (n = 187) | p      |
|--------------------|-----------------|----------------|------------------|------------------|------------------|--------|
| Age                | 46.71 ± 7.61    | 46.95 ± 7.98   | 46.21 ± 7.10     | 40.81 ± 4.02     | 53.18 ± 4.85     | 0.61   |
| Sex (male/female)  | 221/171         | 113/92         | 108/79           | <0.001           | 0.663            | <0.01  |
| BMI                | 23.16 ± 4.64    | 23.58 ± 4.11   | 21.10 ± 4.47     | 23.13 ± 4.75     | 23.19 ± 4.53     | 0.37   |
| Systolic blood pressure | 113.4 ± 17.16 | 114.70 ± 15.82 | 108.2 ± 17.47 | <0.001 | 111.0 ± 15.8 | 0.006 |
| Diastolic blood pressure | 72.48 ± 11.76 | 74.68 ± 11.08  | 69.64 ± 12.04   | 69.96 ± 10.47    | 72.25 ± 12.49    | <0.001 |
| WBC                | 5.19 ± 1.506    | 5.298 ± 1.528  | 5.071 ± 1.472    | 5.209 ± 1.537    | 5.187 ± 1.475    | 0.520  |
| ALT (U/L)          | 22.87 ± 15.42   | 27.84 ± 17.71  | 16.44 ± 8.21     | 23.22 ± 18.02    | 22.49 ± 11.98    | 0.433  |
| TG (mg/dl)         | 96.63 ± 73.58   | 115.40 ± 87.50 | 72.27 ± 38.47    | 87.96 ± 58.84    | 106.10 ± 86.08   | 0.008  |
| LDL-c (mg/dl)      | 131.4 ± 32.95   | 133.1 ± 30.26  | 129.10 ± 36.10   | 127.07 ± 34.70   | 136.20 ± 30.28   | 0.001  |
| Uric acid (mg/dl)  | 5.42 ± 1.41     | 6.21 ± 1.18    | 4.41 ± 0.97      | 5.29 ± 1.47      | 5.56 ± 1.32      | 0.060  |
| HbA1c (%)          | 5.66 ± 0.39     | 5.67 ± 0.45    | 5.63 ± 0.29      | 5.57 ± 0.31      | 5.74 ± 0.44      | <0.001 |
| FPG (mg/dl)        | 99.65 ± 12.42   | 102.8 ± 14.22  | 95.56 ± 7.96     | 98.50 ± 13.39    | 100.90 ± 11.16   | 0.002  |
| MDA (µM)           | 0.23 ± 0.06     | 0.24 ± 0.06    | 0.22 ± 0.05      | 0.230 ± 0.06     | 0.234 ± 0.06     | 0.205  |
| SOD1               | 1.68 ± 3.94     | 1.67 ± 2.84    | 1.68 ± 5.02      | 1.74 ± 4.86      | 1.61 ± 2.58      | 0.474  |
| SOD2               | 0.56 ± 0.55     | 0.65 ± 0.67    | 0.44 ± 0.30      | 0.57 ± 0.63      | 0.55 ± 0.45      | 0.257  |
| GPX1               | 0.48 ± 0.55     | 0.57 ± 0.67    | 0.37 ± 0.29      | 0.49 ± 0.60      | 0.48 ± 0.48      | 0.386  |
| CAT                | 0.44 ± 0.50     | 0.47 ± 0.58    | 0.39 ± 0.36      | 0.46 ± 0.58      | 0.41 ± 0.39      | 0.568  |
| mtDNA              | 2.93 ± 1.14     | 3.14 ± 1.26    | 2.67 ± 0.90      | 2.90 ± 1.15      | 2.98 ± 1.13      | 0.711  |
| Smoking habits (+/-) | 292/100        | 134/87         | 158/13           | 108/14           | 144/43           | 0.275  |
| Exercise habit (+/-) | 169/187        | 79/114         | 90/73            | 86/105           | 83/82            | 0.320  |
| Alcohol consumption (+/-) | 170/222 | 69/152         | 101/70           | 93/112           | 77/110           | 0.334  |

Data are mean ± SEM or number of subjects. BMI, body mass index; WBC, white blood cell; ALT, aminotransferase; TG, triglyceride; LDL-c, low density lipoprotein cholesterol; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose; MDA, malondialdehyde; SOD, superoxide dismutase; mRNA, messenger ribonucleic acid; GPX, glutathione peroxidase; CAT, catalase; mtDNA, mitochondrial deoxyribonucleic acid.
Table 3. Characteristics of several clinical parameters, oxidative stress-related markers, and mRNA of antioxidant enzymes

| Variables                      | No smoking (n = 217) | Past smoking (n = 75) | Present smoking (n = 100) | p     | Exercise habits (−) (n = 169) | Exercise habits (+) (n = 187) | p     | Alcohol (−) (n = 170) | Alcohol (+) (n = 222) | p     |
|--------------------------------|----------------------|----------------------|---------------------------|-------|-----------------------------|-----------------------------|-------|----------------------|----------------------|-------|
| Age (years)                    | 46.52 ± 7.65         | 47.2 ± 7.06          | 46.37 ± 7.92              | 0.448 | 46.71 ± 7.21                | 46.27 ± 7.8                 | 0.361 | 46.55 ± 7.65         | 46.83 ± 7.59         | 0.662 |
| Sex (male/female)              | 74/143               | 60/15                | 87/13                     | <0.001| 79/90                       | 114/73                      | <0.01 | 69/101               | 152/70               | <0.001 |
| BMI (kg/m²)                    | 21.97 ± 4.44         | 24.30 ± 4.21         | 24.90 ± 4.64              | <0.0001| 23.94 ± 5.14                | 23.31 ± 4.47                | 0.385 | 22.36 ± 4.56         | 23.78 ± 4.62         | 0.001 |
| Systolic blood pressure (mmHg) | 111.7 ± 17.72        | 118.30 ± 16.26       | 113.3 ± 15.97             | 0.06  | 112.9 ± 16.96               | 113 ± 17.18                 | 0.839 | 110.4 ± 16.99        | 115.6 ± 16.98        | 0.002 |
| Diastolic blood pressure (mmHg)| 71.63 ± 12.09        | 74.75 ± 10.86        | 72.63 ± 11.57             | 0.06  | 72.50 ± 12.08               | 72.5 ± 11.75                | 0.778 | 70.16 ± 11.31        | 74.26 ± 11.82        | 0.001 |
| WBC (x 10⁹/µl)                 | 4.927 ± 1.223        | 4.999 ± 1.236        | 5.969 ± 1.960             | <0.001| 5.374 ± 1.682               | 5.010 ± 1.291               | 0.049 | 5.182 ± 1.447        | 5.211 ± 1.554        | 0.861 |
| ALT (U/L)                      | 20.11 ± 5.25         | 24.97 ± 14.42        | 27.28 ± 22.18             | <0.001| 23.08 ± 18.91               | 21.57 ± 7.06                | 0.162 | 21.09 ± 12.72        | 24.23 ± 17.11        | 0.011 |
| TG (mg/dl)                     | 80.12 ± 49.86        | 97.83 ± 61.75        | 131.2 ± 106.0             | <0.0001| 101.3 ± 75.07               | 91.35 ± 70.59               | 0.906 | 83.93 ± 46.63        | 106.2 ± 87.58        | 0.016 |
| LDL-c (mg/dl)                  | 129.7 ± 28.60        | 133.5 ± 43.04        | 133.90 ± 33.24            | 0.608 | 132.7 ± 32.63               | 130.6 ± 33.68               | 0.382 | 137.2 ± 34.46        | 126.9 ± 31.10        | 0.002 |
| Uric acid (mg/dl)              | 5.00 ± 1.33          | 5.97 ± 1.13          | 5.97 ± 1.44               | <0.001| 5.35 ± 1.48                 | 5.41 ± 1.36                 | 0.537 | 5.03 ± 1.33          | 5.72 ± 1.40          | <0.001 |
| HbA1c (%)                      | 5.63 ± 0.32          | 5.62 ± 0.24          | 5.72 ± 0.56               | 0.148 | 5.68 ± 0.49                 | 5.62 ± 0.28                 | 0.210 | 5.65 ± 0.29          | 5.65 ± 0.45          | 0.437 |
| FPG (mg/dl)                    | 97.88 ± 12.89        | 101.3 ± 9.45         | 102.3 ± 12.83             | <0.001| 100.4 ± 16.07               | 98.9 ± 8.81                 | 0.855 | 96.94 ± 8.56         | 101.7 ± 14.37        | <0.001 |
| MDA (µM)                       | 0.23 ± 0.05          | 0.24 ± 0.06          | 0.23 ± 0.06               | 0.287 | 0.23 ± 0.06                 | 0.229 ± 0.06                | 0.365 | 0.225 ± 0.05         | 0.230 ± 0.06         | 0.009 |
| SOD1                           | 1.85 ± 4.91          | 1.62 ± 2.77          | 1.36 ± 1.64               | 0.376 | 1.45 ± 2.44                 | 1.95 ± 5.13                 | 0.268 | 1.70 ± 5.00          | 1.66 ± 2.87          | 0.07  |
| SOD2                           | 0.57 ± 0.61          | 0.60 ± 0.46          | 0.52 ± 0.48               | 0.152 | 0.50 ± 0.44                 | 0.64 ± 0.66                 | <0.01 | 0.54 ± 0.65          | 0.58 ± 0.46          | 0.004 |
| GPx1                           | 0.47 ± 0.59          | 0.57 ± 0.60          | 0.45 ± 0.38               | 0.210 | 0.45 ± 0.53                 | 0.53 ± 0.59                 | 0.056 | 0.43 ± 0.50          | 0.53 ± 0.57          | <0.001 |
| CAT                            | 0.44 ± 0.53          | 0.48 ± 0.43          | 0.39 ± 0.45               | 0.097 | 0.41 ± 0.52                 | 0.46 ± 0.48                 | 0.217 | 0.40 ± 0.42          | 0.46 ± 0.55          | 0.09  |
| mtDNA                          | 2.78 ± 1.00          | 3.04 ± 1.00          | 3.20 ± 1.44               | 0.002 | 2.80 ± 1.11                 | 2.99 ± 1.07                 | 0.033 | 2.02 ± 1.28          | 2.97 ± 1.02          | 0.06  |
| Exercise habit (−/+)           | 90/112               | 31/40                | 48/35                     | 0.170 | 78/91                       | 77/110                      | 0.334 | 78/78                | 91/109               | 0.40  |
| Alcohol consumption (−/+)      | 119/98               | 16/59                | 35/65                     | <0.0001| 78/91                       | 77/110                      | 0.334 | 78/78                | 91/109               | 0.40  |

Data are mean ± SEM or number of subjects. *p<0.05, **p<0.01, ***p<0.001 vs nonsmoking group. BMI, body mass index; WBC, white blood cell; ALT, aminotransferase; TG, triglyceride; LDL-c, low density lipoprotein cholesterol; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose; MDA, malondialdehyde; SOD, superoxide dismutase; mRNA, messenger ribonucleic acid; GPx, glutathione peroxidase; CAT, catalase; mtDNA, mitochondrial deoxyribonucleic acid.
**Fig. 2.** Exercise-related changes in mRNA levels of antioxidant enzymes in PBMCs and MDA levels in plasma. (A) Relative mRNA levels of SOD1, SOD2, CAT, and GPX1 in PBMCs. Data are mean ± SEM of 14 individuals. **p<0.01, ***p<0.001 vs pre-exercise. ###p<0.001, vs post-exercise (multiple comparison test). *p<0.05, vs pre-exercise (t test). (B) Lipid peroxidation in plasma. MDA were measured using the fluorescence method. Data are mean ± SEM of 14 individuals. **p<0.001, vs pre-exercise.

**Fig. 3.** Changes in SOD2 mRNA in subjects of the exercise habit and no exercise habit groups. The changes of SOD2 mRNA before and after exercise intervention in the exercise habit group (A) and no exercise habit group (B). *p<0.05, vs pre-exercise, *p<0.05, **p<0.01, vs post-exercise. (C) The comparison of SOD2 mRNA levels between the exercise habit group and no exercise habit group before exercise (at baseline). Data are mean ± SEM of 14 individuals.
Table 4. Correlation among mRNA of antioxidative enzymes, oxidative stress marker with several clinical parameters

|        | SOD1   | SOD2   | GPX1   | CAT    | MDA    | mtDNA   |
|--------|--------|--------|--------|--------|--------|---------|
|        | r      | p      | r      | p      | r      | p       | r      | p      |
| Age    | −0.050 | 0.323  | 0.050  | 0.325  | 0.068  | 0.187  | 0.008  | 0.871  | 0.054  | 0.285  | 0.025  | 0.625  |
| Sex    | −0.092 | 0.069  | −0.231 | <0.001 | −0.206 | <0.001 | −0.092 | 0.069  | −0.174 | 0.001  | −0.242 | <0.001 |
| BMI    | 0.326  | <0.001 | 0.152  | 0.003  | 0.186  | <0.001 | 0.069  | 0.176  | 0.069  | 0.174  | 0.100  | 0.048  |
| SBP    | 0.024  | 0.632  | 0.133  | 0.008  | 0.117  | 0.021  | 0.016  | 0.747  | 0.165  | 0.001  | 0.045  | 0.379  |
| WBC    | −0.013 | 0.802  | 0.051  | 0.313  | 0.128  | 0.011  | −0.012 | 0.807  | 0.144  | 0.004  | −0.005 | 0.917  |
| ALK    | −0.268 | <0.001 | −0.310 | <0.001 | −0.241 | <0.001 | −0.271 | <0.001 | −0.035 | 0.493  | −0.114 | 0.026  |
| ALT    | −0.110 | 0.030  | 0.120  | 0.018  | 0.106  | 0.035  | 0.034  | 0.505  | 0.172  | 0.001  | 0.157  | 0.002  |
| TG     | −0.004 | 0.932  | 0.016  | 0.759  | 0.051  | 0.321  | −0.027 | 0.591  | 0.148  | 0.003  | 0.125  | 0.013  |
| LDL-c  | 0.004  | 0.937  | 0.002  | 0.975  | −0.046 | 0.359  | −0.015 | 0.773  | 0.122  | 0.015  | 0.017  | 0.738  |
| Uric acid | −0.014 | 0.043  | 0.138  | 0.007  | 0.153  | 0.002  | 0.035  | 0.497  | 0.125  | 0.015  | 0.087  | 0.089  |
| HbA1c  | −0.109 | 0.031  | 0.076  | 0.133  | <0.001 | 0.992  | 0.039  | 0.443  | −0.010 | 0.884  | 0.035  | 0.487  |
| FPG    | −0.068 | 0.182  | 0.098  | 0.052  | 0.047  | 0.351  | −0.053 | 0.299  | 0.141  | 0.005  | 0.157  | 0.002  |
| MDA    | 0.064  | 0.209  | −0.300 | <0.001 | −0.254 | <0.001 | −0.202 | <0.001 | 0.126  | 0.012  | 0.148  | 0.003  |
| SOD1   | −0.721 | <0.001 | 0.720  | <0.001 | 0.765  | <0.001 | −0.300 | <0.001 | 0.130  | 0.010  | 0.084  | 0.095  |
| SOD2   | −0.688 | <0.001 | 0.720  | <0.001 | 0.671  | <0.001 | −0.254 | <0.001 | 0.062  | 0.224  | 0.084  | 0.095  |
| GPX1   | −0.736 | <0.001 | 0.765  | <0.001 | 0.671  | <0.001 | −0.202 | <0.001 | 0.848  | 0.095  | 0.126  | 0.012  |
| CAT    | 0.148  | 0.003  | 0.130  | 0.010  | 0.062  | 0.224  | 0.084  | 0.095  | 0.126  | 0.012  | 0.148  | 0.003  |
| mtDNA  | 0.046  | 0.359  | −0.073 | 0.150  | −0.023 | 0.644  | −0.078 | 0.123  | 0.033  | 0.515  | 0.124  | 0.014  |
| Alcohol consumption | 0.092 | 0.068 | 0.147  | 0.004  | 0.209  | <0.001 | 0.085  | 0.092  | 0.078  | 0.122  | 0.097  | 0.055  |
| Exercise habits | 0.068 | 0.198 | 0.147  | 0.006  | 0.102  | 0.055  | 0.066  | 0.217  | 0.006  | 0.864  | 0.114  | 0.003  |

BMI, body mass index; WBC, white blood cell; ALT, aminotransferase; TG, triglyceride; LDL-c, low density lipoprotein cholesterol; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose; MDA, malondialdehyde; SOD, superoxide dismutase; mRNA, messenger ribonucleic acid; GPx, glutathione peroxidase; CAT, catalase; mtDNA, mitochondrial deoxyribonucleic acid.

Table 5. Correlation of SOD2 mRNA with exercise habits

|        | No smoking | Past smoking | Present smoking |
|--------|------------|--------------|-----------------|
|        | r          | p            | r               | p       |
| Total  | 0.171      | 0.015        | 0.078           | 0.527   | 0.094  | 0.399  |
| Male   | 0.136      | 0.282        | 0.069           | 0.613   | 0.011  | 0.929  |
| Female | 0.092      | 0.282        | 0.003           | 1.000   | 0.300  | 0.545  |

Table 6. Odds for exercise habits according to SOD2mRNA

| Tertiles of SOD2 | Q1 (<0.34) | Q2 (0.35–0.54) | Q3 (>0.55) | p for trend |
|------------------|------------|----------------|------------|-------------|
| Model 1          | 1          | 1.369 (0.822–2.278) | 2.007 (1.200–3.356) | 0.008 |
| Model 2          | 1          | 1.259 (0.750–2.113) | 1.781 (1.049–3.024) | 0.032 |
| Model 3          | 1          | 1.265 (0.742–2.155) | 1.903 (1.090–3.232) | 0.024 |
| Model 4          | 1          | 1.126 (0.650–1.952) | 1.592 (0.892–2.842) | 0.116 |

Data are odds ratios and (95% confidence intervals). Model 1: no adjustment. Model 2: adjustment for age and sex. Model 3: adjustment for age, sex, BMI, ALT, TG, LDL-c, HbA1c, systolic blood pressure, MDA, mtDNA, and alcohol consumption. Model 4: smoking habit was added to model 3.

Logistic regression analysis. The logistic regression analysis for exercise habits according to SOD2 mRNA levels are shown in Table 6. SOD2 mRNA associated positively with exercise habit after adjustment for age, sex, BMI, ALT, TG, LDL-c, SBP, MDA, mtDNA, and alcohol consumption in model 3. When smoking habit was added to model 3, the association between SOD2 mRNA and exercise habits disappeared in model 4.

Discussion. The main findings of the intervention study were significant increases in mRNA levels of SOD1, GPX1, and SOD2 in PBMC after two weeks of exercise. The validation cross-sectional study demonstrated that SOD2 mRNA in whole blood cells was a significant independent determinant of exercise habits in nonsmokers. This result also showed that increased SOD2 was
accompanied by decreased lipid peroxidation and increased mitochondrial biogenesis. These indicated that SOD2 mRNA level in the blood might be useful as a phenotypic biomarker of the mechanism of muscle-based health promotion by exercise.

In the intervention study, 30 min of daily jogging was implemented over a 2-week period, and blood samples were taken three times. In our previous intervention study of four weeks of bicycle exercise in a trained population, the mRNA levels of CAT and GPX1, but not SOD1 or SOD2, were significantly elevated the day after the end of intervention. In another study of 30 min of treadmill exercise for 7 days, the mRNA levels of GPX1, SOD1, and SOD2 were all significantly elevated in CD34(+) PBMC on the day after the end of intervention. These differences might be due to the type of exercise (e.g., aerobic or anaerobic) and timing of sample collection. It has been reported that lipid peroxidation rises immediately after acute exercise and returns to normal the following day. Other studies reported that lipid peroxidation in plasma decreased after repeated exercise intervention for more than a week. Although the slight decrease in MDA levels observed in our study the day after the last exercise could be consistent with these reports, it is difficult to explain a significant increase observed two weeks after the exercise intervention. This needs to be examined in future.

What is unique about our data is the elevated mRNA levels of SOD2 in PBMC two weeks after exercise intervention, but not one day after. This finding may explain the results of our validation study, in which only SOD2 but not SOD1 or GPX1, CAT mRNAs were associated with exercise habits. Adaptation of the oxidative stress that first occurs in muscles might gradually spread to affect non-muscular tissues, including blood cells, for a certain period of time. Muscle cells is known to generate cytokines and other muscle fiber-derived peptides, such as myokines. Myokines communicate with other organs such as adipose tissue, liver, bone, and brain to exert the beneficial effects of exercise at the whole-body level.

In the cross-sectional study, the genes candidates for exercise biomarkers in interventinal studies were validated in the blood samples from 392 healthy adults without comorbidities. We also examined mtDNA levels, a marker of mitochondrial biogenesis, and analyzed lifestyle habits including exercise, alcohol consumption, and smoking. In this study, SOD2 was the only significant factor found to be altered by exercise although the reports from other research have shown different types of antioxidant enzymes to be altered by exercise. This discrepancy might be due to the fact that these studies did not take lifestyle into account. The lifestyle such as alcohol consumption, smoking and taking supplementation were reported to affect oxidative stress levels in the blood. The results that exercise habit had the positive correlation with mtDNA and the negative correlation with MDA in our study reinforced the usefulness of SOD2 as a biomarker for exercise. The similar mechanism have been reported regarding the impact of muscles on improving overall health. Interestingly in our study, the level of mtDNA correlated significantly and positively with past and current smoking in addition to exercise, but not with alcohol consumption. This result differs from the previous another report, which showed that mtDNA level was negatively correlated with smoking, and not significantly correlated with exercise. This may be related to differences in how smoking and non-smoking are separated from past smoking, or to the inclusion of disease groups such as diabetes or hypertension; therefore, the medications used by their subjects could have affected the blood levels of oxidative stress markers. In fact, in other studies of healthy subjects except for the patients, mtDNA was reported to increase after exercise. However, another bench research has shown that nicotine increases MDA and decreases SOD activity, which could inhibit mitochondrial biogenesis. Further verification is needed to elucidate the mechanism of SOD2 and mitochondrial function after smoking.

The adaptive increase in antioxidant enzymes in skeletal muscles induced after exercise is involved in ROS production in the mitochondria due to high consumption of O2. However, the mechanism responsible for the induction of antioxidant enzymes in PBMC is not clear yet. Our data suggest unknown inducers of ROS in the mitochondria of PBMC since induction of SOD2 was not observed at the end of the intervention but 2 weeks after the end of the study.

Although our findings are significant, several study limitations should be discussed. First, the sample size was small. Second, the mRNA level was not measured in PBMC but in blood clots in the validation study. Third, causal relationships could not be determined because one of the two studies was a cross-sectional study. Fourth, the intervention study did not include a control study. Fifth, some reporting bias may have been introduced because the information on exercise training was obtained via self-reported questionnaire. Finally, for proper analysis of exercise training, information about clerical staff or physical labor is needed.

Our results suggest that SOD2 mRNA in the blood is a potentially suitable biomarker of exercise training in non-smokers. Future investigation using cohort studies is needed to confirm the usefulness of SOD2 mRNA in blood.

Author Contributions

Study concept and design, KO; acquisition of data, KT, NO; analysis and interpretation of data, KO; drafting of the manuscript, SO; critical revision of the manuscript for important intellectual content, YO and YT; statistical analysis, KO; obtained funding, SO and KO; administrative, technical, or material support, NO and ME; study supervision, NS and KO.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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