Table 2. Frequencies of the genotype and the allele of the β2- and β3-adrenoceptor polymorphisms in the four study groups according to the response in weight loss

Groups	Genotype (%)			χ^2 Test Among Four Genotype	Allele (%)		χ^2 Test Among Two Alleles
Arg16Gly of β2-adrenoceptor	Arg16/Arg16	Arg16/Gly16	Gly16/Gly16		Arg16	Gly16	
Weight loss maintenance group	18 (48.6%)	14 (37.8%)	5 (13.5%)		50 (67.6%)	24 (32.4%)	
Rebound weight gain group	8 (22.2%)	18 (50.0%)	10 (27.8%)	$\chi^2 = 13.88$,	34 (47.2%)	38 (52.8%)	$\chi^2 = 11.36$,
Slow weight loss group	13 (21.7%)	32 (53.3%)	15 (25.0%)	$P = .031$	58 (48.3%)	62 (51.7%)	$P = .010$
Weight loss resistant group	3 (14.3%)	12 (57.1%)	6 (28.6%)		18 (42.9%)	24 (57.1%)	
Gln27Glu of β2-adrenoceptor	Gln27/Gln27	Gln27/Glu27	Glu27/Glu27		Gln27	Glu27	
Weight loss maintenance group	33 (91.7%)	4 (10.8%)	0 (0.0%)		70 (94.6%)	4 (5.4%)	
Rebound weight gain group	34 (94.4%)	2 (5.6%)	0 (0.0%)	$\chi^2 = ---$,	70 (97.2%)	2 (2.8%)	$\chi^2 = 9.86$,
Slow weight loss group	44 (73.3%)	16 (26.7%)	0 (0.0%)	$P = ---$	104 (86.7%)	16 (13.3%)	$P = .020$
Weight loss resistant group	14 (66.7%)	7 (33.3%)	0 (0.0%)		35 (83.3%)	7 (16.7%)	
Trp64Arg of β3-adrenoceptor	Trp64/Trp64	Trp64/Arg64	Arg64/Arg64		Trp64	Arg64	
Weight loss maintenance group	26 (70.2%)	10 (27.0%)	1 (2.7%)		62 (83.8%)	12 (16.2%)	
Rebound weight gain group	28 (77.8%)	8 (22.2%)	0 (0.0%)	$\chi^2 = 7.91$,	64 (88.9%)	8 (11.1%)	$\chi^2 = 5.43$,
Slow weight loss group	32 (53.3%)	27 (45.0%)	1 (1.7%)	$P = .245$	91 (75.8%)	29 (24.2%)	$P = .143$
Weight loss resistant group	13 (61.9%)	8 (38.1%)	0 (0.0%)		34 (81.0%)	8 (19.0%)	

The definitions for the 4 study groups according to the response in weight loss are referred to in the Results, Prevalence of weight loss maintenance, rebound weight gain, and weight loss resistance section.

was 18.5%. The frequency distributions for alleles in our subjects were similar to those in previous studies in Japanese cohorts, but lower than studies in whites.[23,24]

Physical Measurements

The mean age, BMI, BP levels, and heart rates at entry were similar among the four groups (Table 1). However, the entry measurements for total body fat mass and waist-to-hip ratio were significantly lower in the weight loss maintenance group versus the other three groups (weight loss resistant, slow weight loss, or rebound weight gain). At 6 months, the weight loss maintenance group had significantly greater weight loss, body fat loss, and a decrease in the waist-to-hip ratio compared to the weight loss resistant and slow weight loss groups. The BP reductions at 24 months were significantly greater in the weight loss maintenance and slow weight loss groups compared to the weight loss resistant group and the rebound weight gain group (Table 1). Only in the weight loss maintenance group did the heart rates decline at 24 months.

The subjects carrying the Gly16 allele had greater total body fat mass and waist-to-hip ratios at entry and throughout the study (Table 3), and the subjects carrying the Glu27 allele had greater total body fat mass (Table 4).

In all subjects, weight loss and mean BP reduction during 24 months were 8.9 ± 4.4 kg (10.8% ± 5.3%) and 4.5 ± 3.1 mm Hg (4.7% ± 3.2%). Mean BP reductions per amount of weight lost were similar among the four study groups (0.4 ± 0.2 mm Hg/kg in the weight loss maintenance group; 0.3 ± 0.1 mm Hg/kg in the rebound weight gain group; 0.5 ± 0.2 mm Hg/kg in the weight loss resistant group; and 0.5 ± 0.3 mm Hg/kg in the slow weight loss group).

Hormone Levels

Plasma NE and leptin levels, and HOMA-IR decreased with weight loss in the four study groups (Table 1). The most significant finding was that plasma NE and leptin levels were substantially greater in the weight loss resistant group compared to the weight loss maintenance group at entry and throughout the study. In the rebound weight gain group, plasma NE level was significantly greater than in the weight loss maintenance group. The slow weight loss group also had higher plasma NE and leptin levels at entry compared to the groups who succeeded in a significant weight loss at 6 months (weight loss maintenance and rebound weight gain groups), but lower values than the weight loss resistant group (Table 1). Plasma NE and leptin levels in the subjects carrying the Gly16 and Glu27 alleles were higher at entry and throughout the study compared to those without the Gly16 or Glu27 allele. The HOMA-IR in the subjects with the Gly16 allele was higher throughout the study, as previously we reported,[25] whereas that in the subjects with the Glu27 allele was similar (Tables 3 and 4).

Table 3. Characteristics of subjects according the genotype of the Gly16 at entry and during a weight loss program

Genotype	Without Gly16 Allele (Arg16Arg)			With Gly16 Allele (Arg16Gly + Gly16Gly)		
	At Entry	At 6 Months	At 24 Months	At Entry	At 6 Months	At 24 Months
Subjects (*n*)	42	42	42	112	112	112
Age (yr)	36 ± 7	37 ± 7§	38 ± 7§	37 ± 6	37 ± 6§	39 ± 6§
BMI (kg/m^2)	27.3 ± 2.0	23.8 ± 2.1	23.6 ± 2.0†§	27.4 ± 1.8	24.5 ± 2.3§	24.6 ± 1.9§
Total body fat mass (kg)	24.0 ± 5.4*	20.2 ± 4.8*‡	17.6 ± 4.5*§	25.5 ± 4.8	21.7 ± 4.3‡	19.2 ± 5.1§
Waist-to-hip ratio	1.16 ± 0.12*	1.05 ± 0.10‡	0.96 ± 0.13*§	1.24 ± 0.10	1.09 ± 0.12‡	1.04 ± 0.14§
Systolic BP (mm Hg)	132 ± 9	130 ± 10	126 ± 9‡	133 ± 10	132 ± 10	131 ± 9
Diastolic BP (mm Hg)	79 ± 9	79 ± 8	75 ± 6‡	79 ± 10	78 ± 9	75 ± 7‡
Mean BP (mm Hg)	97 ± 10	96 ± 10	92 ± 7‡	97 ± 11	96 ± 9	94 ± 9
Heart rate (beats/min)	69 ± 9	67 ± 7	64 ± 7‡	69 ± 7	68 ± 7	66 ± 7
Norepinephrine (pmol/mL)	1.85 ± 0.39*	1.56 ± 0.33*‡	1.26 ± 0.37*§	2.11 ± 0.35	1.80 ± 0.36‡	1.41 ± 0.40§
Leptin (ng/mL)	8.6 ± 2.9*	6.6 ± 2.7*‡	4.9 ± 2.1*§	9.3 ± 3.0	7.3 ± 2.8‡	5.7 ± 2.9‡
HOMA-IR	2.2 ± 0.7*	2.1 ± 0.4	1.8 ± 0.6‡	2.6 ± 0.6	2.3 ± 0.5	2.0 ± 0.5‡

Data are mean ± SD.
BMI = body mass index; BP = blood pressure; HOMA-IR = the homeostasis model assessment of insulin resistance.
* $P < .05$, † $P < .01$ compared with values in subjects with the Gly16 allele; ‡ $P < .05$, § $P < .01$ compared with values at entry.

Relationship With Weight Loss and BP Reduction

Using linear regression analysis, plasma NE levels at entry and at 24 months correlated significantly with mean BP ($r = 0.54$, $P < .001$, $r = 0.42$, $P < .001$, respectively), heart rate ($r = 0.27$, $P < .05$, $r = 0.21$, P = not significant, respectively), BMI ($r = 0.28$, $P < .05$, $r = 0.25$, $P < .05$, respectively), total body fat mass ($r = 0.36$, $P < .001$, $r = 0.35$, $P < .001$, respectively), and plasma leptin level ($r = 0.42$, $P < .001$, $r = 0.37$, $P < .001$, respectively). Changes in heart rate for 24 months did not correlate with changes in plasma NE.

In multiple linear regression analysis, total body fat mass ($P = .043$), plasma NE ($P = .016$) and leptin levels ($P = .020$), but not heart rate, at entry were significant determinant factors for absolute weight changes for 24 months ($R^2 = 0.337$, F = 3.56, $P = .010$). Mean BP ($P = .050$), total body fat mass ($P = .041$), and plasma NE level ($P = .042$) at entry were significant determinant factors for absolute changes in mean BP for 24 months ($R^2 = 0.301$, F = 2.45, $P = .047$). Changes in total body fat mass ($P = .019$), waist-to-hip ratio ($P = .034$), plasma NE ($P = .033$) and leptin levels ($P = .022$) for 2 years were significant determinant factors for absolute changes in mean BP ($R^2 = 0.381$, F = 5.03, $P = .007$).

Discussion

The present study shows that the initial levels of total body fat mass, plasma NE and leptin levels, and the frequency of the Gly16 allele of the Arg16Gly of the β2-adrenoceptor polymorphism are significantly higher in people resistant to weight loss and those who have rebound weight gain compared to those with successful weight loss maintenance. Thus, measurement of these parameters might predict those subjects who will fail to lose weight in both the short and long term or who will regain weight after an initial success in weight loss as determined in a dietary and exercise weight loss program. In addition, the frequency of the Glu27 allele of the β2-adrenoceptor is higher in subjects who are weight loss resistant and in those with slow weight loss, in people who fail to lose weight in the short term (6 months), compared to those with weight loss maintenance or rebound weight gain, who lose weight in the short term. These findings indicate that sympathetic overactivity as reflected by high plasma NE levels associated with the Gly16 and Glu27 polymorphisms might be linked to mechanisms that explain weight loss resistance and rebound weight gain despite adherence to long-term diet and exercise programs.

A number of studies have demonstrated several BP-lowering mechanisms accompanying weight loss.[6,7] In our weight loss program, average percent reduction in body weight and mean BP for 24 months in all subjects were 10.8% and 4.7%, respectively. These results revealed similar values to those levels in the meta-analysis by Neter et

Table 4. Characteristics of subjects according the genotype of the Glu27 at entry and during a weight loss program

Genotype	Without Glu27 Allele (Gln27Gln)			With Glu Allele (Gln27Glu)		
	At Entry	At 6 Months	At 24 Months	At Entry	At 6 Months	At 24 Months
Subjects (*n*)	125	125	125	29	29	29
Age (yr)	36 ± 6	37 ± 6§	38 ± 6§	37 ± 7	37 ± 7§	39 ± 7§
BMI (kg/m^2)	27.4 ± 1.7	24.0 ± 2.1§	24.1 ± 2.0§	27.3 ± 1.8	24.9 ± 2.2‡	24.5 ± 2.1§
Total body fat mass (kg)	24.5 ± 5.7*	20.5 ± 4.5*§	18.0 ± 4.3*§	25.9 ± 6.0	22.4 ± 3.8‡	20.1 ± 4.7§
Waist-to-hip ratio	1.19 ± 0.13	1.09 ± 0.10	0.98 ± 0.12§	1.22 ± 0.11	1.13 ± 0.09	1.03 ± 0.13‡
Systolic BP (mm Hg)	133 ± 9	131 ± 10	125 ± 9*‡	134 ± 10	133 ± 9	131 ± 10
Diastolic BP (mm Hg)	79 ± 9	79 ± 9	75 ± 9‡	79 ± 9	78 ± 7	75 ± 9
Mean BP (mm Hg)	97 ± 10	96 ± 9	91 ± 8*‡	97 ± 11	96 ± 7	94 ± 10
Heart rate (beats/min)	69 ± 8	67 ± 7	65 ± 7	69 ± 7	68 ± 6	65 ± 7
Norepinephrine (pmol/mL)	1.94 ± 0.33*	1.67 ± 0.41*‡	1.29 ± 0.34*§	2.20 ± 0.33	1.92 ± 0.45	1.51 ± 0.42‡
Leptin (ng/mL)	7.1 ± 2.8*	6.9 ± 2.8*	5.1 ± 2.7*‡	9.5 ± 3.1	8.0 ± 3.0‡	5.8 ± 2.8‡
HOMA-IR	2.4 ± 0.4	2.1 ± 0.7	2.0 ± 0.6	2.5 ± 0.6	2.3 ± 0.6	2.0 ± 0.5

Data are mean ± SD.
Abbreviations as in Table 3.
* $P < .05$, † $P < .01$ compared with values in subjects with the Glu27 allele; ‡ $P < .05$, § $P < .01$ compared with values at entry.

al.[7] And, normalization of BP often occurs before obese subjects reach their ideal weight. Therefore, overweight and obese hypertensive patients should be encouraged to lose even a modest amount of weight as it has pronounced beneficial effects on BP levels and other risk factors.

It is established that weight loss is accompanied by reductions in sympathetic nerve activity (SNA), insulin resistance, plasma leptin levels, and BP levels.[1–3,5] However, few investigations have examined how the sympathetic nervous system, insulin resistance, and leptin level are involved in weight loss resistance and rebound weight gain.[26] More than 20 years ago, Tuck et al found significant reductions in SNA and BP during rapid weight loss and weight loss using a very low calorie diet.[2,3,5] In the present study, we note that plasma NE, leptin, and the HOMA-IR levels track with weight changes and in addition that plasma NE and leptin levels at entry are determinant factors for predicting changes in body weight during a weight loss program, thus further demonstrating that SNA (plasma NE levels) and plasma leptin levels are major control factors for changes in body weight.[5,19,27]

In the present study, we used plasma NE levels as an index of SNA. Tuck,[28] Grassi and Esler,[29] and Rahn et al[30] reviewed that there are different results in SNA values in hypertensive patients depending on the method of SNA measurement including: regional NE spillover, muscle sympathetic nerve activity (microneurography), and plasma NE measurements. Spillover methods are considered as the gold standard for SNA measurements, but in humans these are difficult and invasive measurements. Furthermore , Rumantir et al[31] reported different values for regional sympathetic nerve activity between the kidneys and heart in obesity-related hypertensive subjects. Plasma NE levels are more practical for large population studies, [5,15,19,25] but represent several different process (secretion, clearance, and reuptake of NE) making it difficult to determine whether the defect is overproduction or decreased metabolism.

Pathophysiological involvement of genetic abnormalities in the β2- and β3-adrenoceptor system in obesity are well described.[10–15,22] Among β2- and β3-adrenoceptor polymorphisms, amino acid substitutions, Arg16Gly and Gln27Glu of the β2-adrenoceptor and Trp64Arg of the β3-adrenoceptor polymorphism are considered functionally important in the control of body weight.[10–15,22] In the present study, the weight loss maintenance group have a lower frequency of the Gly16 and Glu27 alleles of the β2-adrenoceptor and lower plasma NE levels, suggesting that the Gly16 and Glu27 alleles are related to a blunted β2-adrenoceptor activity and resultant sympathetic overactivity as shown by higher plasma NE levels.[15] Furthermore, the slow weight loss and weight loss resistance groups in our study during a 24-month period have a higher frequency of the Glu27 allele and higher plasma NE levels compared to the groups who succeed in significant weight loss in the short term. We have reported that the individuals carrying the Gly16 and Glu27 alleles have greater weight gain and BP elevations.[15] Taken together,

one could propose that the characteristics of the Gly16 and Glu27 alleles of the β2-adrenoceptor polymorphisms during weight gain may stabilize body weight even with on-going caloric restriction and exercise causing resistance to weight loss.

Kaye et al[32] found a strong relationship between heart rate and the level of cardiac sympathetic nerve activity measured by the spillover method. Our results show that changes in plasma NE do not correlate with changes in heart rate, whereas heart rate correlates with plasma NE at entry. These findings indicate that the limitation that plasma NE level does not always precisely reflect the response of regional (heart) sympathetic nerve activity to weight change, but we could speculate that the subjects carrying the Gly16 or Glu27 alleles who have less reductions in heart rate might have an impaired response of cardiac sympathetic nerve activity to weight loss through the blunted β2-adrenoceptor sensitivity and resultant cardiac risk through resistance to weight loss. However, further studies are needed to evaluate the differences in the sympathetic-mediated thermogenesis in the subjects carrying the β2-adrenoceptor polymorphisms.

In conclusion, greater adiposity and sympathetic overactivity (high plasma NE levels) might predict those obese individuals who have complete resistance to lose weight during the 24-month period and those who will have rebound weight gain after a successful initial weight loss. The sympathetic overactivity in those subjects who have rebound weight gain and in those who have resistance to weight loss may be associated with the polymorphisms in the Gly16 and Glu27 alleles of the β2-adrenoceptor.

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Association of Hypoadiponectinemia With Smoking Habit in Men

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Abstract—Adiponectin is emerging as an important molecule in obesity, the metabolic syndrome, and cardiovascular disease. On the other hand, smoking habit is well known to be related to cardiovascular disease and hypertension. To examine the association between adiponectin concentration and smoking habit, we performed an epidemiological survey and an acute exposure test in humans and an experiment in adipocytes to elucidate the mechanism underlying the association between adiponectin and smoking. In the epidemiological study, we enrolled a total of 331 male subjects to examine chronic smoking exposure. Plasma adiponectin was significantly lower ($P=0.01$) in current smokers (5.3 ± 0.3 μg/mL) than in never-smokers (6.5 ± 0.4 μg/mL). A significant association between smoking and low adiponectin level was also confirmed in multiple regression analysis including age, body mass index, hypertension, diabetes, hyperlipidemia, and creatinine clearance (never-smokers 6.5 ± 0.4 μg/mL; past smokers 5.6 ± 0.3 μg/mL; current smokers 5.2 ± 0.4 μg/mL; $F=4.52$; $P=0.01$). To examine the acute effect of smoking on adiponectin concentration for 12 hours, we measured plasma adiponectin level in 5 male never-smokers before smoking and 3, 6, and 12 hours after smoking, with the result that adiponectin showed a significant decrease after smoking (12 hours; $-14.5\pm0.6\%$; $P<0.01$). In cultured mouse 3T3-L1 adipocytes, H_2O_2 and nicotine reduced the mRNA expression and secretion of adiponectin in a dose-dependent manner. Smoking habit is associated with adiponectin concentration in men, and its suppressive effect is mediated in part through direct inhibition of smoking on adiponectin expression in adipocytes. (***Hypertension.*** **2005;45:1094-1100.)**

Key Words: smoking ■ oxidative stress ■ risk factors ■ lipids ■ lipoprotein ■ metabolism

Cigarette smoking exacts a continuing toll on public health and is an established risk factor for hypertension and cardiovascular disease, and nonsmoking is a leading preventive strategy against coronary artery disease. Furthermore, cigarette smoking and its cessation are reported to alter lipid metabolism.[1–3] It is well established that smoking stimulates lipolysis in vivo. The lipolytic effect of smoking has been attributed to the nicotine component being mediated via release of catecholamines.[3] Nicotine, a major component of cigarette smoke, promotes inflammation and progression of atherosclerotic lesions.[4–6] Furthermore, nicotine also has a direct effect on human adipose tissue.[7–9] On the other hand, oxidative stress has been shown to be a key phenomenon involved in the effects of smoking. Cigarette smoke contains a large amount of free radicals, which degrade NO released from the endothelium and also produce highly reactive intermediates, resulting in endothelial injury. Oxidative stress can damage many cell components, such as DNA, lipid membranes, and proteins, and lead to apoptosis and cell damage.[10,11]

Adiponectin, an adipose tissue–specific collagen-like factor, is abundantly present in plasma and possesses antiatherogenic properties. Adiponectin is emerging as an important molecule in obesity,[12] the metabolic syndrome,[13–15] cardiovascular disease,[16] lipid metabolism,[15] and hypertension.[17,18] In addition, adiponectin concentration is correlated independently with the vasodilator response to reactive hyperemia, and its concentration could be an independent parameter of endothelial function.[19] Endothelial dysfunction, an early marker of atherosclerosis, has been observed in chronic smokers as well as after acute cigarette smoking.[20,21] These results suggest that adiponectin may be a mediator between smoking and several diseases such as hypertension and coronary artery disease. Furthermore, smoking may directly regulate adiponectin concentration via lipolysis.

Although Miyazaki et al[22] reported that in subjects with coronary artery disease, smoking status was associated with reduced adiponectin concentration, using a small number of subjects, the association between plasma adiponectin and smoking status was evaluated without adjusting for con-

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founding factors and without consideration of the sex difference in adiponectin level.[23] Sex is an important confounding factor for evaluating adiponectin concentration, and the clinical importance of smoking habit in evaluating adiponectin concentration has not been fully elucidated. In the present study, we examined whether smoking habit is associated with a lower adiponectin level. First, we performed a cross-sectional study using a large number of subjects, including only males, to examine the chronic effect of smoking. Second, we performed an acute smoking exposure test in never-smokers and evaluated the effect for 12 hours. Finally, we demonstrated an inhibitory effect of H_2O_2 and nicotine on the expression and secretion of adiponectin in vitro.

Methods

Epidemiological Study (Chronic Effect of Smoking)

A total of 331 male subjects were selected from patients who were admitted and underwent medical investigation including a general check-up at Osaka University Hospital, Japan. All subjects enrolled in this study were Japanese. The study protocol was approved by the ethical committee of Osaka University, and all subjects gave written informed consent to participate in the study. All procedures followed were in accordance with the institutional guidelines of Osaka University. Smoking status was determined by interview on the day of measuring clinical parameters, and the subjects were divided into 3 groups according to smoking habit: never-smokers, past smokers (who had a history of habitual smoking but had quit), and current smokers. As a result, the numbers of never-smokers, past smokers, and current smokers were 79, 136, and 116, respectively. Hypertension was defined as systolic blood pressure (BP) of ≥140 mm Hg or diastolic BP of ≥90 mm Hg on repeated measurements, or receiving antihypertensive treatment. Diabetes mellitus was defined according to World Health Organization criteria.[24] Hyperlipidemia was defined as total cholesterol (T-chol) of >6.22 mmol/L, triglyceride (TG) of >2.26 mmol/L, or HDL cholesterol (HDL-chol) of <0.91 mmol/L. Ischemic heart disease was defined as a ≥75% organic stenosis of ≥1 major coronary artery, as confirmed by coronary angiography or a history of myocardial infarction or percutaneous transluminal coronary angioplasty. Renal failure was defined as fasting serum creatinine (Cr) concentration >176.8 μmol/L. Subjects with ischemic heart disease, chronic renal failure, nephrotic syndrome, overt congestive heart failure, valvular heart disease, secondary hypertension, or atrial fibrillation were excluded. Furthermore, no subjects receiving steroid therapy were included in this study.

Each subject was studied on the day after admission, in the morning after having abstained from alcohol, caffeine, and smoking, as well as food for 8 hours before the study. BP was measured by well-trained physicians, and venous blood was drawn from all subjects. Height and body weight were measured and body mass index (BMI) calculated. Plasma samples for subsequent assay were stored at −80°C. Insulin sensitivity was estimated using the homeostatic model assessment (HOMA) index (ie, plasma glucose level×(plasma insulin level/22.5). Brinkman index was calculated using the formula: number of cigarettes smoked per day×number of years of smoking. Plasma concentration of adiponectin was determined using a sandwich ELISA system (Adiponectin ELISA kit; Otsuka Pharmaceutical Co. Ltd.), as reported previously.[12] The parameters T-chol, TG, HDL-chol, and Cr levels were also determined. Urine samples were collected for 24 hours to evaluate Cr clearance (Ccr).

Acute Smoking Exposure Test

To examine the acute effect of smoking on adiponectin concentration, we measured plasma adiponectin level in 5 healthy volunteers who had never smoked (age 33 to 46 years; BMI 24.0±1.0 kg/m^2). All subjects were male and were coauthors included in this study, and the exclusion criteria of this study were the same as those described previously. After completion of the baseline study, all participants were asked to smoke a cigarette (1.1 mg nicotine; 14 mg tar) and were instructed to inhale. Before and 3, 6, and 12 hours after smoking, venous blood was drawn.

Effect of H_2O_2 and Nicotine on Expression and Secretion of Adiponectin In Vitro

3T3-L1 mouse preadipocytes were grown to confluence and induced to differentiate into adipocytes, as described previously.[25] Seven days after the initiation of differentiation (assessed by this criterion), 85% to 90% of the cells were judged to be differentiated. On day 7, the indicated concentrations of H_2O_2 with/without *N*-acetyl-L-cysteine (NAC) or nicotine (Sigma) were added to the media for 24 hours.

An aliquot of the media after 24 hours of stimulation was subjected to ELISA (Adiponectin ELISA kit; Otsuka Pharmaceutical Co. Ltd.) to detect the amount of adiponectin secreted.

Loss of 3T3-L1 adipocyte integrity was evaluated spectrophotometrically by measurement of lactate dehydrogenase (LDH) activity in the supernatant using a standard kit (LDH-Cyotoxic Test; Wako).

3T3-L1 adipocyte cellular protein samples were isolated using ISOGEN (Nippon Gene) according to manufacturer protocol. Adipocyte protein concentration was determined by colorimetric protein assay (detergent solubilization) using DC Protein Assay (Bio-Rad) according to manufacturer protocol. The relative secretion of adiponectin into the media was normalized to the amount of cellular protein in the same sample.

Total RNA from adipocytes was isolated using ISOGEN, treated with DNAse to prevent contamination with genomic DNA, and finally resuspended in diethylpyrocarbonate-treated MilliQ. Expression levels of adiponectin and 18S mRNA were quantified by real-time quantitative RT-PCR using an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Inc.) according to manufacturer instructions. TaqMan probes and primers for adiponectin and 18S were Assay-on-Demand Gene Expression products (Applied Biosystems, Inc.). We used amplification of 18S ribosomal RNA in each of the stimulated conditions for sample normalization. The relative expression of adiponectin mRNA was normalized to the amount of 18S in the same mRNA sample using the standard curve method described by the manufacturer.

Statistical Analysis

Means or proportions of clinical characteristics and cardiovascular risk factors were computed for each smoking pattern. Continuous variables were expressed as mean±SEM. Differences between smoking status groups for variables including adiponectin concentration were analyzed by 1-way ANOVA and post hoc comparison (Dunnet's procedure). Unpaired *t* test was used to examine the differences in adiponectin between 2 groups. Pearson's correlation coefficients were used to assess the relationships between adiponectin and all other variables. Multiple regression models were used to assess the relationship between adiponectin concentration and smoking status after adjustment for potential confounding factors. The significance of differences in adiponectin levels before and after smoking was evaluated using repeated-measures ANOVA. In the in vitro study, differences were analyzed by unpaired *t* test. All *P* values were 2-sided, and those <0.05 were considered statistically significant. All calculations were performed using a standard statistical package (JMP 4.0; SAS Institute).

Results

Association of Plasma Adiponectin Concentration With Smoking Habit in Humans

The clinical and biochemical characteristics of the study subjects divided into 3 groups according to smoking habit are shown in Table 1. We first examined the association between smoking habit and adiponectin concentration. The concentra-

TABLE 1. Clinical Characteristics of Study Subjects

Variables	Never-Smokers	Past Smokers	Current Smokers
n	79	136	116
Brinkman index	0±0	792±53	742±58
Age, years	58.0±1.2	62.2±0.9*	57.5±1.0
BMI	23.6±0.3	23.7±0.3	23.2±0.3
Adiponectin, μg/mL	6.5±0.4	5.7±0.3	5.3±0.3*
Systolic BP, mm Hg	130±2	134±1	133±2
Diastolic BP, mm Hg	80±1	81±1	85±1*
Hypertension, %	66.7	71.0	73.9
Diabetes, %	10.3	15.9	20.0
Hyperlipidemia, %	27.9	30.0	38.0
T-chol, mmol/L	4.99±0.09	5.18±0.08	5.26±0.10*
TG, mmol/L	1.48±0.12	1.78±0.09	1.64±0.11
HDL-chol, mmol/L	1.48±0.05	1.45±0.04	1.41±0.04
HOMA index	1.7±0.3	2.0±0.3	2.1±0.4
Cr, μmol/L	82.0±2.5	80.6±1.8	76.3±2.2
Ccr, mL/min	85.7±3.7	82.4±2.6	83.5±3.2

Values are given as mean±SEM.
*$P<0.05$ compared with never-smokers for each parameter.

TABLE 2. Clinical Characteristics of Subgroups Without Medication and Diabetes

Variables	Never-Smokers	Past Smokers	Current Smokers
n	27	41	30
Brinkman index	0±0	850±94	554±74
Age, years	58.8±2.5	62.0±2.1	60.1±2.5
BMI	22.6±0.5	22.3±0.4	21.8±0.3
Adiponectin, μg/mL	8.3±0.8	7.1±0.6	6.1±0.7*
Systolic BP, mm Hg	117±4	125±3	128±4
Diastolic BP, mm Hg	74±3	76±2	79±3
Hypertension, %	14.8	17.1	16.1
Hyperlipidemia, %	31.8	29.4	34.8
T-chol, mmol/L	4.99±0.15	5.14±0.15	4.90±0.16
TG, mmol/L	1.49±0.20	1.48±0.18	1.64±0.22
HDL-chol, mmol/L	1.55±0.10	1.53±0.09	1.62±0.11
HOMA index	1.1±0.4	1.4±0.3	1.5±0.7
Cr, μmol/L	72.9±6.7	75.4±4.7	76.6±7.8
Ccr, mL/min	84.0±5.4	80.8±4.1	83.0±5.4

Values are given as mean±SEM.
*$P<0.05$ compared with never-smokers for each parameter.

tion of adiponectin was significantly lower in current smokers than in never-smokers ($P=0.01$). Furthermore, the concentration of adiponectin showed a tendency to be lower in past smokers than in never-smokers ($P=0.06$). Diastolic BP and T-chol in current smokers and age in past smokers were significantly higher than those in never-smokers ($P<0.05$). In addition, the kinds of drugs that influence adiponectin concentration, such as angiotensin II receptor blockers, angiotensin-converting enzyme (ACE) inhibitors, and peroxisome proliferator-activated receptor-γ (PPAR-γ) ligands, were not significantly different among the smoking status.

In the total subjects, adiponectin level was significantly associated with age ($r=0.38$; $P<0.01$), BMI ($r=-0.33$; $P<0.01$), and Ccr ($r=-0.36$; $P<0.01$). Furthermore, adiponectin level was significantly lower in patients with hypertension (5.1±0.2 versus 7.3±0.3 μg/mL; $P<0.01$), diabetes (5.0±0.2 versus 6.2±0.3 μg/mL; $P<0.01$), and hyperlipidemia (4.5±0.3 versus 5.8±0.2 μg/mL; $P<0.01$). We next performed multiple regression analysis including age, BMI, hypertension, diabetes, hyperlipidemia, and Ccr and revealed that adiponectin concentration in never-smokers was ≈1.25× higher than that in current smokers (never-smokers 6.5±0.4 μg/mL; past smokers 5.6±0.3 μg/mL; current smokers 5.2±0.4 μg/mL; $F=4.52$; $P=0.01$).

To exclude the effect of diabetes and drugs on adiponectin concentration, we next examined the effect of smoking habit on adiponectin concentration after excluding subjects with diabetes and subjects receiving any medication. The clinical and biochemical characteristics of these study subjects are shown in Table 2. Adiponectin concentration significantly increased with age ($r=0.41$; $P<0.01$) and HDL-chol ($r=0.43$; $P<0.01$) and decreased with BMI ($r=-0.50$; $P<0.01$), systolic BP ($r=-0.35$; $P<0.01$), diastolic BP ($r=-0.36$; $P<0.01$), TG ($r=-0.30$; $P<0.05$), HOMA ($r=-0.29$; $P<0.05$), and Ccr ($r=-0.41$; $P<0.01$). On the other hand, there was no significant association between adiponectin and T-chol ($r=-0.04$). Although clinical variables other than adiponectin concentration were not significantly different, adiponectin concentration was significantly lower in current smokers than in never-smokers ($P=0.04$).

Brinkman index was not associated with adiponectin concentration in the total subjects ($r=-0.05$) or in subjects without medication or diabetes ($r=-0.19$). However, in current smokers (n=116), the number of cigarettes smoked per day was inversely associated with adiponectin concentration ($r=-0.21$; $P<0.04$).

Effect of Acute Smoking Exposure on Plasma Adiponectin Concentration

The mean adiponectin level before smoking was 7.0±1.5 μg/mL. Percent changes in plasma concentration of adiponectin in response to smoking are shown in Figure 1. Acute smoking exposure produced a significant decrease in plasma level of adiponectin at 3 hours (−9.2±0.7%) and 6 hours (−13.1±1.2%), and the maximum decrease was observed at 12 hours after smoking (−14.5±0.6%; $F=17.3$; $P<0.01$).

Inhibitory Effects of H_2O_2 and Nicotine on Expression and Secretion of Adiponectin in 3T3-L1 Adipocytes

We investigated the effect of H_2O_2 and nicotine on the regulation of adiponectin secretion and gene expression in 3T3-L1 adipocytes. Incubation with H_2O_2 or nicotine reduced adiponectin mRNA expression and adiponectin secretion into the media in a dose-dependent manner (Figures 2 and 3). The effects of H_2O_2 to reduce adiponectin mRNA expression and secretion into the media were antagonized by coincubation with NAC (Figure 2). Secretion of adiponectin into the media was significantly reduced compared with control by nicotine

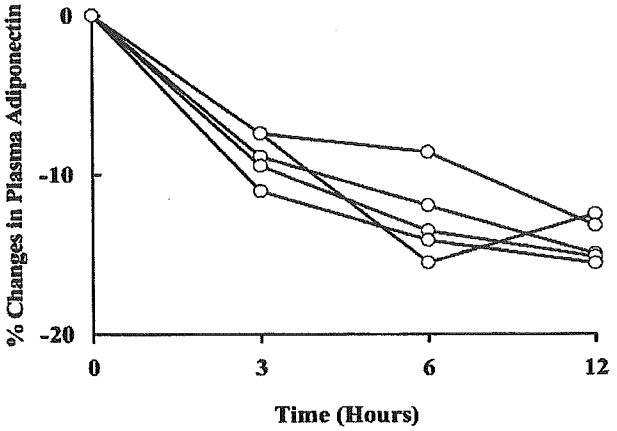

Figure 1. Percent changes in plasma adiponectin levels before and after smoking. Individual changes in adiponectin level were plotted. Adiponectin levels were expressed as percent change from initial values (n=5).

at concentrations $\geq 10^{-8}$ mol/L. We next studied the adipocyte protein concentration; the amount of adiponectin in the media was adjusted by each of the amount of cellular protein. As shown in Figures 2B and 3B, even after adjustment for protein amount, adiponectin secretion was significantly reduced by incubation with H_2O_2 or nicotine in a dose-dependent manner.

Cytotoxicity was also assessed by LDH leakage from adipocytes into the media. As shown in Figure 2C, H_2O_2 (100 μmol/L) significantly increased LDH release from adipocytes. When cultured in the presence of NAC (10^{-2} M), this increase was significantly attenuated. On the other hand, as shown in Figure 3C, treatment with nicotine also significantly increased leakage of LDH from adipocytes at concentrations $\geq 10^{-7}$ mol/L.

Discussion

The present study demonstrated that the plasma adiponectin concentration was significantly lower in male subjects who were current smokers than in never-smokers, and the association was observed even in subjects without diabetes and medication. Furthermore, multiple regression analysis including age, BMI, hypertension, diabetes, hyperlipidemia, and Ccr showed that adiponectin concentration was significantly lower in current smokers. Acute smoking exposure reduced adiponectin concentration significantly at 12 hours after smoking in never-smokers. In cultured 3T3-L1 adipocytes, oxidative stress and nicotine reduced the secretion and expression of adiponectin. These results suggest that smoking may decrease plasma adiponectin concentration in men.

In this study, even in subjects without diabetes and medication, the association between adiponectin concentration and clinical variables was in accordance with previous reports that adiponectin concentration was significantly associated with age,[18,26] BMI,[12] TG,[13] HDL-chol,[27] BP,[18] and insulin resistance indicated by HOMA.[14]

Although adiponectin concentration is decreased in several diseases,[12–14,16,18] the mechanisms that regulate plasma adiponectin concentration have not been fully elucidated. It has been reported that weight reduction[13] and certain drugs such as PPAR-γ ligands,[25] ACE inhibitors, and angiotensin II receptor blockers[28] increased the adiponectin concentration, a cytokine, tumor necrosis factor-α (TNF-α), reduced the expression of adiponectin in adipocytes,[25] and some human mutations of adiponectin affect plasma adiponectin concentration.[18,29] In this study, we demonstrated that smoking habit is also associated with adiponectin concentration. Furthermore, our finding of lower adiponectin levels in chronic smokers is in line with the fact that chronic smokers are insulin resistant.[30] Thus, our results may support investigation of the mechanisms of several disorders induced by smoking.

Smoking is known to be associated with increased oxidative stress. Reactive oxygen species such as H_2O_2 are also normally produced during cellular oxidation reduction processes. Although our results showed significant cytotoxicity in adipocytes incubated with H_2O_2 at a concentration of 100 μmol/L, this cytotoxicity was significantly attenuated when they were cultured with NAC. Furthermore, H_2O_2 decreased the expression and secretion of adiponectin from adipocytes in a dose-dependent manner. Previous reports have shown that oxidative stress disrupts activation of phosphatidylinositol 3-kinase (PI3K),[31,32] which is a key molecule in the secretion of adiponectin in 3T3-L1 adipocytes.[33] Thus, we propose the idea that oxidative stress induced by tobacco smoke decreases the secretion and expression of plasma adiponectin via inhibition of activation of PI3K in adipocytes.

Nicotine activates nicotinic acetylcholine (nACh) receptors, which belong to the family of ionotropic receptors consisting of 5 transmembrane subunits building up ion channels. nACh receptors are widely distributed throughout the central and peripheral nervous system and are involved in signal transmission at the skeletal neuromuscular junction, in autonomic ganglia, and in the brain.[34,35] Functional nACh receptors are expressed in adipocytes in mice,[36] and nicotine exerts direct stimulation of lipolysis via nACh receptors in human adipose tissue.[7] Thus, nicotine has the possibility of regulating adiponectin concentration directly. In our experiments, nicotine had a significant inhibitory effect at concentrations $\geq 10^{-7}$ mol/L, which can be found in the plasma of smokers.[37] Furthermore, our results also showed significant cytotoxicity in adipocytes incubated with nicotine at a concentration of 10^{-6} mol/L. These results could also be in accordance with previous reports that nicotine itself induces lipolysis by activating local nicotinic cholinergic receptors in adipose tissue.[7] Thus, our results indicate that nicotine in tobacco smoke decreases plasma adiponectin via inhibition of the secretion and expression of adiponectin in adipocytes.

Apart from nicotine and oxidative stress, there are several other possible mechanisms by which smoking habit may affect adiponectin concentration. It has been reported that smoking itself and tissue hypoxia elevate TNF-α,[38,39] a powerful proinflammatory cytokine and a mediator of inflammation, which is known to decrease adiponectin concentration.[25] These findings also support the idea that persistent production of TNF-α induced by chronic exposure to cigarette smoke may promote the development of hypoadiponectinemia. Furthermore, nicotine elicits release of the catecholamines epinephrine and norepinephrine,[40] and

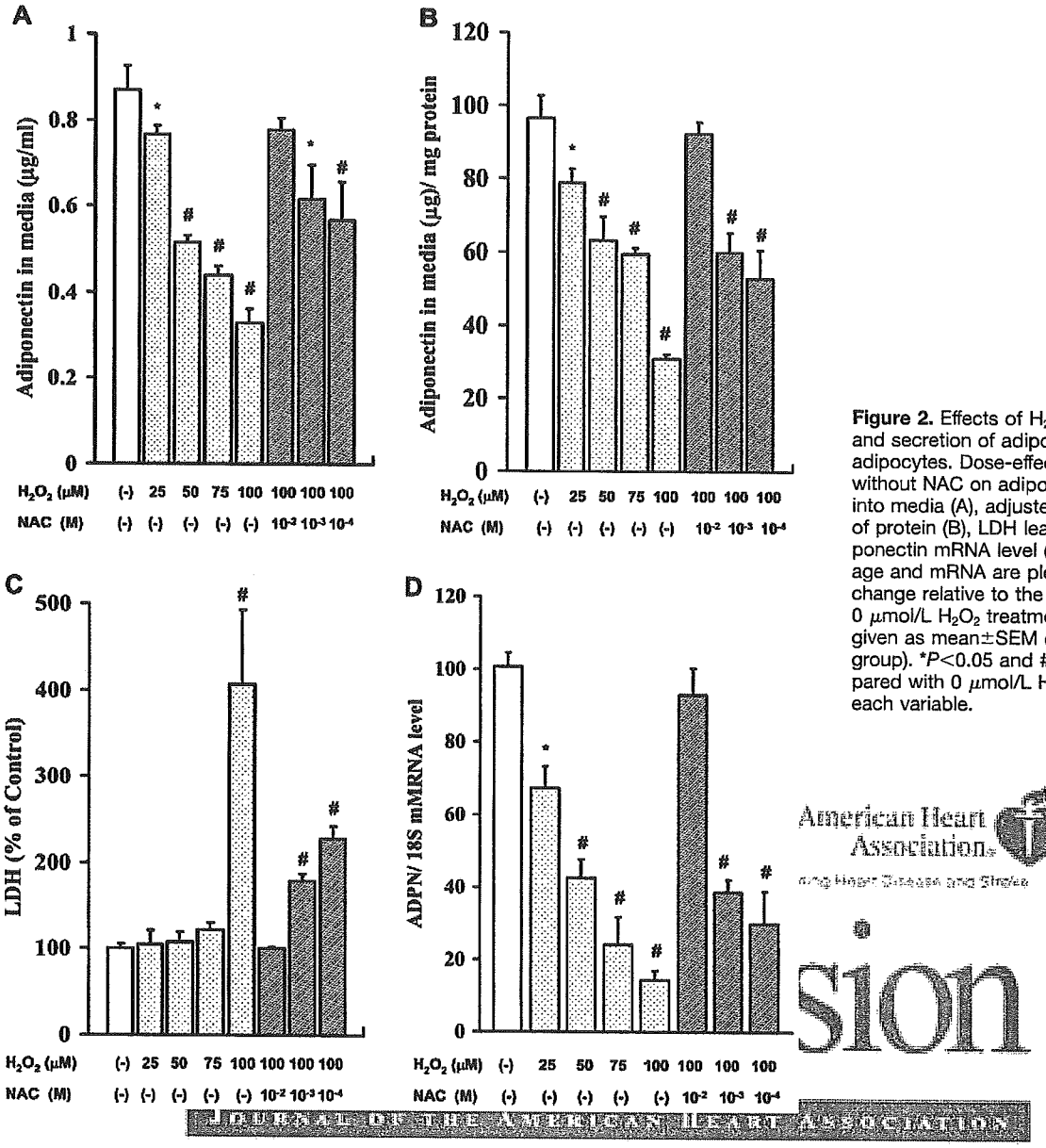

Figure 2. Effects of H_2O_2 on expression and secretion of adiponectin in 3T3-L1 adipocytes. Dose-effect of H_2O_2 with/without NAC on adiponectin secreted into media (A), adjusted by the amount of protein (B), LDH leakage (C), and adiponectin mRNA level (D). All LDH leakage and mRNA are plotted as percent change relative to the level with 0 μmol/L H_2O_2 treatment. Values are given as mean±SEM (n=12 in each group). *P<0.05 and #P<0.01 compared with 0 μmol/L H_2O_2 treatment for each variable.

β-adrenergic stimulation suppresses adiponectin gene expression.[41]

With respect to cessation of habitual smoking, in this study, adiponectin level was between those of nonsmokers and current smokers, even after adjustment for confounding factors. These results suggest that the decreasing effect of smoking on adiponectin concentration might remain even after smoking cessation. Another reason is that even after smoking cessation, smoking-related damage persisted, such as endothelial dysfunction and continuing low-grade inflammation indicated by C-reactive protein,[42] which is known to affect adiponectin concentration.[19,43] To clearly confirm whether smoking cessation affects adiponectin concentration, a cohort study is required.

Because tobacco smoke consists of >4000 chemical constituents, it is impossible to predict the effect of nicotine and oxidative stress within this complex mixture of components. Although we showed that nicotine and oxidative stress have a potent inhibitory effect on adiponectin secretion, there are several other molecules in cigarette smoke that may be toxic to adipocytes (eg, cadmium, cotinine, and thiocyanate).[44] The net effect of cigarette smoke on the function of adiponectin may be quite different from that of nicotine or H_2O_2 alone. Another limitation is that this study was designed as a cross-sectional study rather than a randomized clinical trial or observational study. Furthermore, several important determinants of adiponectin level, such as body fat content and waist circumference, were not measured in our study. Instead of these measurements, we included HOMA and BMI in the analysis of this study. Previous reports have shown that body fat content, especially intra-abdominal fat, is a determinant of adiponectin level.[26] On the other hand, the different localization of fat mass itself influences cardiovascular risk factors such as T-chol, TG, and HDL-chol.[45] In our study, except for T-chol, the clinical characteristics were not significantly different among subjects (Table 1). Furthermore, the subjects

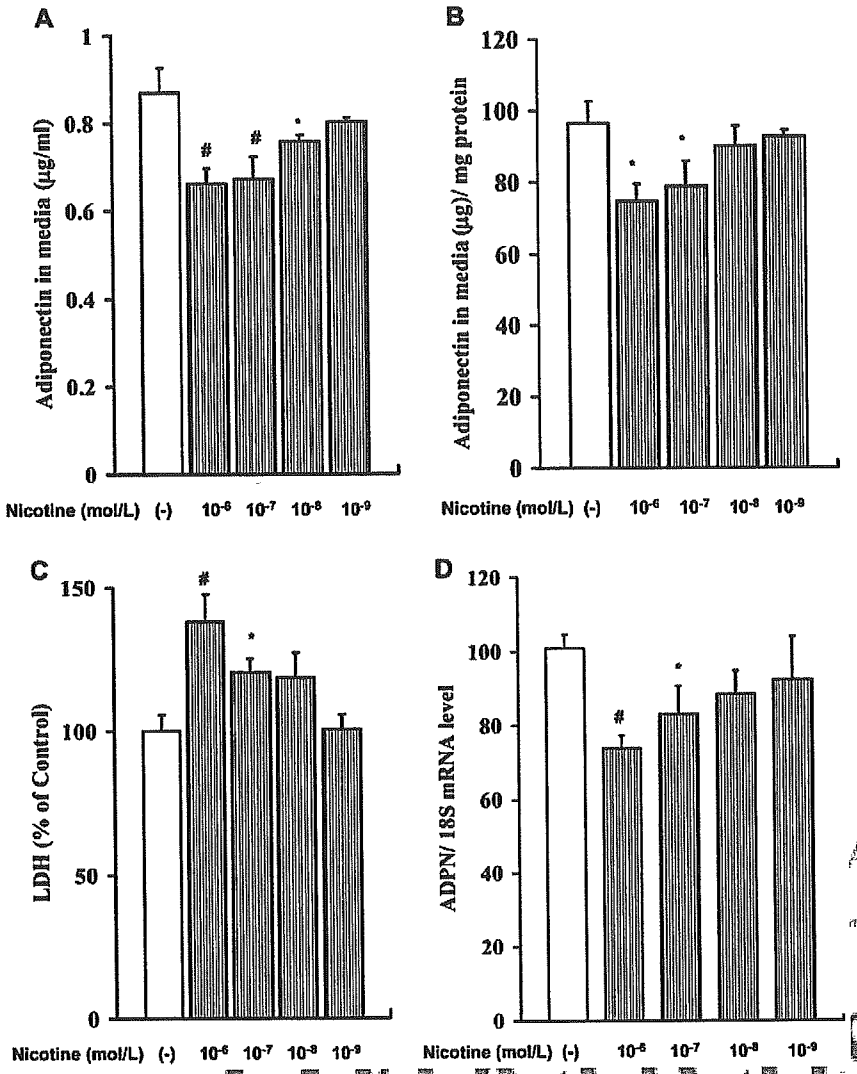

Figure 3. Effects of nicotine on expression and secretion of adiponectin in 3T3-L1 adipocytes. Dose-effect of nicotine on adiponectin secreted into media (A), adiponectin adjusted by the amount of protein (B), LDH leakage (C), and adiponectin mRNA level (D). All LDH leakage and mRNA are plotted as percent change relative to the level with 0 mol/L nicotine treatment. Values are given as mean±SEM (n=12 in each group). *$P<0.05$ and #$P<0.01$ compared with 0 mol/L nicotine treatment for each variable.

included in this study were relatively lean, and obesity (BMI ≥30 kg/m^2) was present in only 2.5% of the total subjects. Thus, the effect of different fat distributions on adiponectin concentration among the groups may be relatively small in this study. On the other hand, our study could not provide a conclusion on the influence of "passive smoking" on adiponectin concentration. Further investigation is required to examine these effects.

In conclusion, our results demonstrated that smoking habit is associated with a lower adiponectin concentration in men. This reduction may be induced through a direct effect of oxidative stress and nicotine on adipocytes.

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ORIGINAL ARTICLE

Usefulness of measuring serum markers in addition to comprehensive geriatric assessment for cognitive impairment and depressive mood in the elderly

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Background: To determine the utility of various serum markers for assessment of cognitive and mental functions in the elderly, we performed a Comprehensive Geriatric Assessment (CGA) in the out-patient clinic in Kyoto University Hospital.

Methods: We measured serum levels of dehydroepiandrosterone (DHEA), DHEA-S, malondialdehyde low-density lipoproteins (MDA-LDL), and high-sensitivity C-reactive protein (hs-CRP) in 145 patients to find the association of these markers with activities of daily living (ADL), cognitive impairment and depressive symptoms.

Results: We found that the levels of hs-CRP were significantly higher in patients with lower scores in Mini-Mental State Examination (MMSE) and Kohs block design test, and higher scores in the button test, indicating that hs-CRP may be associated with the cognitive function in elderly patients. We also found that the levels of DHEA-S were lower in patients with higher scores (9 or over) on the Geriatric Depression Scale-15 (GDS), indicating that DHEA-S may be associated with depressive mode in elderly patients. Total cholesterol, high-density cholesterol (HDL-C), or albumin were not statistically different in each group studied.

Conclusions: Thus, our data indicate that measuring hs-CRP and DHEA-S would be helpful to assess the cognitive function and depressive symptoms in elderly patients.

Keywords: Comprehensive Geriatric Assessment (CGA), cognitive function, C-reactive protein (CRP), depression, dehydroepiandrosterone (DHEA).

Introduction

The Comprehensive Geriatric Assessment (CGA) emerged during the 1980s as an important strategy to improve care for elderly patients with complex, medical, psychosocial and functional problems.[1,2] Earlier studies showed that CGA in inpatient units dramatically improved survival and functional status.[3,4] Therefore, if

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we utilize CGA more intensively for the care of elderly patients in our hospitals, CGA will have a more beneficial effect on outcomes.[5] Although CGA for inpatients is very important to improve the outcomes of elderly patients, the focus of recent investigations has been shifted to outpatient CGA due to high cost of inpatient care in the United States.[6] One study showed that outpatient CGA helps maintain functioning and the ability to perform daily activities.[7] However, its benefits are not consistently demonstrated or recognized.

Although survival is one of the most commonly reported outcomes in clinical studies, Stuck *et al.* reported in a meta-analysis that outpatient CGA did not improve survival compared to usual care despite the fact that significant survival benefits were observed in inpatients and home-based CGA.[8] Two of the four trials of outpatient CGA in the meta-analysis were, however, criticized because the subjects were relatively healthy and not at high risk. Additional studies of outpatient CGA were therefore conducted with greater attention to targeting frail subjects, resulting in improved outcomes in elderly patients including mental health[9–11] and functional status.[6,7] Although outpatient CGA has, so far, no demonstrable benefit for the survival of older, frail patients compared to usual care, outpatient CGA should be a good way to assess elderly patients, to diagnose patients with mild cognitive impairment or depressive symptoms, and to eventually prevent functional decline. However, additional measurement might be required to improve the survival.

Cognitive impairment in elderly patients is sometimes hard to diagnose in the outpatient clinic. Therefore, screening elderly patients by Mini-Mental State Examination (MMSE) is useful to diagnose the initial phase of dementia or mild cognitive impairment, although it takes time to screen all the patients with MMSE. Screening depression is also helpful for the care of elderly patients and a 15-item Geriatric Depression Scale (GDS-15) is commonly used for that purpose. This test also takes time in the outpatient clinic. Finding a marker for early diagnosis of cognitive impairment or depressive mood therefore would be important to select high-risk patients.

Chronic brain inflammation characterizes Alzheimer's disease (AD), the most of common neurodegenerative disease associated with progressive cognitive decline. Certain cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α, are shown to influence a number of different mechanisms that can induce or accelerate the development of neurodegeneration, indicating a correlation of inflammation with cognition.[12,13] Because high-sensitivity C-reactive protein (hs-CRP) can reflect the presence of inflammation and can be induced by these cytokines, we chose hs-CRP as a candidate marker for screening patients with cognitive impairment.

Androstenedione and dehydroepiandrosterone (DHEA) are produced in the biosynthetic pathway of androgen and estrogen. The sulfate ester of DHEA is DHEA-S. The decline of DHEA has been pursued as a major factor in the development of age-associated disorders.[14] Among the studies investigating the effect of DHEA-S on mood in the elderly, some studies show that DHEA-S improves mood[15,16] while others do not.[17,18] Therefore, the effect of these steroids on neurodegenerative diseases remains inconclusive. Malondialdehyde-low density lipoproteins (MDA-LDL) are associated with oxidatively-modified products of LDL and can be associated with atherosclerotic disease.[19] Therefore, if MDA-LDL is associated with cognitive impairment or depressive mood, controlling risk factors for atherosclerotic disease might be important.

From these findings we assessed the activities of daily living (ADL), cognitive functions and depressive symptoms in elderly patients who visited our outpatient clinic for the first time and examined the correlation with various serum markers listed above. We also used 'Get up and go', 'Button scores' and 'Functional reach' to assess neurobehavioral functions in these patients. By these measurements, we should be able to select high-risk patients for cognitive and functional decline and eventually would be able to use outpatient CGA to improve survival of elderly patients.

Methods

Subjects

All elderly (basically 65 years or older) patients who came to Kyoto University Hospital for the first time or had not been seen for the past 6 months in this hospital were asked to attend for health problem screening. We started outpatient CGA in May 2001. One hundred and forty-five consecutive patients aged 62 and older (mean age ± SD: 75.6 ± 0.56) who visited the outpatient clinic from May 2001 through March 2004 were enrolled for this study after written informed consent was taken from each patient or his/her family member. The study protocol was approved by the Ethical Committee of Kyoto University School of Medicine.

Measurements

Comprehensive Geriatric Assessment (CGA) was done on the day of patient visit by experienced speech therapists after history taking and physical examination were done. The CGA included height, body weight, blood pressure, basic activities of daily living (BADL), which was measured with the Barthel Index. For higher-level functional capacity, each subject's independence was rated by the Tokyo Metropolitan Institute of Gerontology (TMIG) Index of competence.[20] This

assessment consists of a 13-item index including three sublevels of competence: (i) instrumental self-maintenance; (ii) intellectual activities; and (iii) social role. MMSE was used to assess cognitive functions. Neurobehavioral functions were assessed by the Kohs block design (KBD) test,[21] 'Get up and go' and 'Button scores'. A cutoff point of 12 for KBD test was used as described.[21] Functional reach was also determined as described.[22] Briefly, each subject was positioned next to the wall with one arm raised 90° with fingers extended, and a yardstick was mounted on the wall at shoulder height. The distance in centimeters that a subject was able to reach forward from an initial upright posture to the maximal anterior leaning posture without moving or lifting the feet was measured by visual observation of the third finger tip against the mounted yardstick. The distances of two trials were averaged as the functional reach score, with a greater distance indicating better balance ability.

We screened depressive symptoms using the Japanese version of GDS-15.[23] Higher scores of GDS-15 indicate a greater degree of depressive mood. In this study we used a cutoff point of 9. Therefore, we defined depression as a GDS-15 score of 9 or more.

'Get up and go'

This test of balance is commonly used to examine functional mobility in elderly subjects.[24] The test requires the subject to stand up, walk 3 m (10 ft), turn, walk back and sit down. The time to complete the test is strongly correlated with functional mobility. Elderly people who can complete the test in less than 20 s are independent in transfer tasks, which are normal activities in daily living.

'Button scores'

'Button scores' evaluate manual dexterity using a panel with combinations of 10 hooks, 10 big buttons and five small buttons. There were three discrete measurements of time recorded for each participant (10 hook-ons, 10 big button-on-and-offs, and five small button-on-and-offs). Total manual dexterity time in seconds, defined as the Button Score, was calculated by adding the average times for one hook-on and one big or small button-on-and-off.[25,26] A cutoff point of 17 was used for the analysis.

Serum marker measurement

Serum levels of DHEA, DHEA-S, MDA-LDL and hs-CRP were measured by SRL (Tokyo, Japan). DHEA and DHEA-S were measured by radioimmunoassay. MDA-LDL was measured by enzyme-linked immunosorbent assay (ELISA). Hs-CRP was measured with CardioPhase kit (Dade Behring, Tokyo, Japan).

Statistical analysis

Differences in continuous variables among the disease groups were determined by one-way analysis of variance (ANOVA). A P-value of less than 0.05 was considered significant. Multiple regression analysis was used to assess the involvement of age and sex.

Results

Table 1 summarizes the patient characteristics in the study population. The mean age in this study group was 75.6 years and the percentage of males was 40%. There was no statistical difference in age between males and females. The ADL of the patients was relatively well preserved. The mean Barthel index (0–100) was 98.3 and was not statistically different between males and females. Instrumental ADL was assessed by the Tokyo Metropolitan Institute of Gerontology Index (TMIG Index) (0–13). The mean value was 10.3 and was not statistically significant between males and females either. We assessed depression by GDS-15 and found that the mean score was 5.23. The GDS scores were slightly higher in females than in males, but the difference was not statistically significant. The mean score was almost comparable to that of community-dwelling elderly people in Japan.[27]

We then determined the cognitive function by MMSE and found that the mean scores were 25.2 (Table 2). We also determined the KBD test to assess spatial recognition and found that the mean score was 22.3. 'Get up and go' and 'Button scores' were assessed and the mean time to be required was 14.9 and 12.1 s, respectively. The mean length of functional reach in these patients

Table 1 Mean age, Barthel index, Tokyo Metropolitan Institute of Gerontology (TMIG) index and Geriatric Depression Scale (GDS) scores in males and females

	n	Age	Barthal index	TMIG index	GDS
Total	145	75.6 ± 0.56	98.3 ± 0.61	10.3 ± 0.25	5.23 ± 0.31
Male	58	74.8 ± 0.90	99.5 ± 0.38	10.5 ± 0.37	4.51 ± 0.45
Female	87	75.9 ± 0.72	98.3 ± 0.98	10.1 ± 0.33	5.70 ± 0.41

Data are expressed as mean ± SEM.

Table 2 Mean MMSE, KBS, button scores, Get up and go, and functional reach in males and females in this population

	MMSE	KBS	Button score	'Get up and go'	Functional reach
n	142	133	134	131	125
Total	25.2 ± 0.46	22.3 ± 1.13	12.1 ± 0.45	14.9 ± 0.46	23.4 ± 0.66
Male	25.7 ± 0.77	24.0 ± 1.88	12.8 ± 0.62	14.1 ± 0.74	26.2 ± 0.84*
Female	25.0 ± 0.56	21.0 ± 1.39	11.6 ± 0.62	15.5 ± 0.57	21.3 ± 0.66*

Data are expressed as mean ± SEM. *$P < 0.01$. *n*, number of patients studied.

Table 3 Mean levels of dehydroepiandrosterone (DHEA), DHEA-S, malondialdehyde-low density lipoproteins (MDA-LDL) and high-sensitivity C-reactive protein (hs-CRP). Difference in male and female patients

	DHEA (ng/ml)	DHEA-S (ng/ml)	MDA-LDL (U/L)	hs-CRP (ig/ml)
Total	2.08 ± 0.10	777 ± 49.3	147 ± 5.84	4.98 ± 1.56
Male	2.02 ± 0.15	995 ± 93.8*	128 ± 8.31**	3.42 ± 1.66
Female	2.12 ± 0.13	625 ± 45.3*	158 ± 7.62**	5.79 ± 2.22

Data are expressed as mean ± SEM. *$p < 0.01$, **$p < 0.01$, male vs female.

was 23.4 cm. These values were also comparable to the data of community-dwelling elderly in Japan.[28]

We next measured the serum levels of DHEA, DHEA-S, MDA-LDL and hs-CRP in this population. The mean value of DHEA, DHEA-S, MDA-LDL and hs-CRP were 2.08 ng/mL, 777 ng/mL, 147 U/L and 4.98 μg/mL, respectively (Table 3). DHEA-S was higher and MDA-LDL was lower in males than in females. However, there was no statistical difference in DHEA or hs-CRP in males and females. Figure 1 A shows the age-dependent decrease of DHEA-S in this population. DHEA-S and age were negatively correlated (the coefficient was –0.4). DHEA also showed an age-dependent decline in this population, but the coefficient was –0.2 (Fig. 1b). MDA-LDL and hs-CRP did not show age-dependent changes in this population (data not shown).

To determine the association of hs-CRP with the cognitive function in the elderly, we examined the correlation to MMSE, KBD and 'Button scores'. We divided the patients into two groups according to the points of MMSE (cutoff; 24), KBD (cutoff; 12), and Button scores (cutoff; 17). We found that the level of hs-CRP was significantly higher in the patients with lower MMSE and KBD, and higher button scores (Fig. 2a). These differences were significant by multiple regression analysis after adjusting for age and sex. These results indicate the association of hs-CRP with cognitive and functional impairment. However, the level of total cholesterol, high-density cholesterol (HDL-C) or albumin was not statistically different between each group studied (data not shown). Although the level of hs-CRP was also higher in the patients who took longer time to complete 'Get up and go', the difference was not statistically significant. The levels of DHEA, DHEA-S or MDA-LDL

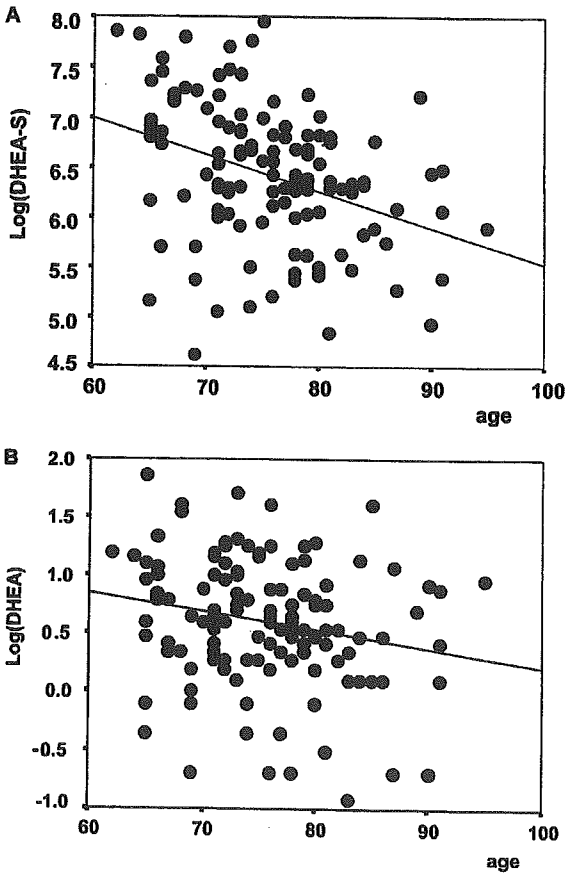

Figure 1 Age-dependent decrease of dehydroepiandrosterone (DHEA)-S and DHEA in elderly patients. Relationship between age and serum levels of (A) DHEA-S or (B) DHEA in the study patients is shown. The Y-axis is shown as natural log of (A) DHEA-S or (B) DHEA.

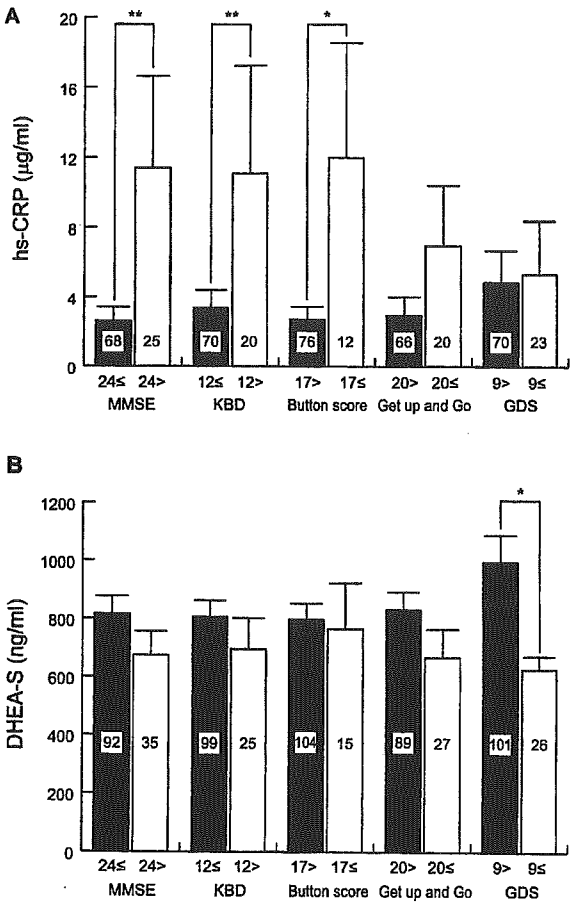

Figure 2 Levels of high-sensitivity C-reactive protein (hs-CRP) and DHEA-S in study patients. (A) Hs-CRP and (B) DHEA-S were measured in patients at the first visit to Kyoto University hospital after informed consent was taken. Patients were divided into two groups according to the level of each test. Patients were divided into two groups according to the score of Mini-Mental State Examination (MMSE); 24 and more, and less than 24, time for Kohs block design (KBD); less than 12 and 12 and more, 'Button scores'; less than 17 and 17 and more, the time required for 'Get up and go'; less than 20 and 20 and more, Geriatric Depression Scale (GDS); less than 9 and 9 and more. Values are the mean ± SEM. Number of the patients in each group is shown in each column. $^{*}P < 0.05$, $^{**}P < 0.01$.

were, however, not associated with these tests (Table 4, Fig. 2b).

In contrast, the levels of DHEA-S were significantly lower in the patients with higher GDS scores (9 or over). These differences were also significant by multiple regression analysis after adjusting for age and sex ($P < 0.05$). In contrast, the other markers, including hs-CRP, were not associated with GDS scores (Fig. 2b).

Among the patients with lower MMSE (less than 24), 52.6% had dementia while only 4.1% had dementia among the patients with normal MMSE (24 or over). As

Table 4 Mean dehydroepiandrosterone (DHEA) and malondialdehyde-low density lipoproteins (MDA-LDL) levels in each group of patients

	MMSE		KBD		'Button score'		'Get up and go'		GDS	
	≤ 24	> 24	≤ 12	> 12	≥ 17	< 17	> 20	≤ 20	> 9	≤ 9
DHEA (ng/ml)	2.07 ± 0.11	2.11 ± 0.22	2.11 ± 0.11	2.12 ± 0.23	2.08 ± 0.10	1.98 ± 0.16	2.14 ± 0.11	1.78 ± 0.18	2.10 ± 0.11	2.04 ± 0.21
MDA-LDL (U/L)	146 ± 6.67	150 ± 12.0	151 ± 7.00	134 ± 11.3	150 ± 6.54	145 ± 17.2	152 ± 7.38	141 ± 12.0	147 ± 6.56	145 ± 13.1

Data are expressed as mean ± SEM. MMSE, Mini-Mental State Examination; KBD, Kohs block design; GDS, Geriatric Depression Scale.

a risk factors for stroke, hypertension was found in 26.3% of the patients with lower MMSE, while 32.0% of the patients with normal MMSE had hypertension. Other risk factors, such as diabetes mellitus and hyperlipidemia were found in less than 5% of the patients in both groups. In terms of GDS scores, 37.9% of the patients with high scores (nine or over) were diagnosed with depression, while only 5.4% of the patients with low scores (less than 9) were diagnosed with depression. The incidence of dementia was 20.7% and 15.2% in each group, respectively.

Discussion

In this study we demonstrate that hs-CRP could be a marker to predict the cognitive impairment in elderly patients in outpatient clinic. Our study also indicates that DHEA-S is lower in patients with depressive mood in the elderly. Thus, measuring these markers in the outpatient clinic might be very useful to assess cognitive and functional impairment as well as depressive mood in elderly patients in addition to the assessment by CGA.

Comprehensive Geriatric Assessment is a very effective way to assess cognitive and functional impairment in the elderly and to find geriatric problems to improve their quality of life (QOL). However, most of hospitals have not utilized this assessment at their outpatient clinics because it is time consuming and unprofitable. Therefore, most geriatricians assess inpatients with CGA, which is getting more and more popular in Japan. Studies with outpatient CGA have not been successful in terms of survival so far. Therefore, by utilizing outpatient CGA and serum markers we would be able to select patients with potential risk for the future decline of cognitive functions and to eventually improve survival of frail elderly patients, although Bradly *et al.* indicated that the improvement of mental health may be an appropriate and realistic goal for outpatient CGA.[29]

Findings from epidemiological studies and some small clinical trials that non-steroidal anti-inflammatory drug (NSAID) users have a lower risk of AD, with indications of dose effects, has drawn much interest in inflammatory mechanisms in AD.[30,31] As our data show that the patients with cognitive impairment or potential decline have higher levels of hs-CRP, we might be able to select those patients to treat with NSAID to prevent the progression of cognitive impairment. To rationalize this treatment, we need a larger scale of study to prove whether or not the decline in cognitive function is faster in patients with higher hs-CRP levels.

Plasma DHEA shows a progressive age-related decline in men and women. DHEA and androstenedione have been shown to inhibit IL-6 secretion from human mononuclear cells *in vitro*,[32] suggesting a connection between aging of endocrine and immune systems. DHEA has also been shown to suppress IL-4, IFN-γ and astrocytic TNF-α and IL-6 production.[33,34] Despite its interesting inverse association with IL-6 levels and beneficial effects on senescence and cognition, a recent Cochrane Systematic Review found only limited evidence of an improved sense of well-being with DHEA supplementation.[35] Clinical benefit of DHEA supplementation should wait for other ongoing trials.

Association between DHEA-S levels and degenerative disorders of the nervous system, such as dementia and cognitive decline, have been controversial.[17,36–38] Some reports did not show the association of low serum DHEA-S levels with AD and other forms of cognitive dysfunction,[39,40] while others suggest a role of DHEA-S in depression, dementia and impaired cognitive performances in the elderly.[41,42] Although our study did not show the association of DHEA or DHEA-S with MMSE, KBD, 'Get up and go' or functional reach, a significant association of low DHEA-S with depressive mood was shown in our patient group. Our study is a cross-sectional study and the number of the patients is relatively small. Therefore, a longitudinal study will be necessary to determine whether or not the patients with low DHEA-S have a higher risk for the development of depression and whether or not treatment of those patients with DHEA-S can prevent the development of depression. Since the levels of DHEA-S declined according to age, the age-related increase of depression might be explained by a decrease of sex hormone, such as DHEA-S. In this study, we used 8/9 as cutoff for GDS. We used this cutoff point because it was appropriate in terms of sensitivity and specificity (Wada *et al.* unpubl. data). When we used 5/6 as cutoff for GDS, we found a lower level of DHEA-S in patients with GDS scores of 6 or over, but could not find a statistical significance.

Our data indicate higher incidence of dementia in patients with low MMSE and higher hs-CRP. We also demonstrated higher incidence of depression in patients with higher GDS scores and lower DHEA-S levels in elderly patients with relatively preserved ADL. Risk factors for stroke such as hypertension did not seem to be involved in these markers. With these cross-sectional data in hand, we think that it is important to follow these patients to determine whether or not high levels of hs-CRP results in a decrease in cognitive and functional impairment and whether or not low levels of DHEA-S predicts future depression. If low levels of DHEA-S are associated with the development of depression in the elderly, supplementation of DHEA might be beneficial to improve their QOL. It is also important to determine the cutoff point of these markers to select patients with high risk for cognitive decline or depression. A larger scale of study is necessary to address this issue.

In summary, our study indicates that measuring serum markers such as hs-CRP and DHEA-S would be useful to assess elderly patients along with CGA.

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