Relationship among sRAGE, AGE and CRP according to *RAGE* Gly82Ser and obesity status in Korean men

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사랑스런 나의 세 아들 – 한결, 경찬, 영환아! 너희에게 멋진 아빠가 되도록 늘 노력하마.

마지막으로 언제나 저를 사랑해 주시고, 제 길을 인도해 주시는 하나님께 모든 영광을 돌립니다.

“ 너희는 먼저 그의 나라와 그 의를 구하라 그리하면 이 모든 것을 너희에게 더하시리라”
– 마태복음 6장 33절 -

2007. 1월 조 석 현

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ABSTRACT

Relationship among sRAGE, AGE and CRP according to *RAGE* Gly82Ser and obesity status in Korean men

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We studied the relationship among souble receptor for advanced glycation end products (sRAGE), AGE and inflammatory markers according to *RAGE* Gly82Ser gene polymorphism and obesity, a low-grade inflammatory state. We measured circulating concentrations of sRAGE, AGE and inflammatory markers (CRP and IL-6) in a group of Korean men. A total of 1096 men aged 30~70 years with body mass index≥18.5kg/m^2 were recruited. Anthropometric parameters, lipid profiles, glucose, *RAGE* G82S polymorphism, sRAGE, AGEs, and inflammatory markers (CRP and IL-6) were measured. sRAGE concentrations were lowest in those with homozygous mutation, 'S/S' (G/G: 1036.3±40.3, G/S: 807.0±49.6 and S/S: 443.0±47.8pg/ml, p<0.001), which maintained after adjusted for age, BMI, cigarette smoking and alcohol drinking (p<0.001). Stepwise regression showed that RAGE Gly82Ser genotype (β-coefficient=-0.384, p<0.001) and

BMI (β-coefficient=-0.168, $p=0.001$) were major influencing factors on sRAGE concentration. Obese subjects (BMI≥25kg/m^2) had significantly lower levels of sRAGE (831.7±36.7pg/ml) than non-obese subjects (1022.7±47.8pg/ml) ($p=0.009$). In Obese subjects, the 'S/S' group had lower concentrations of sRAGE (439.5±57.7pg/ml) than the G/G (917.5±45.7 pg/ml) or the G/S group (768.8±56.1pg/ml) ($p<0.001$). On the other hand, the obese subject with the S/S genotype showed higher concentrations of AGE and CRP comparing with those with the G/G or G/S genotype ($p=0.012$ and $p=0.006$, respectively). In conclusion, sRAGE is influenced by *RAGE* G82S polymorphism and obesity status as a result of enhanced AGE-RAGE binding affinity. Inflammatory condition such as obesity not only decreases sRAGE levels but also increases AGE and CRP levels particularly in subjects with *RAGE* 82 S/S genotype.

Key Words : soluble RAGE, AGE, CRP, *RAGE* Gly82Ser polymorphism, Obesity, AGE-RAGE binding affinity and inflammation

I. INTRODUCTION

Receptor for advanced glycation end products (RAGE), a multi-ligand member of the immunoglobulin superfamily of cell surface molecules (1) interacts with a diverse class of ligands including AGE (2), thereby resulting in oxidative stress and cellular dysfunction and bringing about the generation of oxygen free radicals and the cellular activation of NF-κb, an oxidative stress marker (3,4). Nawroth et al reported the interaction of AGE-RAGE mediates in the development of diabetic microvascular complication (5). In addition, animal study showed that RAGE expression was upregulated in atherosclerotic plaques of diabetic animals (6) and the human studies implicated the involvement of RAGE in diabetic vasculopathy (7,8).

RAGE has a C-truncated secretory isoform of the receptor protein, termed soluble RAGE (sRAGE) produced by alternative splicing of RAGE mRNA and present in the circulation, which may neutralize the AGEs-mediated damage by acting as a decoy (9, 10, 11). Related to this mechanism, a number of studies have shown that sRAGE is inversely associated with chronic inflammatory state, diabetes, atherosclerosis and metabolic syndrome (12, 13). According to Falcone et al, low levels of sRAGE in plasma are independently associated with the presence of coronary artery disease (CAD) in nondiabetic men (12). Koyama et al. also showed that sRAGE is a novel and potential factor for the metabolic syndrome and atherosclerosis (13).

A functional amino acid change (glycine to serine) at codon 82 in exon 3 of the *RAGE* within putative ligand binding domain (*RAGE* G82S) is also involved in the RAGE expression inflammatory responses (14, 15). Hofmann et al reported that *RAGE* 82S allele upregulates the inflammatory response upon engagement of ligand and might contribute to enhance proinflammatory mechanisms in immune/inflammatory diseases (15).

sRAGE is highlighted as a pivotal protein to block inflammation pathway resulting from RAGE-AGE axis. However, it is unclear which factors affect expression of sRAGE. We assume that there is some mechanism to decrease expression of sRAGE in inflammatory state. Furthermore there were no study for the relationship among sRAGE, AGE and inflammatory markers according to *RAGE* gene polymorphism and obesity. Therefore, we measured circulating concentrations of sRAGE, AGE and inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL6) in Korean men and observed the change of sRAGE concentration and other markers according to G82S *RAGE* gene polymorphism and the status of obesity.

II. SUBJECTS AND METHODS

2.1. Study Subjects

Study participants were all men recruited from the Health Service Center in the course of a routine check-up visit or by a newspaper announcement for health examination. The newspaper announcement briefly described the study design and invited Korean men aged 30~70 years with body mass index≥18.5kg/m^2 to participate in the Cardiovascular Genome Center study as control subjects. Excluded were subjects with orthopedic limitations, weight loss/gain over the previous 6 months or any diagnosis of disease such as diabetes mellitus, cardiovascular disease, liver disease, renal disease, thyroids disease or cancer. None of them were taking any medication. Written informed consent was obtained from all subjects and the protocol was approved by the Institute of Review Board of Yonsei University.

Current cigarette smokers were defined as subjects reporting at least one cigarette per day. Current alcohol drinkers were defined as those reporting at least one portion of alcohol per day. Finally, a total of 1252 male subjects were recruited and they were un-related.

2.2. Anthropometries and blood pressure measurements

Anthropometric measurements included height, body weight and waist and hip circumferences. Body weight and height were measured in the morning, light clothed without shoes. The body mass index (BMI) was calculated as

body weight in kilograms divided by height in square meters (kg/m^2). Body fat percentages were measured with a TBF-105 body fat analyzer (Tanita Corp. Tokyo, Japan). Waist circumference was measured with a flexible tape midway between the lower rib margin and the iliac crest in the standing position after normal expiration. Blood pressure was read from the left arm of seated patients with an automatic blood pressure monitor (TM-2654, A&D, Tokyo, Japan) after 20 minutes of rest. The average of three measurements was recorded for each subject. In this study, presence of obesity was defined by body mass index ≥25 kg/m2 as reported for Korean population (16).

2.3. Fasting blood collection

To reduce the influence of circadian variation, all blood specimens were collected between 08:00 and 10:00 after the subjects had fasted overnight. Venous blood specimens were collected in EDTA-treated and plain tubes. The tubes were placed on ice until they arrived at the laboratory room (within 1-3 hours) and stored at –70℃ until analysis after plasma and serum were separated.

2.4. Genotyping of *RAGE* Gly82Ser

Genomic DNA was prepared from peripheral blood samples using a Puregene® DNA purification kit (Gentra, Minneapolis, MN, U.S.A.), following the manufacturer's protocol. A *RAGE* SNP (single nucleotide polymorphism) [rs17846805, Gly82→Ser (G82S)] was genotyped in 1252

subjects. G82S genotyping was performed by SNP-IT™ assays using SNPstream 25K® System (Orchid Biosciences, Princeton, NJ, USA).

Briefly, the genomic DNA region spanning the polymorphic site was PCR-amplified using one phosphothiolated primer and one regular PCR primer. The amplified PCR products were then digested with exonuclease (Amersham Biosciences, Uppsala, Sweden). The 5´ phosphothiolates were used in this study to protect one strand of the PCR-product from exonuclease digestion. The single-stranded PCR template generated from exonuclease digestion was overlaid onto a 384 well plate that precoated covalently with SNP-IT™ primers (Intergrated DNA Technologies (IDT) Inc., Coralville, IA, USA). These SNP-IT™ primers were designed to hybridize immediately adjacent to the polymorphic site. After hybridization of template strands, SNP-IT™ primers were then extended by a single base with DNA polymerase at the polymorphic site of interest. The SNP-IT™ primer is extended for a single base with DNA polymerase and mixture of appropriate acycloterminator which is labeled with either FITC (Fluorescein isothiocyanate) or biotin (Perkin-Elmer Asia Singapore) and complementary to the polymorphic nucleotide. The final single base incorporated was identified with serial colorimetric reactions with anti-Fluorescein-AP (Roche, Basel, Switzerland) and streptavidin-HRP (Pierce, Rockford, IL, U.S.A.), respectively. The results of blue and/or yellow color developments were analyzed with an ELISA reader and the final genotyping (allele) calls were made with the QCReview™ program.

2.5. Serum lipid profiles and glucose

Serum total cholesterol and triglyceride were measured with commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). After precipitation of serum chylomicrons, very low density lipoprotein (VLDL), and low density lipoprotein (LDL) using dextran sulfate-magnesium, high density lipoprotein (HDL) cholesterol left in the supernatant was measured using an enzymatic method (17). LDL cholesterol was estimated indirectly using the Friedwald formula, i.e., LDL cholesterol = total cholesterol – {HDL cholesterol + (triglycerides/5)}, for subjects with serum triglyceride concentration <400 mg/dL (<4.52 mol/L) (18). In subjects with serum triglyceride concentration ≥4.52 mol/L, LDL cholesterol was measured directly. Fasting glucose was measured by the glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA, U.S.A.).

2.6. Measurement of sRAGE and AGE

The plasma full sRAGE (soluble receptor for advanced glycation endproduct) was measured using a commercially available enzyme-linked immunosorbent assay kit (R&D systems, Minneapolis, MN, USA). The resultant color reaction was read at 450nm using a Victor^2 (Perkin Elmer life sciences, Turku, Finland) and wavelength correction was set to 540nm.

Measurement of AGEs (advanced glycation endproducts) in serum was performed by a non-competitive enzyme-linked immunosorbent assay

(ELISA) according to the method of Horiuchi et al (19). A 96-well microplate was coated at 4℃ overnight with serially diluted AGE-BSA as standards, or diluted serum samples. Unbound sites were blocked with 2% skim milk, 0.5% BSA and 0.5% gelatin in 0.05M carbonate buffer for 1 hour and washed with washing buffer (PBS, 0.05% Tween-20). Each well was incubated for 2 hour with anti-AGE monoclonal antibody (dilution 1:1000, Transgenic Inc., Kumamoto, Japan). The plate was washed again and incubated with HRP-labeled goat anti-mouse IgG antibody (dilution 1:2000, Zymed Inc., San Francisco, CA, U.S.A.) for 1 hour. The unbound antibodies were removed by washing and bound antibodies were detected by incubation with 3,3',5,5'-tetramethylbenzidine (TMB) substrate for 30 minutes. After reaction was stopped by 1M sulfuric acid, the resultant color reaction was read at 450nm using a Victor^2 (Perkin Elmer life sciences, Turku, Finland).

2.7. Serum levels of C-reactive protein and interleukin-6

Serum high-sensitivity C-reactive protein (hs-CRP) levels were measured using a commercially available high-sensitivity kit, CRP-Latex (II) X2 supplied by Seiken Laboratories Ltd. (Tokyo, Japan) that allowed detection of CRP levels as low as 0.001 mg/dL and as high as 32 mg/dL. The assay principle is that latex microparticles coated with monoclonal antibodies against CRP reacts with the CRP in the added samples for immunoagglutination reactions. The measurements of these immunoagglutination reactions were performed on Express Plus auto-analyzer

(Chiron Diagnostics Co., MA, USA) using reaction buffer. The absorbance change was calculated at 572 nm for 3 min.

Serum interleukin-6 (IL-6) was measured using an enzyme immunoassay (R&D Systems, MN, USA). Sample was added to a monoclonal anti-IL-6 antibody which was pre-coated onto a microplate. After washing away any unbound substances, an enzyme-linked polyclonal anti-IL-6 antibody was added. A substrate solution was added to develop color in proportion to the amount of IL-6 bound. The resultant color reaction was read using a Victor^2 (Perkin Elmer Life Sciences, Turka, Finland) at 490 nm and wavelength correction was set to 650 nm. Quantification of IL-6 was performed using the peak area ratio.

2.8. Statistical analysis

Statistical analyses were performed with SPSS version 12.0 for Windows (Statistical Package for the Social Science, SPSS Ins., Chicago, IL, U.S.A.). Hardy Weinberg Equilibrium (HWE) was examined using the Executive SNP Analyzer 1.0 (http://www.istech.info/SilicoSNP/ index.html). One-way analysis of covariance followed by a Bonferroni test and a general linear model (GLM) for adjustment of potential covariates such as age, BMI, cigarette smoking and alcohol drinking were performed to compare the differences in biomarkers among genotype groups. Each variable was examined for normal distribution patterns and significantly skewed variables were log-transformed. For descriptive purposes, mean values are presented

using untransformed and unadjusted values. Results are expressed as mean±S.E. Proportions of cigarette smoking and alcohol drinking were tested by χ^2. A two tailed value of $P<0.05$ was considered statistically significant.

III. RESULTS

3.1. Frequency of the *RAGE* G82S polymorphism

The *RAGE* G82S genotype distribution among the 1252 subjects examined was as follows: 904 subjects examined were homozygous for the G allele (G/G), 319 were heterozygous for the S allele (G/S), and 29 were homozygous for the S allele (S/S). These frequencies did not deviate significantly from Hardy-Weinberg equilibrium. The S allele frequency was 0.177, much greater than that reported in whites (≈0.05) (20,21).

3.2 **Anthropometric and basic biochemical characteristics according to the *RAGE* G82S genotype**

Table 1 shows the general characteristics of the subjects according to the *RAGE* G82S genotype. There were no significant G82S genotype-related differences with respect to age (p=0.161), BMI (p=0.253), waist circumference (p=0.236), body fat % (p=0.932) systolic and diastolic blood pressures (p=0.447 and p=0.991), lipid profiles such as triglyceride (p=0.636) total cholesterol (p=0.935), HDL-cholesterol (p=0.278), LDL-cholesterol (p=0.710), serum glucose (p=0.813) and proportions of cigarette smoking (p=0.587) and alcohol consumption (p=0.658).

Table 1. Anthropometric and biochemical parameters according to *RAGE* G82S

	G/G (n=904)	G/S (n=319)	S/S (n=29)
Age (yrs)	51.4±0.36	51.8±0.55	47.9±2.05
Body mass index (kg/m^2)	24.9±0.09	24.6±0.16	25.2±0.42
Waist circumference (cm)	88.7±0.24	87.9±0.44	88.7±1.13
% body fat	23.3±0.18	23.2±0.30	23.1±0.77
Systolic BP (mmHg)	123.2±0.55	122.2±0.94	125.6±2.44
Diastolic BP (mmHg)	78.9±0.35	78.9±0.59	78.9±1.84
Triglyceride (mg/dL)[1]	157.3±3.13	163.4±8.17	167.9±18.5
Total cholesterol (mg/dL)[1]	192.4±1.27	193.3±2.26	191.9±4.55
HDL-cholesterol (mg/dL)[1]	44.2±0.40	42.1±0.62	42.5±1.71
LDL-cholesterol (mg/dL)[1]	117.7±1.18	119.4±2.08	115.3±4.93
Glucose (mg/dL)[1]	96.5±0.88	96.0±1.47	93.7±3.04
Interleukin-6 (pg/mL)[1]	3.21±0.83	3.05±0.33	5.23±1.74
Current smoker %	45.5	44.2	41.4
Current drinker %	67.1	68.9	55.2

Data are presented as mean±S.E and or percentage.

[1] tested after log-transformed. Determined by one-way analysis of covariance followed by a Bonferroni test

Values significantly different ($p<0.05$) are indicated by different alphabets

3.3. sRAGE, AGE and inflammatory markers according to the *RAGE* G82S genotype

sRAGE concentrations were highest in subjects with wild type ‘G/G’ and lowest in those with homozygous mutation, ‘S/S’ (G/G: 1036.3±40.3 pg/ml and G/S: 807.0±49.6 pg/ml, S/S: 443.0±47.8 pg/ml, $p<0.001$), which maintained after adjusted for age, BMI, cigarette smoking and alcohol drinking ($p<0.001$) (Fig. 1). AGE concentrations were higher in S/S genotype group than the other two groups but, it was not statistically significant ($p0=0.069$, $p1=0.091$).

In association with inflammatory markers, CRP concentrations showed an increasing tendency when carrying 82S allele ($p<0.063$), which turned statistically significant after the adjustment ($p=0.035$) (Figure 1). However, IL-6 concentrations were not significantly different among three genotype groups (Table 1).

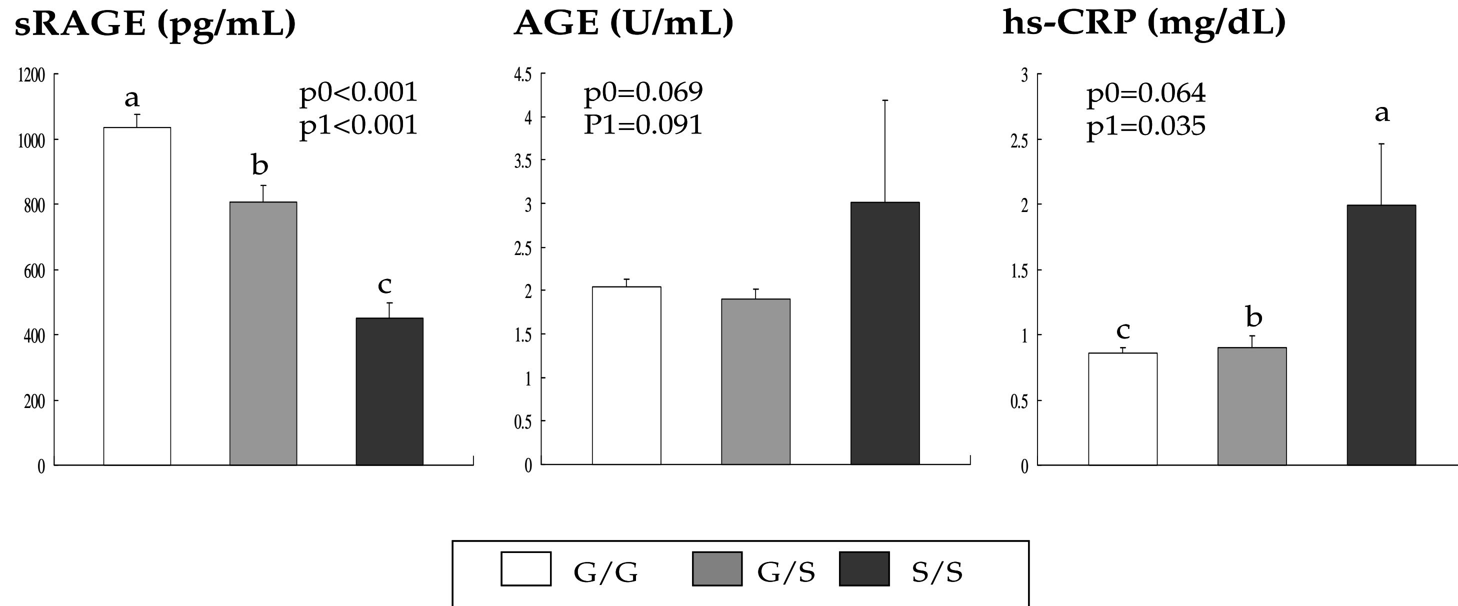

Figure 1. Concentrations of sRAGE, AGE and hs-CRP in Korean men according to *RAGE* G82S polymorphism.

Mean±S.E.

All data were tested after log-transformed. Tested by one way-analysis of variance and general linear model (GLM) followed by Bonferroni method with adjustment (P0: p-value before adjustment, P1: p-value adjusted for age, BMI, cigarette smoking and alcohol drinking).

Values significantly different ($p<0.05$) are indicated by different alphabets by GLM test.

3.4. Stepwise regression to identify major factors influencing sRAGE concentrations

Besides *RAGE* G82S, to find other major factors influencing sRAGE levels among anthropmetric and basic biochemical parameters, we performed a stepwise regression with sRAGE as a dependent variable and age, BMI, waist, blood pressure, triglyceride, HDL-cholesterol, fasting glucose, cigarette smoking and alcohol drinking as independent variables.

We found that *RAGE* G82S is the first influencing factor (standardized β-coefficient = -0.384, $p<0.001$) and BMI is the second one (standardized β-coefficient = -0.168, $p=0.001$) (Table 2). Therefore, we subdivided study subject into two groups 'Non-obese' group ($BMI<25kg/m^2$) and 'Obese' group ($BMI\geq25kg/m^2$) according to their BMI level, and observed the RAGE G82S genotype-associated phenotype in each BMI group.

Table 2. Stepwise regression to identify major factors influencing sRAGE concentration

model		Unstandardized coefficient		Standardized coefficient	p-value	R	p-value
		B	Std.Error	B			
1	Constant	6.858	0.038		<0.001		
	RAGE G82S	-0.317	0.055	-0.385	<0.001	0.385	<0.001
2	Constant	7.640	0.308		<0.001		
	RAGE G82S	-0.317	0.054	-0.384	<0.001	0.420	<0.001
	Body mass index	-0.032	0.012	-0.168	0.011		

Independent variable: *RAGE* G82S, age, BMI, waist, systolic and diastolic blood pressures, triglyceride*, HDL-cholesterol*, glucose*, cigarette smoking and alcohol drinking, **Dependent variable:** sRAGE* * Tested after Log-transformed

3.5. sRAGE, AGE and inflammatory makers according to obese status

We found that age and basic anthropometric parameters such as BMI, waist circumference, body fat % and blood pressure were higher in obese group (BMI≥25kg/m^2) that in non-obse group (BMI<25kg/m^2) (Table 3). Fasting glucose and lipid profile except LDL-cholesterol were also higher in obese group. Related to inflammatory markers, CRP concentration was significantly higher in obese group compared with non-obese group ($p<0.001$), however IL-6 was not statistically significantly different between two groups. Obese subjects had significantly lower levels of sRAGE (831.7±36.7 pg/ml) than non-obese subjects (1022.7±47.8 pg/ml) ($p=0.009$), but AGE concentration was not significantly different between two groups ($p=0.752$) (Table 3).

Table 3. Anthropometric and biochemical parameters between non obese subjects and obese subjects

	Non-obese (n=694)	Obese[2] (n=558)
Age (yrs)	52.0 ± 0.40	50.7 ± 0.45*
Body mass index (kg/m^2)	22.9 ± 0.06	27.2 ± 0.08***
Waist circumferences (cm)	84.5 ± 0.22	93.6 ± 0.24***
% body fat	20.8 ± 0.17	26.3 ± 0.19***
Systolic BP (mmHg)	120.7 ± 0.62	125.9 ± 0.68***
Diastolic BP (mmHg)	77.1 ± 0.38	81.1 ± 0.46***
Triglyceride (mg/dL)[1]	142.0 ± 3.21	180.4 ± 5.57***
Total cholesterol (mg/dL)[1]	190.2 ± 1.40	195.6 ± 1.70*
HDL-cholesterol (mg/dL)[1]	45.9 ± 0.47	41.5 ± 0.44***
LDL-cholesterol (mg/dL)[1]	116.7 ± 1.31	119.9 ± 1.57
Glucose (mg/dL)[1]	95.0 ± 0.92	98.0 ± 1.20*
C-reactive protein (mg/dL)[1]	0.72 ± 0.05	1.10 ± 0.07***
Interleukin-6 (pg/mL)[1]	2.97 ± 0.23	3.50 ± 0.54
Soluble RAGE (pg/mL)[1]	1022.7 ± 47.8	831.7 ± 36.7**
AGE (U/mL)[1]	2.03 ± 0.10	2.02 ± 0.13
Current smoker %	44.4	46.0
Current drinker %	68.7	65.5

Data are presented as mean±S.E and or percentage.

[1] tested after log-transformed. [2]Obesity: Body mass index≥$25kg/m^2$

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ between two groups by independent t-test

3.6. sRAGE, AGE, inflammatory markers according to obese status and *RAGE* G82S polymorphism

We observed the *RAGE* G82S related-phenotype according to obese status. We could not find any statistically significant differences in age, BMI, waist circumference, body fat %, blood pressure and proportions of cigarette smoking and alcohol consumption according to *RAGE* G82S genotype in each of non obese group or obese group.

In non obese subjects (BMI<25kg/m^2), we found the strong association between sRAGE concentration and 82S allele. Subject with the G/G shows the highest levels of sRAGE and those with the S/S genotype had the lowest levels (G/G: 1115.5±58.4 pg/ml and G/S: 832.1±73.7 pg/ml, S/S: 457.1±96.2 pg/ml, p=0.002) (Fig 2). On the other hand, concentrations of AGE and CRP were not significantly different according to *RAGE* G82S genotype (p=0.743 and p=0.436, respectively) (Fig 2).

In obese subjects (BMI≥25kg/m^2), we also found that subjects with the S/S had lower concentrations of sRAGE (439.5±57.7 pg/ml) than in those with the G/G genotype (917.5±45.7 pg/ml) or G/S (768.8±56.1 pg/ml) (p<0.001) (Fig 2). Unlikely shown in non-obese group, obese subjects with 'S/S' genotype had higher concentrations of AGE and CRP compare with those with the G/G or G/S genotype (p=0.012 and p=0.006, respectively) (Fig 2).

On the other hand, we could not find any significant differences in age, basic anthropometric parameters and lipid profile, IL-6 levels and percentages

of current smokers and current drinkers according to *RAGE* G82S in both non-obese and obese groups (Table 4 and 5).

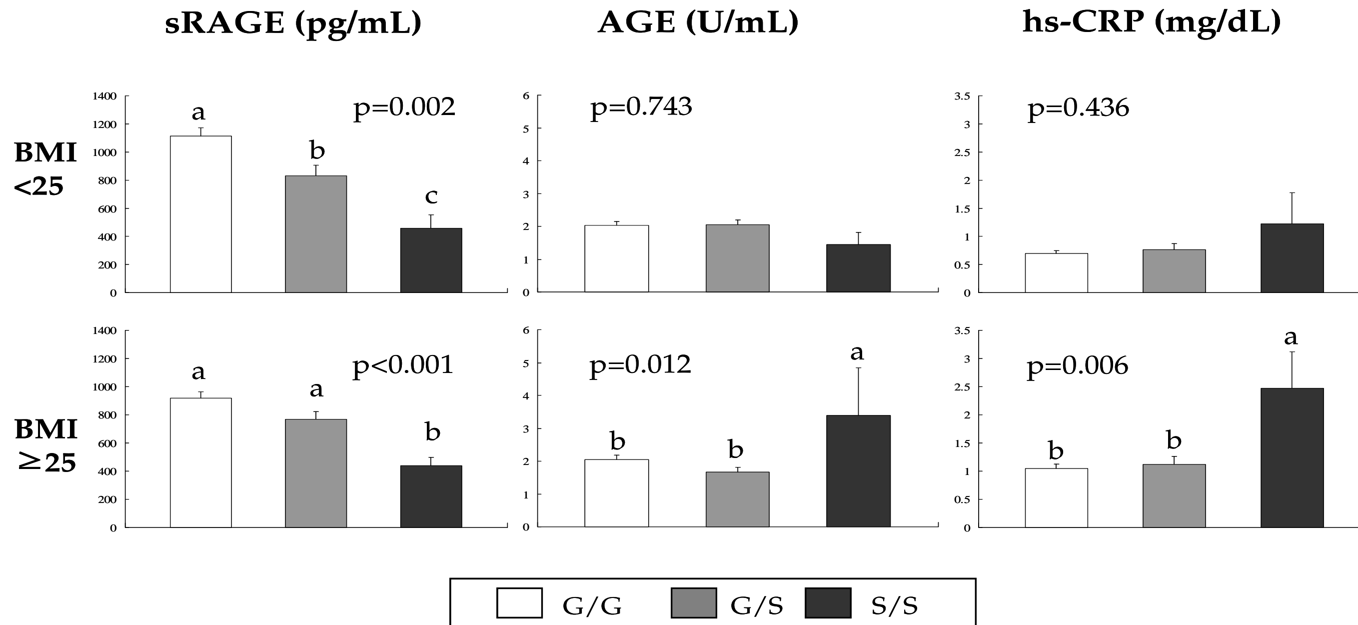

Figure 2. Concentrations of sRAGE, AGE and hs-CRP in Korean men according to obese status and *RAGE* G82S polymorphism.

Mean±S.E.
All data were tested after log-transformed.
Tested by one way-analysis of variance followed by Bonferroni method.
Values significantly different ($p<0.05$) are indicated by different alphabets.

Table 4. Anthropometric and biochemical parameters in nonobese subjects (Body mass index<25) according to *RAGE* G82S

	G/G (n=495)	G/S (n=188)	S/S (n=11)
Age (yrs)	52.2 ± 0.48	51.7 ± 0.75	47.6 ± 3.36
Body mass index (kg/m2)	22.9 ± 0.07	22.8 ± 0.11	22.9 ± 0.47
Waist circumferences (cm)	84.7 ± 0.26	83.9 ± 0.46	83.5 ± 1.18
% body fat	20.8 ± 0.20	20.7 ± 0.35	19.9 ± 1.08
Systolic BP (mmHg)	120.8 ± 0.74	120.1 ± 1.23	124.1 ± 4.00
Diastolic BP (mmHg)	77.0 ± 0.44	77.4 ± 0.76	77.8 ± 2.31
Triglyceride (mg/dL)[1]	140.5 ± 3.67	146.9 ± 6.78	126.5 ± 15.6
Total cholesterol (mg/dL)[1]	190.2 ± 1.70	190.2 ± 2.58	188.5 ± 7.68
HDL-cholesterol (mg/dL)[1]	46.5 ± 0.57	44.3 ± 0.84	45.1 ± 1.95
LDL-cholesterol (mg/dL)[1]	116.4 ± 1.59	117.5 ± 2.39	118.2 ± 8.63
Glucose (mg/dL)[1]	95.7 ± 1.12	93.2 ± 1.66	89.8 ± 5.60
Interleukin-6 (pg/mL)[1]	3.00 ± 0.26	2.91 ± 0.46	2.83 ± 1.04
Current smoker %	45.5	42.0	36.4
Current drinker %	68.1	71.1	54.5

There were no significant differences in variables according to genotype.

Data are presented as mean±S.E and or percentage.

[1] tested after log-transformed. Determined by one-way analysis of covariance followed by a Bonferroni test

Values significantly different ($p<0.05$) are indicated by different alphabets

Table 5. Anthropometric and biochemical parameters in obese subjects (BMI≥25) according to *RAGE* G82S

	G/G (n=409)	G/S (n=131)	S/S (n=18)
Age (yrs)	50.5 ± 0.54	52.0 ± 0.81	48.0 ± 2.67
Body mass index (kg/m2)	27.2 ± 0.09	27.3 ± 0.20	26.6 ± 0.30
Waist circumferences (cm)	93.6 ± 0.27	93.7 ± 0.56	91.8 ± 1.15
% body fat	26.2 ± 0.23	26.2 ± 0.34	25.3 ± 0.65
Systolic BP (mmHg)	126.1 ± 0.81	126.1 ± 1.42	126.5 ± 3.15
Diastolic BP (mmHg)	81.1 ± 0.54	81.2 ± 0.91	79.5 ± 2.64
Triglyceride (mg/dL)[1]	177.7 ± 5.13	187.1 ± 17.2	193.2 ± 29.9
Total cholesterol (mg/dL)[1]	195.1 ± 1.91	197.7 ± 4.06	193.9 ± 5.74
HDL-cholesterol (mg/dL)[1]	41.5 ± 0.52	41.5 ± 0.90	40.9 ± 2.46
LDL-cholesterol (mg/dL)[1]	119.5 ± 1.75	122.3 ± 3.79	113.5 ± 6.08
Glucose (mg/dL)[1]	97.5 ± 1.40	99.9 ± 2.65	96.0 ± 3.51
Interleukin-6 (pg/mL)[1]	3.44 ± 0.71	3.26 ± 0.43	6.60 ± 2.59
Current smoker %	45.6	47.3	44.4
Current drinker %	65.9	65.6	55.6

There were no significant differences in variables according to genotype.

Data are presented as mean±S.E and or percentage.

[1] tested after log-transformed. Determined by one-way analysis of covariance followed by a Bonferroni test

Values significantly different ($p<0.05$) are indicated by different alphabets

IV. DISCUSSION

In this present study, we found that *RAGE* G82S polymorphism and obesity status influence plasma sRAGE concentration. Particularly, the genotype effect on the levels of AGE and CRP, a inflammatory marker were shown only in obese status; sRAGE concentrations were lower in subject with 82S allele than those G/G homozygotes regardless of obesity status. On the other hand, concentrations of AGE and CRP were significantly higher in only obese subjects with S/S genotype that those with major G allele.

RAGE is thought to be activated by AGEs to induce intracellular MAP kinase activity (22) leading to the nuclear translocation of NF-κb (3,4,23) and activation of several secondary messenger systems that increase the production of pro-inflammatory cytokines and adhesional molecules (24). The activation of NF-κb also results in increased RAGE expression and increases the number of ligand binding sites, thereby prolonging NF-κb activation (4,5). This enhanced AGE-RAGE binding affinity is suggested to cause the decrease in sRAGE concentration by cellular signaling pathways. sRAGE is known to be produced by alternative splicing of RAGE mRNA (11). Carboxy terminal proteolytic cleavage of RAGE is thought to be another mechanism of sRAGE

production in humans (25). Levels of sRAGE may be regulated by *RAGE* G82S polymorphism. Actually, cells bearing the *RAGE* 82S allele displayed the increased ligand affinity, thereby relatively less producing sRAGE and enhancing proinflammatory mechanisms (15).

Another considerable confounding factor is obesity status and the related inflammatory response. In our study, we found that *RAGE* G82S and BMI were major influencing factors on sRAGE concentration. Obese subjects (BMI$\geq$25kg/m^2) also showed higher levels of CRP as well as lower levels of sRAGE compared with nonobese ones. As already reported, obesity is related to a low-grade inflammatory state (26) and weight status is positively correlated with hsCRP (27). C-reactive protein, a key proinflammatory cytokine that is highly elevated in atherosclerotic patients, not only serves as a biomarker for the risk of cardiovascular disease but also functions as an active mediator of atherosclerosis by promoting arterial endothelial activation (28). Zhong, et al. reported that CRP not only enhanced RAGE ligand binding, but also increased *RAGE* gene expression (29) in human endothelial cells at both the protein and mRNA level. In this aspect, we could find significantly differences in CRP levels, inversely correlated with sRAGE according to *RAGE* G82S genotypes, particularly in obese status.

However, we could not find any significant differences in AGE concentrations according to obesity status. We observed the significantly higher AGE concentrations only in obese subjects with 82S/S genotype than those with G allele, even though the concentration tended to be higher in S/S genotypes among whole subjects ($p<0.01$). This pattern is a little bit similar with that of CRP. AGE concentrations shown in our study might be more closely associated with CRP levels rather than *RAGE* G82S or obesity itself. As we mentioned above, CRP might enhance AGE-RAGE interaction (29); we assumed that CRP itself or its cellular signaling pathway affects AGE-RAGE axis and RAGE- NF-kb axis thereby forming more AGE. In addition, Tan et al. elevated plasma CRP levels are associated with increased serum concentrations of AGEs in patients with type 2 diabetes and serum concentration of AGEs could also be an independent determinant of plasma CRP levels (30).

On the other hand, any significant differences were not shown in IL-6 levels according to *RAGE* G82S or obesity status, which is quite different from the results shown in CRP levels. We may consider a few things; obesity status in this study subjects were relatively less severe (average BMI of obesity group: $27.2\pm0.08kg/m^2$) than Western population and IL6 may not respond to intrinsic and extrinsic

stimulation as acute as CRP, a general inflammatory marker.

In conclusion, sRAGE concentration regulated by enhanced AGE-RAGE binding is shown to be significantly influenced by not only *RAGE* G82S polymorphism but also obesity status. Particularly, significant genotype effect on the levels of AGE and CRP were shown in obese status. It indicates that stimulating condition such as obesity increases CRP levels, which may activate *RAGE* gene expression more and strengthen the binding of AGE-RAGE in subject with 82S allele thereby less producing sRAGE.

However, this study has some limitations. It is performed only in men without any type of diagnosed disease under cross-sectional design. Therefore we need further study to find out how CRP affects AGE-RAGE axis in more detail through the weight reduction study in both men and women and TO measure the diverse AGEs and signaling cascades identified in RAGE-mediated cellular signaling.

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국문 요약

Relationship among sRAGE, AGE and CRP according to *RAGE* Gly82Ser and obesity status in Korean men

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저자는 AGE-RAGE binding이 강화되는 RAGE Gly82Ser genotype 및 obesity 같은 상황에서 sRAGE, AGE, CRP 의 혈중 농도의 변화를 측정하여 이들간의 상호 관계를 알아보고자 하였다. 30-70세 사이의 BMI≥18.5kg/m^2 를 가진 총 1096명의 남성 이 실험에 모집되었고, anthropometrics, lipid profiles, glucose, *RAGE* G82S polymorphism, sRAGE, AGEs, inflammatory markers (CRP and IL-6) 등을 측정하였다. soluble RAGE는 *RAGE* Gly82Ser genotype에 의해 영향을 받았으며 82 S/S genotype 군에서 현저하게 낮았다 (G/G: 1036.3±40.3 pg/ml and G/S: 807.0±49.6 pg/ml, S/S: 443.0±47.8 pg/ml, p<0.001). Stepwise analysis를 통해

RAGE Gly82Ser genotype과 BMI가 sRAGE에 영향을 주는 것으로 나타났다 (standardized β-coefficient = -0.384, p<0.001, standardized β-coefficient = -0.168, p=0.001). sRAGE는 obese group에서 현저하게 낮았으며 (S/S:439.5±57.7 pg/ml G/G:917.5±45.7 pg/ml G/S (768.8±56.1 pg/ml) (p<0.001)), Obesity가 동반된 82 S/S geotype 군에서 soluble RAGE가 낮았을 뿐더러 CRP의 상승 및 AGE의 상승도 관찰할 수 있었다 (p=0.012 and p=0.006, respectively). sRAGE는 AGE-RAGE binding affinity가 강화되는 *RAGE* Gly82Ser genotype과 비만에 의해 영향을 받는 것으로 사료 되며 82 S/S genotype군에서 비만과 같이 inflammation이 증가되어 지는 상황이 동반되었을 때 soluble RAGE의 감소와 더불어 AGE와 CRP의 상승하게 되는 것으로 보인다.

핵심 단어 : sRAGE, AGE, CRP, *RAGE* Gly82Ser polymorphism, Obesity, AGE-RAGE binding affinity and inflammation