SERUM COMPONENTS AND LIFESTYLE FACTORS - IV

Relationship of Serum Superoxide Dismutase Activity and Lifestyle in Healthy Japanese Adults

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Abstract

Superoxide dismutase (SOD) is an antioxidant enzyme that acts to degrade superoxide, a major causitive factor for oxidative stress associated with cancer, cardiovascular disease, and various other ailments. Here, to assess an association between antioxidants and lifestyle factors related to cancer risk, we analyzed serum SOD activity among the subjects within a large-scale cohort study in Japan. As results, significant differences in serum SOD activity were found between the sexes (lower in males), among female age groups (lower in younger individuals), and in males with the BMI (lower in those with a high BMI). Linear increase in serum SOD activity with aging and decrease with BMI were observed in females. Significantly low SOD activity was evident in male heavy smokers. In contrast, elevation was noted in female frequent drinkers. In conclusion, our findings do suggest associations between serum SOD activity and lifestyle factors. However, for further study, establishment of a standard measurement method for SOD activity should be a high priority.

Keywords: Superoxide dismutase - lifestyle - aging - BMI - smoking - drinking alcohol - antioxidant intake

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Introduction

Evidence has been presented that oxidative stress causes cancer, cardiovascular disease, and various other ailments (Kinnula et al., 2004; Weinberg et al., 2009; Fearon et al., 2009). The main causal factor is excess generation of reactive oxygen species (ROS), for example in cigarette smoke, which may lead to carcinogenesis (Valavanidis et al., 2009). One of the most important ROS species is superoxide, and therefore superoxide dismutase (SOD), the enzyme that catalyzes breakdown of superoxide into oxygen and hydrogen peroxide, is regarded as a critical protective factor in cells exposed to oxygen. Many *in vitro* studies have shown SOD overexpression in cell lines to exert inhibitory effects on cancer cell growth (Kinnula et al., 2004). Levels of SOD activity in human tumor cells may also be elevated, although some studies have shown opposite results (Kinnula et al., 2004). Further data are thus needed to clarify the roles of SOD in development of cancer.

In the present study, to assess possible associations between antioxidants and lifestyle factors related to cancer risk, serum SOD activity was analyzed in subjects within a large-scale cohort study in Japan.

Materials and Methods

Study Population and Serum Samples

This study was conducted as a cross-sectional study about lifestyle factors within the Japan Collaborative Cohort Study (JACC Study), the details of which were described previously (Ohno et al., 2001; Tamakoshi et al., 2005). A baseline survey was conducted from 1988 to 1990. Thirty-five percent of the all cohort participants (39,242 subjects aged 40 to 79 at baseline) donated blood samples, and these were stored at -80ºC until analyses were performed.

Causes and dates of death were determined by reviewing all death certificates (official) in each study area until 1997. Those who had died in this period or who had been diagnosed as suffering from cancer by 1994 were regarded as cases for nested case-control studies. For each case, we randomly selected 3 or 4 controls, matching them for gender, age and residential area. For

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the purpose of the present study, we excluded all cases. According to this criterion, 10,350 (5,388 males and 4,962 females) controls were selected as subjects.

The study design and use of serum were approved by the Ethical Board at the Nagoya University School of Medicine, where the central office of the JACC Study was located at that time.

Categorization of Lifestyle Factors

Body mass index (BMI) was calculated based on individual's height and weight collected through self-reporting. BMI was classified into three categories as follows; less than 18.5 kg/m^2 (regarded as underweight by World Health Organization), from greater than or equal to 18.5 to less than 25 kg/m^2 (normal), from greater than or equal to 25 to less than 30 kg/m^2 (overweight), and from greater than or equal to 30 kg/m^2 (obese).

Subjects who answered "yes" to the question for smoking habit were categorized into the "Current" group, and those who answered "quit" or "never" were categorized into the "Former" or the "Never" groups. Male smokers were further categorized by the number of cigarettes smoked per day.

Subjects who answered more than once a week to the question for drinking habit were categorized into the ">4 (more than four) times per week", or the "1-4 (once to four times per week)" groups. Those who answered less than once a week or "seldom" were categorized into the "<1 (less than once per week)" group. Those who answered "quit" were categorized into the "Former(quit)" group. Frequent male drinkers were further categorized by the average amount of alcohol intake per day.

Laboratory Assays

Serum samples were assayed in 1999 and 2000 as described previously (Ito et al., 2005). Serum SOD activity was estimated from decreasing rate of nitrite produced by hydroxylamine and superoxide anions by improved nitrite methods (Oyanagi, 1984). The assay range was 0.1-10.0 U/mL, and intra- and interassay coefficients of variation were 4.02-6.79%, and 2.79-5.82%, respectively (Ito et al., 2005).

Statistics

Male and female data were separately analyzed because of differences in lifestyle factors and the ranges of serum SOD activity. Comparisons of continuous data between groups were performed by t test or one-way ANOVA with a post-hoc Dunnett's test. Characteristics and lifestyle-adjusted mean levels of serum SOD activity among different BMI and lifestyle groups were compared by analysis of covariance (ANCOVA) with a post-hoc Dunnett's test. A test for linear trend was performed by a polynomial contrast analysis. All statistical analysis were performed by SPSS 15.0J (SPSS Inc.).

Results

The mean level of serum SOD activity in male group is 3.21 (95%CI: 3.14-3.28), and in female group is 3.52 (95%CI: 3.45-3.60). There was a significant difference

Table 1. Mean Values for Serum SOD Activity (U/mL) and 95% Confidence Intervals (CIs) for Different Age Groups

Sex	Age	Number	Mean	95% CI	P value*
Male	40-49	288	3.42	(3.07-3.77)	0.864
	50-59	1,191	3.09	(2.96-3.22)	0.062
	60-69	2,858	3.20	(3.11-3.28)	0.347
	70-79	1,051	3.32	(3.17-3.46)	Ref.
	P for trend		0.684		
Female	40-49	421	3.22	(2.96-3.48)	0.002
	50-59	1,062	3.52	(3.36-3.67)	0.106
	60-69	2,150	3.44	(3.32-3.56)	0.004
	70-79	1,329	3.76	(3.59-3.93)	Ref.
	P for trend		0.002		

* Compared with the reference category by Dunnett's test

Table 2. Crude and Adjusted Mean Levels of Serum SOD Activity (U/mL) and 95%CI among Different BMI Groups

Sex	BMI (kg/m^2)	Number	Mean Crude	Mean Adjusted*	95% CI	P value+
Male	≤18.4	287	3.58	3.52	(3.23-3.85)	0.039
	18.5-24.9	4,052	3.19	3.21	(3.10-3.31)	Ref.
	25.0-29.9	836	3.13	3.10	(2.91-3.30)	0.302
	≥30.0	45	3.05	3.15	(2.37-3.93)	0.887
				P for trend		0.321
Female	≤18.4	313	3.87	3.94	(3.46-4.41)	0.438
	18.5-24.9	3,285	3.53	3.80	(3.45-4.16)	Ref.
	25.0-29.9	1,019	3.47	3.70	(3.32-4.08)	0.307
	≥30.0	93	3.04	3.27	(2.57-3.96)	0.091
				P for trend		0.047

CI: confidence interval; *Age, smoking and drinking adjusted; +Compared with the reference category by the Dunnett's test

Table 3. Serum SOD Activity (U/mL) and 95%CIs among Lifestyle Groups

Lifestyle/Sex Status	Number	Mean Crude	Mean Adjusted*	95% CI	P value+
Smoking	Male				
≥21	407	3.13	3.01	(2.77-3.25)	0.046
11-20	1,233	3.09	3.05	(2.91-3.20)	0.019
≤10	357	3.42	3.43	(3.18-3.69)	0.387
Former	1,226	3.28	3.24	(3.10-3.38)	0.568
Never	912	3.25	3.30	(3.14-3.47)	Ref.
	Female				
Current	121	3.69	3.80	(3.26-4.33)	0.839
Former	72	3.32	3.68	(3.00-4.37)	0.621
Never	4,066	3.49	3.85	(3.60-4.10)	Ref.
Drinking	Male				
>4 days per week					
≥30g	285	3.21	3.25	(2.97-3.54)	0.981
20-29g	835	3.21	3.27	(3.10-3.44)	0.854
≤19g	1,057	3.28	3.26	(3.11-3.41)	0.926
1-4 days	674	3.27	3.27	(3.08-3.46)	0.874
<1 day	1,024	3.23	3.25	(3.10-3.40)	Ref.
Former	260	2.96	2.95	(2.66-3.25)	0.079
	Female				
>4 days	502	3.72	3.68	(3.33-4.02)	0.035
1-4 days	333	3.37	3.32	(2.93-3.71)	0.656
<1 day	3,387	3.48	3.39	(3.11-3.67)	Ref.
Former	37	4.27	4.71	(3.78-5.64)	0.004

* Age, BMI, smoking and drinking adjusted; +Compared with the reference category by the Dunnett's test

between each sex (P<0.001). The mean levels of serum SOD activity in different age groups stratified by sex are shown in Table 1. Significant differences and a linear trend in serum SOD activity were observed in female age groups (lower in younger).

The crude and adjusted mean levels of serum SOD activity in different BMI groups stratified by sex are shown in Table 2. We observed a significant difference in serum SOD activity in male BMI groups (Underweight vs. normal BMI) and a linear trend in female BMI groups (lower in higher BMI). The crude and adjusted mean levels of SOD activity in different lifestyle groups are shown in Table 3. Significant lower SOD activity was observed in male heavier smokers. Also, significant higher SOD activity was observed in female frequent drinkers.

Discussion

We here observed a linear increase in serum SOD activity with aging, particularly in females. While many studies have focused on the relationship between aging and antioxidants, the results have been controversial. One of these studies was in line with our findings (Mecocci et al., 2000), whereas others showed opposite results or no correlation (Andersen et al., 1997; Andriollo-Sanchez et al., 2005; Lubkowska et al., 2009).

Some studies reported that exercise or diet programs for weight loss can increase the level of SOD activity (Skrha et al., 2005; Shih et al., 2006). With the metabolic syndrome, oxidative stress and endothelial dysfunction are accompanied by increased ROS production capacity, and down-regulation of SOD isoforms (Roberts et al., 2006). Consistent with these reports, SOD activity in higher BMI groups was found to be lower in our study.

An influence of smoking on SOD activity in blood has been variously reported in previous studies (Bolzan et al., 1997; Mulholland et al., 1999; Kocyigit et al., 2001; Orhan et al., 2005; Garg et al., 2006). There has been an inconsistency in how SOD activity is regulated by smoking. Here, although an obvious dose dependence was not observed, our data do suggest that smoking can down-regulate serum SOD activity in males. Since cigarette smoke contains a lot of ROS, direct smoke exposure should increase SOD demand in cells. Correspondingly, it has been reported that antioxidant gene expression including SOD in lung cells is higher in smokers (Gilks et al., 1998; Harju et al., 2004). Therefore, if it is true that SOD activity in blood is down-regulated, there may be an indirect regulation by smoking.

Our results show that lower alcohol intake tends to down-regulate serum SOD activity in female. In other studies, a significant difference in SOD activity was not found between drinkers and non-drinkers in healthy females (Casado et al., 2008), or the serum SOD activity in alcoholic groups was lower (Peng et al., 2005; Huang et al., 2009). As such, because there are few reports that support our results about the effect of alcohol to SOD, we need further studies to clarify this point.

Limitations of this study need to be mentioned. It is unclear whether serum SOD activity represents the general oxidative condition in human body, because cellular SOD activity (such as in erythrocytes or lung cells) has been commonly measured in past studies. Nevertheless, according to one study in which both methods were performed, similar patterns were observed in common, such as increase in SOD activity with aging (Mecocci et al., 2000). Next, the number of female smokers and drinkers were too small to be compared with the other groups. In addition, since measurement methods of SOD activity have not been standardized, it is difficult to compare results across different studies. Ranges of SOD activity in the reports cited above therefore mostly differ from each other.

In conclusion, our findings suggest an association between serum SOD activity and some lifestyle factors. However, there is still insufficient evidence to determine the significance of SOD for lifestyle related diseases. In our next study, we will also assess other lifestyle factors, such as exercise. For further investigations, establishment of a standard measurement method for SOD activity should be a high priority.

Member list of the JACC Study Group

The present members of the JACC Study who co-authored this paper together with their affiliations are as follows: Dr. Akiko Tamakoshi (present chairperson of the study group), Aichi Medical University School of Medicine; Drs. Mitsuru Mori & Fumio Sakauchi, Sapporo Medical University School of Medicine; Dr. Yutaka Motohashi, Akita University School of Medicine; Dr. Ichiro Tsuji, Tohoku University Graduate School of Medicine; Dr. Yosikazu Nakamura, Jichi Medical School; Dr. Hiroyasu Iso, Osaka University School of Medicine; Dr. Haruo Mikami, Chiba Cancer Center; Dr. Michiko Kurosawa, Juntendo University School of Medicine; Dr. Yoshiharu Hoshiyama, University of Human Arts and Sciences; Dr. Naohito Tanabe, Niigata University School of Medicine; Dr. Koji Tamakoshi, Nagoya University Graduate School of Health Science; Dr. Kenji Wakai, Nagoya University Graduate School of Medicine; Dr. Shinkan Tokudome, National Institute of Health and Nutrition; Dr. Koji Suzuki, Fujita Health University School of Health Sciences; Dr. Shuji Hashimoto, Fujita Health University School of Medicine; Dr. Shogo Kikuchi, Aichi Medical University School of Medicine; Dr. Yasuhiko Wada, Kansai Rosai Hospital; Dr. Takashi Kawamura, Kyoto University Center for Student Health; Dr. Yoshiyuki Watanabe, Kyoto Prefectural University of Medicine Graduate School of Medical Science; Dr. Kotaro Ozasa, Radiation Effects Research Foundation; Dr. Tsuneharu Miki, Kyoto Prefectural University of Medicine Graduate School of Medical Science; Dr. Chigusa Date, Faculty of Human Environmental Sciences, Nara Women's University; Dr. Kiyomi Sakata, Iwate Medical University; Dr. Yoichi Kurozawa, Tottori University Faculty of Medicine; Dr. Takesumi Yoshimura, Fukuoka Institute of Health and Environmental Sciences; Dr. Yoshihisa Fujino, University of Occupational and Environmental Health; Dr. Akira Shibata, Kurume University School of Medicine; Dr. Naoyuki Okamoto, Kanagawa Cancer Center; and Dr. Hideo Shio, Moriyama Municipal Hospital.

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