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Serum Adiponectin Is Associated with Smoking Status in Healthy Korean Men

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Abstract. Objective: To measure the association between smoking and serum adiponectin, taking into consideration insulin resistance and obesity. Material and Methods: The cross-sectional study was carried out in Seoul, Korea in 2006. Waist circumference (WC), body mass index (BMI), and serum adiponectin were measured in 2,500 healthy Korean men. Multiple linear regression models were used to assess the association of smoking status with serum adiponectin level. WC, BMI, and homeostasis model assessment (HOMA) were classified into two groups according to median values. Results: The mean adiponectin concentrations were 6.6 μg/ml and 7.3 μg/ml in current smokers and non-smokers. After adjusting for age, BMI, and alcohol consumption, mean log adiponectin levels decreased by 0.064 μg/ml in current smokers compared with non-smokers (P = 0.0190). Mean log adiponectin levels also decreased by 0.030 and 0.095 μg/ml in moderate and heavy smokers compared to non-smokers. The relationship between adiponectin and smoking was similar between the high and low insulin resistance, BMI, and WC groups. Conclusions: These results suggest that serum adiponectin levels are associated with smoking status. These data also support that lower serum adiponectin concentrations in smokers may not be dependent on insulin resistance status or obesity.

Key words: Smoking, Adiponectin, Obesity, Insulin resistance

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ADIPONECTIN has been reported as a new risk factor for the development of diabetes. Exclusively secreted by adipose tissue, adiponectin is a 244-amino acid protein that regulates the metabolism of lipids and glucose, and circulates quite abundantly in plasma [1–5]. Adiponectin decreases insulin resistance and body weight by increasing lipid oxidation in muscle and in other organs, such as the pancreas and liver [6]. Hotta *et al.* [7] showed that plasma adiponectin concentrations were reduced among individuals with obesity, diabetes mellitus, or coronary heart disease [7, 8], implying that adiponectin plays a role in these conditions.

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Many studies have reported on hypoadiponectinemia in smokers [9–13]. However, the underlying mechanisms of this relationship are not yet fully understood. Because the prevalence of insulin resistance may also be increased in smokers [14, 15], it is not clear whether hypoadiponectinemia in these individuals is due to smoking, or to the coexistence of insulin resistance. However, relevant literature on the relationship between serum adiponectin and smoking status independent of insulin resistance is sparse. Thus, the purpose of this study was to measure the association between smoking and adiponectin, taking into consideration the effects of insulin resistance and obesity. We hypothesized that adiponectin in smokers is associated with smoking status regardless of insulin resistance and obesity.

Material and Methods

Study subjects

The study population consisted of 3,820 male subjects who participated in the Korean Metabolic Syndrome Research Initiative and had routine health examinations at the Health Promotion Center in university hospitals from January to December 2006. The participants who volunteered to undergo the health examinations were informed of the purpose and contents of this research project. Recruitment of these volunteer subjects only took place after their informed consent had been obtained. Participating hospitals are listed in the appendix [16]. The analysis excluded subjects with missing information on waist circumference (WC), body mass index (BMI), or adiponectin levels, and those who had a history of cancer, cardiovascular disease, stroke, diabetes, or hypertension (n = 565). We also excluded those who had been on medication for either hypertension or diabetes, and subjects with fasting blood sugar>110 mg/dl or hypertension (SBP ≥140 or DBP≥90 mmHg) (n = 755). Finally, 2,500 subjects, aged 24 to 87 years, were selected for subsequent analysis. The Institutional Review Board of Human Research of Yonsei University approved the study, and written informed consent was obtained from all subjects.

Data collection

Each participant was interviewed using a structured questionnaire to collect history of cigarette smoking (non-smoker, ex-smoker, or current smoker) and alcohol consumption (non-drinker or drinker of any amount of alcohol), as well as other demographic characteristics such as age, gender, and family history of diabetes. Both current and ex-smokers were asked to report the average number of cigarettes they smoke or had smoked per day. WC was measured midway between the lower rib and the iliac crest. Weight and height were measured while the participants were wearing light clothing. BMI was calculated as weight (kg) divided by the square of height (m^2). Blood pressure was measured with the subject in a seated position by a registered nurse or blood pressure technician using a standard mercury sphygmomanometer or automatic manometer. Both systolic and diastolic blood pressures were measured after a 15 minute rest.

Measurement of biomarkers

For the clinical chemistry assay, serum was separated from peripheral venous blood sample that was obtained from each participant after 12 hours of fasting, and was stored at –70°C for two hours. Metabolic syndrome biomarkers such as fasting blood glucose, total cholesterol (TC), triglycerides (TG), and high density lipoprotein cholesterol (HDL-C) were measured using the Hitachi-7600 analyzer (Hitachi Ltd., Tokyo, Japan). For subjects with available serum, adiponectin levels were measured using an enzyme-linked immunosorbent assay (Mesdia Co., Ltd., Seoul, Republic of Korea). Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR). Homeostasis model assessment (HOMA) indices were calculated as follows: HOMA = fasting insulin (μl U/ml) × fasting glucose (mmol/l) / 22.5. The intra- and inter-assay variances for adiponectin were 6.3% to 7.4% and 4.5% to 8.6%, respectively [16]. Data quality control was performed in accordance with the procedures of the Korean Association of Laboratory Quality Control.

Statistical analyses

All biomarkers were seen to have a normal distribution except adiponectin. Therefore, log transformation was used to analyze adiponectin levels. Analysis of variance (ANOVA) was used to analyze the statistical differences among the characteristics of the study participants. Mean of serum adiponectin levels were calculated for each category of smoking status. Multiple linear regression models were used to assess the association of smoking status with serum adiponectin level. Current smokers were further divided into three groups of 1–10, 11–20, and 20 or more cigarettes per day. Analyses were adjusted for age at enrollment (continuous variable), BMI, and alcohol drinking status.

To examine the association between smoking status and adiponectin, stratified by insulin resistance (HOMA-IR), BMI, and WC, we divided our study samples into two groups (by median value) of HOMA-IR (<0.750 and ≥0.750), BMI (<24.2 and ≥24.2), and WC (<84 and ≥84). All analyses were conducted using SAS statistical software, version 9.0 (SAS Institute Inc, Cary, NC). All statistical tests were two-sided, and statistical significance was determined as $p<0.05$.

Table 1. General characteristics of the Korean male study population according to smoking status in Seoul, Korea, 2006

N	Smoking Status			P-value
	Non-smokers	Ex-smokers	Current smokers	
	647 Mean ± SD	822 Mean ± SD	1031 Mean ± SD	
Age, year	44.5 ± 8.8	46.8 ± 9.1	42.8 ± 7.9	<0.0001
WC, cm	83.3 ± 6.9	85.3 ± 6.9	84.6 ± 7.5	<0.0001
BMI, kg/m^2	24.0 ± 2.5	24.6 ± 2.4	24.4 ± 2.8	<0.0001
Adiponectin, μg/ml	7.3 ± 4.4	7.0 ± 3.8	6.6 ± 3.7	0.0010
Log Adiponectin, μg/ml	2.5 ± 0.6	2.5 ± 0.5	2.4 ± 0.6	0.0006
FBS, mg/dL	90.7 ± 9.1	92.2 ± 9.6	91.1 ± 9.5	0.0048
SBP, mmHg	119.7 ± 10.5	119.9 ± 11.6	118.5 ± 10.4	0.0090
DBP, mmHg	74.8 ± 8.0	75.0 ± 8.0	73.5 ± 7.9	<0.0001
HDL cholesterol, mg/dL	50.6 ± 10.9	50.7 ± 11.1	48.8 ± 10.9	0.0001
LDL cholesterol, mg/dL	113.1 ± 28.2	116.5 ± 26.3	116.3 ± 30.1	0.0394
Triglyceride, mg/dL	119.2 ± 67.1	139.5 ± 95.0	151.3 ± 104.9	<0.0001
C-reactive protein, mg/dL	0.15 ± 0.3	0.16 ± 0.4	0.15 ± 0.2	0.7646
HOMA	0.9 ± 0.6	1.0 ± 0.7	0.9 ± 0.7	0.0012
	%	%	%	
Family history of diabetes	18.0	15.9	14.0	0.0752
Alcohol drinking	80.8	90.0	93.2	<0.0001

P-value refers to differences between groups as determined by ANOVA and X^2 test for continuous and categorical variables, respectively.
FBS: fasting blood sugar, BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, HOMA: homeostasis model assessment, HDL: high density lipoprotein, LDL: low density lipoprotein

Table 2. Age-adjusted Pearson correlation coefficients between serum adiponectin and clinical parameters in Korean men in Seoul, Korea, 2006

	Subjects (n = 2500)	
	r	P
BMI, kg/m^2	–0.1930	<0.0001
WC, cm	–0.2236	<0.0001
FBS, mg/dL	–0.1690	<0.0001
SBP, mmHg	–0.1168	<0.0001
DBP, mmHg	–0.0170	0.4023
HDL cholesterol, mg/dL	0.2307	<0.0001
LDL cholesterol, mg/dL	–0.0114	0.5736
Triglyceride, mg/dL	–0.1554	<0.0001
C-reactive protein, mg/dL	–0.0201	0.3228
HOMA	–0.1666	<0.0001

FBS: fasting blood sugar, BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, HOMA: homeostasis model assessment, HDL: high density lipoprotein, LDL: low density lipoprotein

Table 3. Multiple linear regression model of mean log adiponectin levels in healthy Korean men in Seoul, Korea, 2006

Variables		Model 1		Model 2	
		β	P value	β	P value
Age, year		0.005	0.0001	0.004	0.0040
Smoking status	Ex	–0.038	0.1889	–0.002	0.9397
	Current	–0.089	0.0012	–0.064	0.0190
BMI, kg/m2				–0.042	<0.0001
Alcohol drinking				–0.085	0.0142

BMI: body mass index

Results

The characteristics of participants in relation to their smoking status are shown in Table 1. The mean age differed significantly according to smoking status. Current smokers were the youngest and ex-smokers the oldest. Mean adiponectin concentrations were 6.6 μg/ml and 7.3 μg/ml in current smokers and non-smokers, respectively. C-reactive protein levels were not associated with smoking status (P = 0.7646). Alcohol consumption were associated with smoking status in that current or ex-smokers were more likely to consume alcohol frequently.

Table 4. Multiple linear regression model* of mean log adiponectin levels according to HOMA, BMI, and WC in healthy Korean men in Seoul, Korea, 2006

Variables	Subgroups	N	Light smokers (1–9/day)		Moderate smokers (10–19/day)		Heavy smokers (≥20/day)	
			β	P value	β	P value	β	P value
	All	2500	–0.001	0.9891	–0.030	0.4375	–0.095	0.0028
HOMA	<0.750	1249	0.083	0.2729	–0.089	0.0981	–0.090	0.0432
	≥0.750	1251	–0.062	0.3677	0.031	0.5692	–0.101	0.0292
BMI, kg/m2	<24.2	1252	0.069	0.3304	–0.066	0.2044	–0.110	0.0124
	≥24.2	1248	–0.077	0.2996	–0.001	0.9961	–0.087	0.0629
WC, cm	<84	1138	0.112	0.1473	–0.033	0.5331	–0.076	0.0967
	>=84	1362	–0.074	0.2817	–0.039	0.4809	–0.108	0.0147

Adjusted for age, BMI, and alcohol drinking.
BMI: body mass index, WC: waist circumference, HOMA: homeostasis model assessment
* Reference group: non-smokers

Levels of adiponectin were inversely associated with BMI, WC, and triglycerides, while directly associated with HDL cholesterol (P<0.001) (Table 2). Mean log adiponectin levels were decreased by 0.089 μg/ml in current smokers compared with non-smokers after adjusting for age (P = 0.0012), and decreased by 0.064 μg/ml after adjusting for age, BMI, and alcohol drinking (P = 0.0190) (Table 3).

Current smokers were grouped according to the quantity of cigarettes smoked per day (1–10, 11–20, 20 or more) in order to examine the relationship between smoking intensity and adiponectin levels (Table 4). Mean log adiponectin levels decreased by 0.030 and 0.095 μg/ml in moderate smokers and heavy smokers, respectively, in comparison to non-smokers. The age-adjusted Pearson correlation coefficient between serum adiponectin and the number of cigarettes/day was also –0.0597 and statistically significant (p = 0.0087) (data not shown). Next, to control potential confounding by insulin resistance and obesity, the data were further stratified by BMI, WC, and HOMA (Table 4). The relationship between adiponectin and smoking was similar among insulin resistance (HOMA) groups. When compared to non-smokers, mean log adiponectin levels decreased by 0.090 μg/ml and 0.101 μg/ml in both heavy smokers with HOMA<0.750 (p-value = 0.0432) and with HOMA≥0.750 (p value = 0.0292), respectively. Similar associations of adiponectin levels with smoking status were seen in the BMI and WC groups.

Discussion

Our study demonstrates that smoking status is associated with lower levels of adiponectin, which supports findings of previous studies [9–13]. In a recent study in a healthy Japanese population, a significant dose-response relationship was found between the number of cigarettes and adiponectin concentrations in men [9]. The present study also showed a dose-dependent relationship between smoking amounts and adiponectin levels in Korean men. The relationship remained even in subgroup analyses by HOMA-IR.

Because the prevalence of insulin resistance is increased in smokers [14, 15], reports of lower adiponectin concentrations in smokers may not be related to smoking, but to the concomitant presence of insulin resistance in smokers [10]. However, in a recent cross-sectional study consisting of 30 smokers and 30 non-smokers, the difference in mean adiponectin concentrations between smokers and non-smokers did not depend on the insulin resistance statuses of the subjects [10]. Since the study by Abbasi used a small sample size, with a bigger population we also looked at the association between adiponectin and smoking. We also found that the lower plasma adiponectin concentrations in smokers were not dependent on the insulin resistance statuses of the subjects. These results support that reports of lower adiponectin concentrations in smokers may be related to smoking, not to the concomitant presence of insulin resistance in smokers.

Cigarette smoke contains a large amount of free radicals, resulting in endothelial injury. Oxidative

stress can damage many cell components, including DNA, lipid membranes, and proteins, and may lead to apoptosis and cell damage [17, 18]. On the other hand, nicotine, a major component of cigarette smoke, promotes inflammation and has a direct effect on human adipose tissue [19, 20]. In a study using cultured mouse 3T3-L1 adipocytes, Iwashma *et al.* [11] reported that H_2O_2 and nicotine reduced mRNA expression and secretion of adiponectin in a dose-dependent manner. This provides a biologically plausible explanation for our findings in that we also observed a dose-response relationship between smoking amounts and adiponectin.

However, in contrast to the impact of smoking on serum adiponectin concentrations, C-reactive protein (CRP) concentrations were not associated with smoking status in the present study. Some previous studies have suggested an adverse effect of smoking on CRP concentration [21, 22]. However, recent cross-sectional studies found no difference of plasma CRP levels between smokers and non-smokers, as seen in our study [10, 23].

The main strength of our study is that to our knowledge this may be the first study enrolling a large number of healthy individuals that examined associations between serum adiponectin levels and smoking, while taking into consideration insulin resistance and obesity. This study has also several limitations. Due to its cross-sectional design, this study cannot elucidate mechanisms or determine the direction of causality. A single assessment of adiponectin levels may be susceptible to short-term variation, which would bias the results toward the null. Another limitation is that this study was done in only men because there are few female smokers in Korea. However, Pischon *et al.* [24] reported that intra-individual adiponectin levels are reasonably stable over time, with an intra-class correlation coefficient of 0.85 for adiponectin levels measured within the same participants one year apart.

In conclusion, our findings suggest that adiponectin concentrations are lower in smokers, and this association may not be due to the concomitant presence of insulin resistance and obesity. Further studies including other populations such as women or other ethnic groups should be performed to confirm the association between adiponectin and smoking status regardless of insulin resistance.

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Appendix

List of participating hospitals in Korean Metabolic Syndrome Research Initiatives:

Severance Hospital, Yonsei University; Ewha Women's University; Seoul National University; Korea University.

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