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Relation of Serum Oxygen Radical Absorbance Capacity with Metabolic Risk Factors in Human Volunteers

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ABSTRACT

Oxygen radical absorbance capacity(ORAC) is known to be a sensitive and simple method to determine total antioxidant capacity(TAC) in biological samples. While TAC has received great attention with its relation to pathogenesis in the progression of several diseases, little is known about association of ORAC with metabolic risk factors. The aim of this study is to evaluate the relationship between ORAC and serum lipid profiles, fasting glucose and anthropometric measures. One hundred seventeen volunteers participated in the study. Perchloric acid treated serum was used to determine $ORAC_{pca}$. Mean $ORAC_{pca}$ of subjects whose serum total cholesterol(TC) concentrations were $\geq$ 200 mg/dl was significantly($P < 0.05$) higher than that of subjects whose TC concentrations were < 200mg/dl. There were significantly positive correlations between $ORAC_{pca}$ and serum concentrations of TC($P < 0.05$) and low density lipoprotein (LDL) cholesterol($P < 0.01$). The positive relation between cholesterol concentrations and $ORAC_{pca}$ in serum may suggest an elevated TAC against oxidative stress associated with the cardiovascular risk factors. (*J Community Nutrition* 7(4) : 215~219, 2005)

KEY WORDS : oxygen radical absorbance capacity · cholesterol · triglyceride · body mass index · human.

Introduction

Oxygen Radical Absorbance Capacity(ORAC) has been employed for years to determine plasma or serum total antioxidant capacity(TAC) in animals(Chen et al. 2004 ; Ninfali, Aluigi 1998) and humans(Cao et al. 1998 ; Mazza et al. 2002 ; Fernandez-Pachon et al. 2005). Among several analytical methods for measuring TAC, ORAC is known to be a relatively simple but sensitive method(Prior, Cao 1999). Plasma or serum ORAC has been shown to respond positively to diets high in fruits and vegetables(Cao et al. 1998), red wine intake(Fernandez-Pachon et al. 2005) and serum anthocyanin concentration following blueberry consumption(Mazza et al. 2002). In addition, plasma or serum components such as protein and uric acid have been shown to account for great proportions of the overall plasma or serum ORAC values and positively correlated with ORAC values(Antolini et al. 2004 ; Fernandez-Pachon et al. 2005 ; Ninfali, Aluigi 1998). The large contribution of these plasma or serum components to ORAC may result in unchanged ORAC values even after dietary antioxidant intake (Chen et al. 2004 ; Engler et al. 2004) and changed ORAC values in several disease conditions without dietary modifications (Sofic et al. 2002). Especially, in renal disease, accumulation of uric acid in serum may explain an increased antioxidant capacity measured by ORAC(Antolini et al. 2004 ; Sofic et al. 2002).

The role of free radicals in the pathogenesis and progression of many diseases has been given great attention and several assays measuring TAC have been often employed to determine in vivo free radical trapping power. Especially, increased oxidative stress and associated oxidative damage have often considered as mediators of vascular injuries in cardiovascular pathologies including atherosclerosis and hypertension(Touyz, 2003). In a study by Vassalle et al.(2004), cardiovascular risk factors including dyslipidemia and hypertension tended to be related with TAC measured by commercialized total antioxidant power(PAO) test. However, the

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relations of TAC to various metabolic risk factors have not been widely investigated and knowledge is still limited whether individual variation of total antioxidant capacity is associated with anthropometric variation. Therefore, in the present study, we employed $ORAC_{pca}$ as a method to determine TAC and investigated the relation of $ORAC_{pca}$ with several metabolic risk factors and anthropometric measures.

Subjects and Methods

One hundred and seventeen human volunteers(18 males and 99 females) living in the Seoul area participated in this study. A single blood sample from each subject was collected in vacutainer tubes after an overnight fast. Serum samples were separated from whole blood for lipid and glucose analysies and the sample aliquots were stored at -80℃ until ORAC analysis.

Subjects' weight, height, and waist and hip circumferences were measured by trained technicians. The BMI was calculated as weight(kg) divided by height(m^2). Waist circumference to hip circumference ratio(WHR) was calculated as the ratio between waist and hip circumferences. Trained technicians measured blood pressure with a semiautomatic device, with subjects in the sitting position after at least 2 min of rest. Serum total cholesterol(TC), low-density lipoprotein(LDL) cholesterol, high density lipoprotein(HDL) cholesterol, triglyceride(TG) and glucose concentrations were measured by ADVIA® 1650 chemistry system(Bayer HealthCare LLC, Germany). The TAC of serum was assessed by ORAC assay using a Victor multilabel plate reader(PerkinElmer Life and Analytical Sciences, Inc., USA) and a slight modification of the method described by Huang et al.(2002). Since the contribution of albumin to ORAC in whole serum($ORAC_{total}$) is reported to be the greatest(27.8%)(Cao and Prior 1998), in the current study, we used nonprotein fraction extracted with perchrolic acid for the $ORAC_{pca}$ assay.

Statistical analyses were done with using SPSS for windows(version 11.0, SPSS Inc, Chicago). Means and standard deviation of measures were calculated for all subjects, and male and female subjects separately. Subjects were divided into two groups by their TC levels(<200mg/dl and ≥ 200 mg/dl) and means and standard deviation of measures were calculated for each group. Differences between the groups were assessed by independent two-tailed t-tests. Pearson's correlation coefficients were used to assess the relationship between $ORAC_{pca}$ and other measures. Differences with $P < 0.05$ were considered significant.

Results

General characteristics and biochemical measures of subjects are shown in Table 1. Mean $ORAC_{pca}$ was significantly higher in male than female subjects($P < 0.05$). Mean BMI and WHR of male volunteers were also higher than females ($P < 0.05$). While mean HDL-cholesterol in serum was lower in males than in females($P < 0.05$), mean serum TG concentration was higher in males than females($P < 0.05$). Mean TC and LDL-cholesterol concentrations tended to be

Table 1. General Characteristics and biochemical measures of subjects

	All Subjects(N = 117)	Males(N = 18)	Females(N = 99)
Age(y)	45.09 ± 12.81	46.61 ± 10.83	44.81 ± 13.16
$ORAC_{pca}$[2] (μmol/L Trolox Eq)	1085.72 ± 189.24[1]	1169.04 ± 195.44*	1070.57 ± 185.08
Height(cm)	161.70 ± 9.23	172.17 ± 5.12	159.78 ± 8.52
Weight(kg)	58.66 ± 10.87	73.78 ± 10.08	55.92 ± 8.53
BMI[3] (kg/m^2)	22.32 ± 3.15	24.88 ± 3.15*	21.85 ± 2.93
WHR[4]	0.85 ± 0.08	0.92 ± 0.06*	0.83 ± 0.08
SBP[5] (mmHg)	110.82 ± 14.51	121.94 ± 17.08*	108.75 ± 13.07
DBP[6] (mmHg)	71.73 ± 10.20	80.83 ± 10.04*	70.04 ± 9.34
Total cholesterol(mg/dl)	199.40 ± 35.19	212.61 ± 33.00	197.00 ± 35.20
Triglyceride(mg/dl)	119.93 ± 84.50	178.00 ± 98.58*	109.37 ± 77.68
HDL-cholesterol(mg/dl)	60.45 ± 12.90	51.58 ± 9.11*	62.06 ± 12.86
LDL-cholesterol(mg/dl)	114.96 ± 30.18	125.43 ± 36.35	113.06 ± 28.72
Glucose(mg/dl)	92.44 ± 18.94	105.56 ± 32.34*	90.05 ± 14.35

[1] mean ± SD [2] Oxygen Radical Absorbance Capacity of perchrolic acid treated serum, [3] Body mass index, [4] Waist circumference to hip circumference ratio, [5] Systolic blood pressure, [6] Diastolic blood pressure
*: Significantly different from females, P <0.05

Table 2. General characteristics and biochemical measures of subjects grouped by the level of total cholesterol

	Total cholesterol ≥ 200 (N = 56, Male: 10, Female: 46)	Total cholesterol <200 (N = 61, Male: 8, Female: 53)
Age(y)	47.68 ± 11.53	42.70 ± 15.53
$ORAC_{pca}$[2] (μmol/L Trolox Eq)	1121.82 ± 195.53[1]*	1052.57 ± 178.48
Height(cm)	162.50 ± 6.81	160.94 ± 11.01
Weight(kg)	60.88 ± 9.90	56.63 ± 11.40
BMI[3] (kg/m2)	23.03 ± 3.15*	21.66 ± 3.02
WHR[4]	0.87 ± 0.08*	0.82 ± 0.08
SBP[5] (mmHg)	113.02 ± 16.87	108.73 ± 11.62
DBP[6] (mmHg)	73.20 ± 11.02	70.34 ± 9.23
Total cholesterol(mg/dl)	227.50 ± 25.80	173.61 ± 19.27
Triglyceride(mg/dl)	142.86 ± 96.84*	98.89 ± 65.33
HDL-cholesterol(mg/dl)	62.88 ± 14.35	58.21 ± 11.06
LDL-cholesterol(mg/dl)	136.04 ± 25.47*	95.61 ± 19.24
Glucose(mg/dl)	93.38 ± 16.10	91.57 ± 21.32

[1] mean ± SD [2] Oxygen Radical Absorbance Capacity of perchrolic acid treated serum, [3] Body mass index, [4] Waist circumference to hip circumference ratio, [5] Systolic blood pressure, [6] Diastolic blood pressure
*: Significantly different from the group of total cholesterol <200mg/dl, P <0.05

Table 3. Correlation coefficient between $ORAC_{pca}$ and metabolic risk factors[1]

	BMI	WHR	SBP	DBP	TC	TG	HDL-C	LDL-C	Glucose
All subjects(N = 117)	0.151	0.017	0.123	0.074	0.232*	0.151	- 0.138	0.245**	0.033
Males(N = 18)	- 0.068	0.308	0.188	0.174	0.545*	- 0.218	0.459	0.498*	- 0.226
Females(N = 99)	0.125	- 0.033	0.031	- 0.039	0.150	0.177	- 0.160	0.160	0.057
TC ≥ 200(N = 56)	0.351**	0.095	0.187	0.111	0.271*	0.247	- 0.372**	0.297*	0.097
TC <200(N = 61)	- 0.130	- 0.138	- 0.044	- 0.034	- 0.025	- 0.094	0.076	- 0.005	- 0.029

[1] BMI: Body mass index, WHR: Waist circumference to hip circumference ratio, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TC: Total cholesterol, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol
*: Significant at P <0.05
**: Significant at P <0.01

higher in males than in females.

Among subjects, only 2 individuals had HDL-cholesterol lower than 40mg/dl and 11 individuals had fasting glucose higher than 110mg/dl. In addition, 16 and 30 out of 117 subjects showed their TG and LDL-cholesterol level ≥ 200 mg/dl and ≥ 130mg/dl, respectively. However, almost half of the subjects had TC greater than 200mg/dl. Since there are evidences that serum cholesterol and cardiovascular risk factors including dyslipidemia are associated with TAC (Nieto et al. 2000 ; Vassalle et al. 2004), subjects were divided into two groups by their serum TC concentration (< 200mg/dl and ≥ 200mg/dl) to compare their $ORAC_{pca}$ and other measures between groups. Each group consisted of 61 and 56 subjects, respectively. General characteristics and biochemical measures of the two groups whose respective serum TC concentration <200mg/dl and ≥ 200mg/dl are presented in Table 2. Mean $ORAC_{pca}$, BMI and WHR were significantly higher in the group with TC concentration at ≥ 200mg/dl than the group with TC concentration at < 200mg/dl (P < 0.05). In addition, mean LDL-cholesterol and TG in serum were higher in the group with TC ≥ 200mg/dl than in the group with TC <200mg/dl (P <0.05).

Table 3 shows the Pearson's correlation coefficients between $ORAC_{pca}$ and measures in all volunteers and each group. $ORAC_{pca}$ of all subjects was significantly positively correlated with serum TC (P <0.05) and LDL-cholesterol (P < 0.01), while it tended to be negatively correlated with serum HDL-cholesterol. Females whose mean serum TC was <200 mg/dl (Table 1) showed no significant association between $ORAC_{pca}$ and other measures. However, males whose mean serum TC was >200mg/dl showed significant correlations between $ORAC_{pca}$ and serum TC and LDL-cholesterol concentrations (P <0.05). As similar to females, the group with serum TC <200mg/dl showed no significant correlations between $ORAC_{pca}$ and other measures. In the group with serum TC ≥ 200mg/dl, $ORAC_{pca}$ showed significant posi-

tive correlations with LDL-cholesterol($P < 0.05$), TC($P < 0.05$) and BMI($P < 0.01$), and negative correlation with HDL-cholesterol($P < 0.01$). Overall blood pressure and fasting glucose of subjects were in normal range(Table 1, Table 2), and showed no significant association with serum $ORAC_{pca}$(Table 3).

Discussion

The lack of knowledge about the relationship between TAC and metabolic risk factors led us to investigate whether there is any association between $ORAC_{pca}$ and serum lipids, BMI, blood pressure and fasting serum glucose. This study suggests that increased levels of serum cholesterol are positively related with serum TAC as measured $ORAC_{pca}$.

In addition to the effect of diet-derived antioxidants in serum on TAC(Mazza et al. 2002), serum constituents, such as albumin, bilirubin and uric acid have been shown to be great contributors to serum TAC measured by ORAC and other analytical methods(Cao et al. 1993 ; Cao, Prior 1998). $ORAC_{pca}$ fraction preserves the water-soluble antioxidants within the sample and about 39.2% of $ORAC_{pca}$ was shown to be contributed by uric acid(Cao, Prior 1998). Based on the finding by Cao and Prior(1998), we assume that the uric acid may be a great factor that contributes $ORAC_{pca}$ values in the current study.

ORAC has been employed to measure TAC in several disease states, and found to be changed with various abnormal conditions(Sofic et al. 2002). In patients with renal disease, serum antioxidant capacity measured by ORAC has shown to be higher than controls and significantly correlated with uric acid concentrations(Antolini et al. 2004). In a study by Nieto et al.(2000), ORAC from protein free serum was significantly higher in atherosclerosis cases than controls and positively correlated with serum cholesterol concentrations. This finding is comparable with the observation of the current study. As similar to Nieto et al.(2000), we also tested ORAC from protein free serum and found a positive association between cholesterol concentrations and $ORAC_{pca}$ values among individuals with relatively high cholesterol levels. Unlike cholesterols, other metabolic risk factors such as blood pressure and fasting glucose did not show any association with $ORAC_{pca}$. Based on the levels of risk factors defined in Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults(2001), only 8.5 and 9.4% of subjects in the current study reached their levels of blood pressure and fasting glucose to the levels of risk factor, respectively, while 48 and 26% subjects had equal or higher than borderline high levels of TC and LDL-cholesterol, respectively. This may explain why fasting glucose and blood pressure did not show their significant relation to $ORAC_{pca}$ in the present study, while TC and LDL-cholesterol were significantly correlated with $ORAC_{pca}$.

In a trial with atherosclerosis cases, Nieto et al.(2000) emphasized not only the relation between ORAC and serum cholesterol concentration but also the association between ORAC and elevated serum uric acid concentration. Uric acid, in addition to its relation to ORAC, has shown to be associated with various metabolic risk factors. In a study done by Ishizaka et al.(2005), 8144 Japanese individuals were tested and serum uric acid was significantly correlated positively with BMI, blood pressure, TC and TG, and negatively with HDL-cholesterol. According to the uric acid quartile, BMI and TG showed a graded increase and HDL-cholesterol showed a graded decrease. Three hundred fifty two middle-aged Brazilian men showed proportionally increased uric acid levels when the number of metabolic risk factors, high blood pressure, obesity, hypertriglyceridemia, low HDL and hyperglycemia, increased as 1, 2 and ≥ 3(Desai et al. 2005). Although these findings of the relationship between uric acid and cardiovascular risk factors have not been fully consistent (Brand et al. 1985), the associations continually shown in recent studies are worth to be noted.

Due to lack of uric acid data and dietary information, we may not be able to strongly assume the factors directly affecting $ORAC_{pca}$ values. In addition, the increased $ORAC_{pca}$ in serum may be due to some confounding by unknown factors contributing $ORAC_{pca}$ values. However, when we consider the fact that the great contribution of uric acid to $ORAC_{pca}$(Cao, Prior 1998), the significant association between $ORAC_{pca}$ and some metabolic risk factors such as BMI, TC, LDL-cholesterol and HDL-cholesterol may be in part explained by changes in uric acid concentration along with increased metabolic risk factors. Increased uric acid, an effective antioxidant, along with cardiovascular risk factors might be a consequence of body's compensatory mechanism to counteract increased free radicals associated with the pathogenesis and progression of diseases(Ames et al. 1985). Therefore, we assume that the unexpected elevation of $ORAC_{pca}$ among individuals with relatively high TC may be due

to increased body's antioxidant defense system against oxidative stress related to cardiovascular risk factors. In addition, future studies should evaluate other serum constituents including uric acid and dietary intake of antioxidants to clarify the factors affecting TAC in serum.

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