Table 1
Correlations of fasting or non-fasting serum triglycerides versus other metabolic parameters

		Age	BMI	TC	HDL-C	PG	HbAlc
Fasting TG	Men $N=4676$	−0.100	0.307	0.314	−0.434	0.126	0.100
	Women $N=8314$	0.306	0.329	0.356	−0.431	0.161	0.236
Non-fasting TG	Men $N=3547$	−0.135	0.315	0.360	−0.375	0.092	0.095
	Women $N=7840$	0.316	0.339	0.388	−0.433	0.200	0.253

All of the correlation coefficients showed $p<0.0001$.
TG, triglycerides; BMI, body mass index; TC, total cholesterol; PG, plasma glucose.

0.39 mmol/l, respectively. In those for non-fasting TG, age, BMI, PG, HbA1c, TC, TG and HDL-C were 58±15 years, 22.9±3.3 kg/m^2, 5.99±1.89 mmol/l, 5.3±0.9% ($n=5483$), 5.08±0.91, 1.45±0.95 and 1.50±0.39 mmol/l, respectively.

Since TG levels, unlike cholesterol levels, do not distribute normally, we measured median, the first and the third quartiles and quartile deviations of TG values in fasting and non-fasting states. The distribution of fasting TG values are shown in Fig. 1A. In men and women combined, the median, the 1st and the 3rd quartile of fasting TG levels were 1.01, 0.73 and 1.41 mmol/l, respectively. In men alone, the median, the first and the third quartile of fasting TG levels were 1.13, 0.82 and 1.62 mmol/l, respectively, whereas in women those values were 0.95, 0.70 and 1.30 mmol/l, respectively. The distribution of non-fasting TG values are shown in Fig. 1B. In men and women combined, the median, the first and the third quartile of non-fasting TG levels were 1.22, 0.86 and 1.76 mmol/l, respectively. In men alone, the median, the first and the third quartile of non-fasting TG levels were 1.36,0.95 and 1.99 mmol/l respectively, whereas in women those values were 1.16, 0.82 and 1.65 mmol/l, respectively.

In men and women combined, median plus twice the quartile deviation for fasting TG value was calculated to be 1.68 mmol/l, being almost equal to the decision point of fasting TG levels of 1.69 mmol/l. Similarly, in men and women combined, median plus twice the quartile deviation for non-fasting TG was calculated to be 2.12 mmol/l, which could be used as decision point of non-fasting serum TG levels.

Iso et al. [1] conducted a 15.5-year prospective study ending in 1997 of 11,068 Japanese aged 40–69 years and found that relative risk of coronary heart disease adjusting for coronary risk factors and time since last meal associated with a 1 mmol/l increase in TG was 1.29 for men and 1.42 for women. They also showed the baseline data for TG distribution in men and women in non-fasting states. On the other hand, since TG levels for Japanese especially for men has increased drastically during the past decade [10], updated data might be more desirable to determine how TG distributes in Japanese population currently.

In this study, we also analyzed the relationships of TG with other metabolic parameters (Table 1). Overall, between fasting and non-fasting states, TG values showed similar associations with age, BMI, TC, HDL-C, PG and HbA1c. When men and women were separately considered, age had positive relations with TG levels both in fasting and non-fasting states in women, whereas not in men. Also, the associations of TG with parameters for glucose metabolism were weaker in men than in women. In conclusion, we have shown the distribution of fasting and non-fasting TG levels in a large number of individuals who underwent annual medical checkup in Ishikawa prefecture Japan.

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We do not have any conflict of interest.

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Original Article

Low Adiponectin Level in Young Normotensive Men with a Family History of Essential Hypertension

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Circulating level of adiponectin, an adipocyte-derived protein, is reduced in states of insulin resistance such as obesity and type 2 diabetes. We have previously shown that hypoadiponectinemia is related to insulin resistance in essential hypertension. Recent studies have shown that normotensive subjects with a positive family history of essential hypertension (FH+) have decreased insulin sensitivity compared to subjects with a negative family history of essential hypertension (FH−). We here examined the association between adiponectin concentration and insulin sensitivity in FH+ and FH−. Thirty young, non-obese and normotensive men without a family history of diabetes mellitus were enrolled. A total of 15 subjects were FH+, and the remaining 15 subjects were FH−. Insulin sensitivity index (ISI) was evaluated by the euglycemic hyperinsulinemic glucose clamp technique. Concentrations of adiponectin and other metabolic variables were measured. The FH+ group had significantly lower levels of ISI and adiponectin than did the FH− group. In all of the subjects, ISI was positively correlated with adiponectin concentration and high-density lipoprotein (HDL) cholesterol level and was negatively correlated with insulin level. Adiponectin concentration was the only independent determinant of ISI in a multiple regression analysis. Our results showed that adiponectin level was significantly decreased and that this was accompanied by reduced insulin sensitivity in young, non-obese and normotensive men with a family history of essential hypertension. Phenotype of reduced adiponectin level as an earlier penetrance may be especially useful in genetic analyses of insulin resistance and essential hypertension. (*Hypertens Res* 2005; 28: 141–146)

***Key Words*: adiponectin, essential hypertension, family history, insulin resistance**

Introduction

Adipose tissue was once thought to be simply a depot for fuel storage in the form of triglyceride. However, it is now known that adipocytes secrete a variety of proteins that are implicated in a wide range of biological effects. Adiponectin, an adipocyte-derived protein, has been independently identified and characterized by several groups (*1–5*). In humans, adiponectin is one of the most abundant gene transcript proteins in adipocytes, corresponding to 0.01% of all proteins (*2*). In contrast with other adipocyte-derived proteins, the circulating adiponectin level is reduced in patients with coronary artery disease and in states of insulin resistance such as obesity and type 2 diabetes (*6–8*). It has also been suggested that patients with type 2 diabetes not only have a decreased adiponectin level in the basal state but also impaired utilization of adiponectin in the tissue (*9*). Moreover, we have previously

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Table 1. Basal Characteristics and Metabolic Variables of the Studied 30 Normotensive Men

Variables	Family history of hypertension	
	−	+
n	15	15
Age (years)	23.5±0.9 (19–32)	23.7±1.1 (18–32)
Body mass index (kg/m^2)	24.3±0.9 (19.4–29.4)	24.6±0.8 (20.3–29.5)
SBP (mmHg)	113.6±3.3 (96–138)	116.5±3.5 (96–138)
DBP (mmHg)	74.7±2.0 (58–86)	76.4±1.9 (62–86)
Mean blood pressure (mmHg)	87.7±2.5 (70.7–105.0)	89.8±2.4 (73.3–106.7)
High-normal blood pressure	4	4
Pulse rate (beats/min)	59.7±1.6 (52–72)	60.7±2.3 (46–76)
Fasting plasma glucose (mmol/l)	4.6±0.1 (4.1–5.1)	4.8±0.1 (4.1–5.3)
Fasting plasma insulin (pmol/l)	22.2±1.5 (18.0–36.0)	25.7±2.3 (16.8–45.0)
Total cholesterol (mmol/l)	4.2±0.2 (3.0–5.5)	4.1±0.2 (3.0–5.5)
HDL cholesterol (mmol/l)	1.0±0.1 (0.7–1.3)	0.9±0.1 (0.7–1.4)
Triglyceride (mmol/l)	0.9±0.2 (0.3–2.3)	1.2±0.1 (0.4–2.3)
Free fatty acid (mmol/l)	0.4±0.1 (0.1–0.7)	0.4±0.1 (0.1–0.8)

Values are *n* or the means±SEM (range). SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein.

shown that hypoadiponectinemia is related to insulin resistance in essential hypertension, and that blockade of the renin-angiotensin system increases adiponectin levels with improvement in insulin sensitivity, at least in part *via* a decrease in adipocyte size (*10*, *11*). Adiponectin has also been suggested to enhance insulin sensitivity and prevent atherosclerosis in animal experiments (*12*, *13*). In fact, it has been reported that adiponectin concentration is negatively correlated with pulse wave velocity (PWV), which is measured as an index of atherosclerosis, and that adiponectin was a significant determinant of PWV in a multiple regression analysis (*14*).

In recent years, growing attention has been paid to the role of genetic factors in linking insulin resistance and essential hypertension. Essential hypertension has a familial predisposition, but the phenotype of elevated blood pressure has delayed penetrance. Insulin resistance sometimes exists before the onset of hypertension, and genetically and environmentally determined insulin resistance and/or compensated hyperinsulinemia might contribute to the development of hypertension (*15–18*). It has been shown that approximately 40% of essential hypertensives are insulin-resistant (*18*, *19*). Recent studies have shown that normotensive offspring of patients with essential hypertension have decreased insulin-stimulated glucose uptake compared to subjects with no family history of hypertension, suggesting that low insulin sensitivity may be a primary factor in the development of hypertension (*18*, *20–25*).

Although it has been demonstrated that adiponectin level is significantly reduced in non-obese relatives of type 2 diabetic subjects with a high propensity for type 2 diabetes (*26*), there have been no studies dealing with the relationship between adiponectin concentration and insulin sensitivity in relation to the family history of essential hypertension. We therefore examined the association between adiponectin concentration and insulin sensitivity assessed by the euglycemic hyperinsulinemic glucose clamp technique in young, non-obese and normotensive men with and without a family history of hypertension.

Methods

Thirty young normotensive men (mean age: 23.6±0.7 years) from the student body of our university were enrolled as volunteers in this study. All subjects were 18–32 years of age, had a body mass index (BMI) of <30 kg/m^2 and blood pressure of <140/90 mmHg, and were not taking any drugs. A total of 15 subjects had a positive family history of essential hypertension (FH+), and the remaining 15 subjects had a negative family history of essential hypertension (FH−). Family history was ascertained by a self-report questionnaire sent to the parents and by records of the parents' physicians. Subjects whose parents were both being treated with antihypertensive medication for essential hypertension were classified as FH+, whereas those without such a history and whose blood pressure was <140/90 mmHg at any recent health check on an annual basis were classified as FH−. Using records of parents' physicians or annual health checkups, parental blood pressure was evaluated on the basis of at least three measurements by a sphygmomanometer performed on different days. Subjects for whom a family history of hypertension was not certain and those with a family history of diabetes mellitus in any relatives were excluded. All of the subjects were hospitalized and were put on a regular diet (2,000 kcal/day) that included 310 g of carbohydrate, 50 g of fat, 80 g of protein, 120 mmol of sodium, and 75 mmol of potassium. Insulin sensitivity was evaluated by the euglycemic hyperinsulinemic glucose clamp technique. Before the clamp study, blood pres-

Table 2. Multiple Regression Analysis

Independent variables	Insulin sensitivity index		Adiponectin	
	β	p	β	p
Adiponectin	0.509	0.010	—	—
Family history of hypertension	-0.215	0.18	-0.102	0.51
Body mass index	0.124	0.44	-0.321	0.028
HDL cholesterol	0.110	0.49	0.092	0.55
Fasting insulin	-0.193	0.22	—	—
Insulin sensitivity index	—	—	0.519	0.003

Data are expressed as standardized regression coefficient (β) and p value. HDL, high-density lipoprotein.

sure and pulse rate were measured and blood samples were obtained from all subjects. Systolic blood pressure of 130–139 mmHg or diastolic blood pressure of 85–89 mmHg was defined as high-normal blood pressure. The concentrations of adiponectin, glucose, insulin, and lipid variables were measured. There were no dropout subjects. This study was performed with the approval of the ethics committee of our institution, and informed consent was obtained from all of the subjects.

Euglycemic Hyperinsulinemic Glucose Clamp Technique

A 2-h euglycemic hyperinsulinemic glucose clamp was performed according to the method described by DeFronzo *et al.* (*27*). A vein in a forearm was cannulated for blood glucose monitoring. During the glucose clamp, blood was continuously drawn at 2.0 ml/h through a catheter. In addition, a contralateral antecubital vein was cannulated with a plastic cannula for the infusion of insulin and glucose. Continuous insulin infusion, monitoring of glucose concentration, and infusion of various amounts of glucose in order to clamp glucose levels in the basal state were performed with a model STG-22 artificial endocrine pancreas (Nikkiso Corp., Tokyo, Japan). The infusion rate of insulin (humalin R U-40; Shionogi Pharmaceutical Co., Osaka, Japan) was 40 mU/m^2/min. During insulin infusion, euglycemia was maintained by infusion of a 20% glucose solution. The mean rate of glucose infusion for the last 30 min of the clamp was used as the M value (mg/kg/min). The insulin sensitivity index (ISI, mg/kg/min per μU/ml) was taken as the M value divided by the steady state plasma insulin concentration during the clamp.

Laboratory Investigations

Serum adiponectin level was measured using a commercially available sandwich enzyme-linked immunosorbent assay kit (Otsuka Pharmaceuticals Co., Ltd., Tokushima, Japan) as previously reported (*5*). Fasting plasma glucose was determined by the glucose oxidase method. Fasting plasma insulin was measured by a radioimmunoassay method (Insulin RIA bead; Dianabot, Tokyo, Japan). Serum lipid profiles, including total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride and free fatty acid, were estimated by enzymatic methods.

Statistical Analysis

Numeric variables are expressed as the means±SEM. The Mann-Whitney U test was used for comparisons between two unpaired variables. Spearman's rank correlation test was used for analysis of correlations between two variables. Multiple linear regression analysis was performed using ISI and adiponectin level as dependent variables and using family history of hypertension (yes: 1; no: 0) and the variables with a significant correlation or a tendency of correlation in univariate regression analysis as independent predictors. Stepwise regression analysis was also performed in a forward direction with F for the entry set to 4, showing the percentage of variance in the adiponectin concentration that significantly independent variables explained (r^2). A p value of <0.05 was considered statistically significant.

Results

As shown in Table 1, the two groups were well matched for age and BMI. There were no significant intergroup differences in blood pressure, number of subjects with high-normal blood pressure, pulse rate, or the levels of glucose, total cholesterol, HDL cholesterol, triglyceride, or free fatty acid. The insulin level in the FH+ group was higher, but not significantly higher, than that in the FH− group. The FH+ group had significantly lower levels of ISI and adiponectin than did the FH− group (Fig. 1).

In all of the subjects, the ISI was positively correlated with adiponectin concentration (r=0.64, p=0.0006) and HDL cholesterol level (r=0.39, p=0.038) and negatively correlated with fasting insulin level (r=−0.34, p=0.034). BMI was not significantly correlated with ISI (r=−0.19, p=0.19). Multiple regression analysis showed that adiponectin concentration was independently related to ISI (Table 2). Stepwise regression analysis also revealed that adiponectin was the only independent predictor of ISI, explaining a total of 43% of the variance in this measure (r^2 = 0.43).

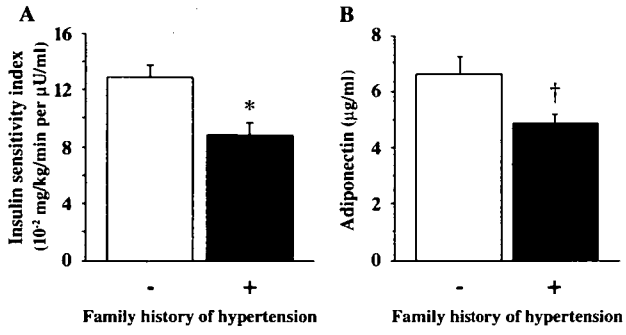

Fig. 1. *Bar graphs show the insulin sensitivity index (ISI) (A) and adiponectin level (B) in young normotensive men without (open bars) and with (closed bars) a family history of essential hypertension. Values are presented as the means±SEM.* *$p<0.01$, †$p<0.05$.

On the other hand, the adiponectin concentration was positively correlated with ISI ($r=0.64$, $p=0.0006$), as stated above, and HDL cholesterol level ($r=0.38$, $p=0.041$) and negatively correlated with BMI ($r=-0.48$, $p=0.0096$) and insulin level ($r=-0.42$, $p=0.0097$). Multiple regression analysis showed that ISI and BMI were independently related to adiponectin concentration (Table 2). Stepwise regression analysis also revealed that ISI and BMI were independent determinants of adiponectin concentration, explaining a total of 53% of the variance in this measure ($r^2 = 0.53$).

Discussion

Three notable findings were obtained in the present study. First, in normotensive offspring of essential hypertensives who had not yet developed high blood pressure, insulin sensitivity was already impaired. This result is in accordance with previous findings (*18*, *20–25*). Second, adiponectin level was significantly correlated with degree of insulin sensitivity in the whole body estimated by the glucose clamp study. In multiple regression analyses, adiponectin was the only determinant of ISI, and the ISI was also a predictor of adiponectin concentration independently of BMI even in young, nonobese and normotensive men, although it has been well established that adiponectin level is decreased in insulin-resistant states such as obesity, type 2 diabetes, and essential hypertension (*7–10*). The third and most important finding in the present study is that serum adiponectin concentration was significantly lower in the group of young normotensive men with a family history of essential hypertension than in the control group of age- and BMI-matched normotensive men with no family history of hypertension. Although it has been demonstrated that adiponectin level is significantly reduced in insulin-resistant first-degree relatives of type 2 diabetic subjects (*26*), this is the first report, to the best of our knowledge, on the relationship between adiponectin concentration and insulin sensitivity in relation to genetic predispositions for the development of essential hypertension.

Decreased adiponectin concentration and reduced insulin sensitivity might be early-penetrance phenotypes in the course of development of human genetic hypertension, because they are already present even in still-normotensive offspring of essential hypertensives. The early appearance of these phenotypes in still-normotensive FH+ subjects suggests that adiponectin concentration and insulin resistance play pathophysiological roles in the later response to high blood pressure.

Since obesity influences insulin sensitivity and adiponectin level (*5*, *15–17*), we enrolled non-obese subjects in the present study. In fact, there was no significant difference in BMI between the two groups. Therefore, the differences in ISIs and adiponectin concentrations between the two groups cannot be explained by a difference in the degree of obesity. Moreover, the subjects were selected so as to exclude the potentially confounding impact of other factors known to influence insulin sensitivity and adiponectin concentration, namely, family history of diabetes mellitus (*26*) and treatment with drugs such as thiazolidinedions (*28*, *29*) and renin-angiotensin system-blocking agents (*10*). Age-matching is also considered very important when comparing groups with positive and negative family histories of essential hypertension, since not only blood pressure but also insulin sensitivity is influenced by phenomena related to the aging process (*30*). The two groups in the present study were well matched for age, and the selected subjects were young (aged 18–32 years). In addition, there was no significant difference in blood pressure or the number of subjects with high-normal blood pressure between the two groups, although it has been reported that young men with high-normal blood pressure have lower serum adiponectin concentrations (*31*).

The precise mechanisms underlying reduced adiponectin levels in normotensive men with a family history of hypertension are unclear. It has been reported that insulin infusion during a glucose clamp study leads to a decrease in adiponectin concentration (*32*), suggesting that chronic hyperinsulinemia associated with an insulin-resistant state leads to a decrease in adiponectin concentration. The decrease in serum adiponectin level could be the result of impaired insulin sensitivity. However, the insulin level, but not ISI, in the FH+ group was not significantly higher than that in the FH− group in the present study as previously reported (*18*, *21–23*), although some previous studies showed significant fasting hyperinsulinemia in the offspring of hypertensive patients (*24*, *25*). Since the subjects in the present study were young, it is possible that insulin sensitivity evaluated by the euglycemic hyperinsulinemic glucose clamp method was decreased but that compensated hyperinsulinemia due to insulin resistance had not yet developed. Even at this time, adiponectin level had already decreased significantly. These findings support the idea that adiponectin primarily influences insulin sensitivity and that decreased insulin sensitivity results in compensated hyperinsulinemia.

Decreased adiponectin concentration may simply be an indicator of insulin resistance, as demonstrated by the direct association of adiponectin with ISI. Alternatively, the observation of decreased adiponectin level in still-normotensive individuals at genetic risk of hypertension is consistent with an early, perhaps pathogenic, role in the subsequent development of hypertension and may point to a logical target, in appropriately stratified patients, for early intervention in the pathogenesis of the disease.

One limitation of this study is the small number of subjects enrolled. Prospective studies using larger numbers of subjects are needed to determine whether adiponectin or a family history of hypertension is a major determinant in the subsequent development of hypertension. In addition, because we did not perform oral glucose tolerance tests in the present study, we could not investigate the presence and influence of impaired glucose tolerance in the subjects. Furthermore, it has been shown that adiponectin concentration is sex-related, being higher in females than in males (*8*, *33*). Although we enrolled only male subjects in the present study to adjust for confounding factors, it is important to confirm our findings by studies using female subjects.

In conclusion, our results showed that adiponectin level was significantly decreased and that this was accompanied by reduced insulin sensitivity in young, non-obese and normotensive men with a family history of hypertension. The reduction in insulin sensitivity coupled with a decrease in adiponectin concentration might precede the development of high blood pressure. Phenotype of reduced adiponectin level as an earlier penetrance may be especially useful in genetic analyses of insulin resistance and hypertension. Our findings might offer a new approach to identifying those at risk of hypertension in addition to novel metabolic strategies for early intervention.

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CLINICAL STUDY

Influence of gender, age and renal function on plasma adiponectin level: the Tanno and Sobetsu study

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Abstract

Design: The aim of this study was to determine the association between aging and adiponectin level from the aspect of the influence of renal function and sex hormones in humans.
Methods: Serum adiponectin and blood urea nitrogen (BUN) levels were measured in 964 subjects (372 males) aged 60.3±12.5 years. Testosterone and free testosterone levels were measured in 123 males, and estrone and estradiol levels were measured in 114 females. The subjects were divided into two age groups; 65 years of age or older (Age ≥65 group) and less than 65 years of age (Age <65 group).
Results: Adiponectin level increased linearly with aging in males, whereas it increased dramatically in females until their 50s. The patterns of changes in adiponectin were similar to those in BUN. In multiple-regression analysis using adiponectin as a dependent variable BUN was selected as a significant independent variable in all subjects and in subjects in the Age ≥65 group, whereas bioactive sex hormones were not selected.
Conclusions: A decrease in adiponectin clearance in the kidney may be the cause of high levels of adiponectin in the elderly. Adiponectin level seems to be influenced more strongly by BUN than by sex hormones and to be increased by a decline in renal function with aging.

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Introduction

Adiponectin is a 244-amino-acid plasma protein (1) that was identified from a gene, *apM1*, specifically expressed in fat tissue. Adiponectin has been shown to circulate as a trimer, hexamer or higher-molecular-mass form in the blood of healthy subjects and to be present at a high level of 5–10 μg/ml (2–6). It has been shown that the ratios among these forms determine their activity (7–9). There are also significant sex differences in the circulating concentrations of adiponectin and in the ratios of their subunits (7, 10). Differences between adiponectin levels were found in normotensive and hypertensive men with abnormal renal function, but not in women (10). It has been reported that the level is low in subjects carrying excessive organ fat and that the level increases with a reduction in body weight and is correlated negatively with body mass index (BMI) (3). In addition, adiponectin level has been shown to be correlated negatively with blood pressure and triglyceride level and positively with high-density lipoprotein (HDL) level and to be decreased in patients with hypertension (11) and hyperlipidemia (12, 13). It has also been shown to be correlated negatively with fasting plasma glucose (FPG) level, plasma glucose level 2 h after a meal and fasting insulin concentration (14, 15), and to be closely associated with insulin resistance (16–20).

On the other hand, it has been reported that adiponectin levels are elevated in the elderly (21, 22). This seemingly contradictory finding that levels of adiponectin, which has anti-atherosclerotic properties, were elevated in elderly subjects who were presumed to have developed atherosclerosis due to the accumulation of risk factors is intriguing. Previous studies showed that there is an inverse relationship between adiponectin and creatinine clearance in essential hypertensives and that adiponectin level was increased in patients with a combination of decline of renal function and hypertension (10). It has also been reported that adiponectin level was increased in patients with end-stage renal disease (23) and that adiponectin level was positively associated with impaired renal function, assessed by urinary albumin-to-creatinine ratio, in patients with diabetes (24). However, the mechanisms by which adiponectin is metabolized and excreted are not known,

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and the relationship between renal function and adiponectin level in humans who are relatively healthy has not been determined. Most of serum testosterone binds to albumin and sex-hormone-binding globulins, and serum free testosterone, which accounts for 1–2% of total serum testosterone, exhibits biological activity in humans (25). However, the mechanisms by which androgen affects adiponectin level have also not been determined, and there has been little investigation of the relationship between free testosterone and adiponectin levels.

In this study, we examined the association between aging and adiponectin level from the aspect of the influence of a decline of renal function or sex hormones in participants in mass-screening tests for residents in a region of Hokkaido, Japan.

Subjects and methods

Of 1519 participants in mass-screening tests for the residents of the towns Tanno and Sobetsu in Hokkaido, Japan, in 2003, 964 males and females with an average age of 60.3±12.5 years (372 males with an average age of 62.8±12.4 years and 592 females with an average age of 58.8±12.3 years) were selected after exclusion of patients undergoing treatment for hypertension, diabetes and hyperlipidemia (subjects from the first selection), and 237 males and females with an average age of 58.3±16.2 years (123 males with an average age of 59.8±16.7 years and 114 females with an average age of 56.6±15.6 years) were randomly selected from seven 10-year age brackets (30s to 90s) in males and from six 10-year age brackets (30s to 80s) in females, with a maximum of 21 subjects from each bracket, after exclusion of patients undergoing treatment for hypertension, diabetes and hyperlipidemia (subjects from the second selection). Since the number of subjects in the 90s bracket in males was only four, they were included in the 80s bracket in males. Patients with reproductive organ disease that might affect sex hormones were not included in this study.

The mass-screening tests were carried out between 0600 and 0800 h in the morning. Height and body weight were measured before blood-pressure measurement, and blood was collected from the subjects under fasting conditions before breakfast. Blood pressure was measured more than once from the right arm after resting for several minutes in a sitting position, and the average was calculated. Blood was collected from the median cubital vein in a sitting position with a vacuum tube. The items measured were systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI, FPG, total cholesterol, triglyceride, HDL, blood urea nitrogen (BUN), serum creatinine and serum adiponectin concentrations. Serum was stored in a freezer at −20 °C. The frozen serum was used to measure testosterone and free testosterone concentrations in males and estrone (E1) and estradiol (E2) concentrations in females after 4 months. Biochemical data were assayed as follows: FPG, the glucose-oxidase electrode method; total cholesterol, the cholesterol oxidase enzymatic assay method; triglyceride, the enzymatic colorimetric method; HDL, the direct liquid-stable assay; BUN, urease- glutamate dehydrogenase method; serum creatinine, Jaffe reaction method; adiponectin, the sandwich ELISA method (human adiponectin ELISA kit; Otsuka Pharmaceutical Co., Tokyo, Japan); testosterone and free testosterone, solid-phase RIA method (Coat-A-Count TotalTestosterone and Coat-A-Count Free Testosterone Diagnostic Products Corp., Los Angeles, CA, USA); E1, the double-antibody RIA method (ESTRONE RIA; Diagnostic Systems Laboratories, Inc., Webster, TX, USA); and E2, solid-phase RIA method (Coat-A-Count Estradiol; Diagnostic Products Corp.). The minimum detectable values for testosterone, free testosterone, E1 and E2 were < 5.0 ng/dl (0.17 nM), < 0.5 pg/ml (1.73 pM), < 15.0 pg/ml (55.5 pM) and < 8.0 pg/ml (29.4 pM), respectively.

The subjects from the first selection were divided into two age groups, 65 years of age or older (Age ≥ 65 group) and less than 65 years of age (Age < 65 group), to compare indices in middle-aged and elderly subjects. Multiple-regression analysis was performed with adiponectin as a dependent variable for both data from subjects from the first selection and data from subjects from the second selection.

The present study was carried out in accordance with the Declaration of Helsinki (1981) of the World Medical Association, and the study protocol was approved by the Research Committee of Sapporo Medical University, Sapporo, Japan. Written, informed consent was obtained from each subject after full explanation of the purpose, nature and risk of all procedures used.

Statistical analysis was performed with Windows SPSS version 12.0 in Japanese (SPSS Japan). Since adiponectin showed an F distribution, natural logarithmic-transformed values (LnAdipo) were used, and each value is presented as a mean±S.D. The unpaired t-test was used to compare data in two groups. A P value of less than 0.05 was considered statistically significant.

Results

The characteristics of subjects from the first selection are shown in Table 1. Adiponectin concentrations were 6.02±3.33 μg/ml in males and 8.91±4.20 μg/ml in females, the concentration being significantly higher in females than in males. LnAdipo correlated positively with age, HDL and BUN and negatively with BMI, DBP, FPG, total cholesterol and triglyceride in males and correlated positively with age, HDL and BUN and negatively with BMI, FPG and triglyceride in females. Age, BMI, SBP, DBP, FPG, triglyceride, BUN and serum creatinine were

Table 1 Background of subjects from the first selection (mean values and correlations related to adiponectin).

	Males ($n = 372$)		Females ($n = 592$)	
	Mean±S.D.	r	Mean±S.D.	r
Age (years)	62.8±12.4*	0.359†	58.8±12.3	0.175†
BMI (kg/m^2)	23.8±3.3*	−0.314†	23.1±3.2	−0.248†
SBP (mmHg)	133.5±21.1*	0.020	129.2±23.2	0.031
DBP (mmHg)	75.9±11.9*	−0.120†	73.0±12.2	−0.004
FPG (mg/dl)	97.2±16.5*	−0.122†	93.3±16.4	−0.200†
TC (mg/dl)	193.2±33.0*	−0.162†	205.3±32.7	0.038
TG (mg/dl)	115.4±75.4*	−0.346†	89.3±43.5	−0.181†
HDL (mg/dl)	51.4±11.6*	0.285†	59.3±13.5	0.201†
BUN (mg/dl)	16.5±4.1*	0.179†	15.0±4.0	0.147†
Cr (mg/dl)	1.10±0.33*	0.082	0.89±0.26	0.071
Adipo (μg/ml)	6.02±3.33*		8.91±4.20	

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; BUN, blood urea nitrogen; Cr, serum creatinine; Adipo, adiponectin.
r, versus LnAdipo, Pearson's correlation coefficient.
* $P < 0.05$ versus females, unpaired t-test.
† $P < 0.05$ versus LnAdipo, Pearson's correlation.
Conversion factors: FPG, mM = mg/dl × 0.05551; TC, mM = mg/dl × 0.02586; TG, mM = mg/dl × 0.01129; HDL, mM = mg/dl × 0.02586; BUN, mM = mg/dl × 0.3570; Cr, μM = mg/dl × 88.40.

significantly higher in males than in females, and total cholesterol and HDL were significantly lower in males than in females.

The mean values of adiponectin and BUN in relation to age are shown in Figs 1 and 2. Adiponectin increased linearly with aging in males, whereas in females it increased sharply until the 50s age bracket with a convex curve and then increased gradually (Fig. 1). The patterns of changes in adiponectin were similar to the patterns of changes in BUN (Figs 1 and 2).

In multiple-regression analysis of sex differences, age, BMI, SBP, FPG, total cholesterol, triglyceride, HDL and BUN with LnAdipo as a dependent variable, BUN was selected as a significant independent variable as well as sex differences, age, BMI, FPG, triglyceride and HDL (Table 2). SBP, BUN and adiponectin were significantly higher and BMI and triglyceride were significantly lower in males in the Age ≥65 group than in males in the Age <65 group, and BMI, SBP, DBP, FPG, total cholesterol, triglyceride, BUN, serum creatinine and adiponectin were significantly higher and HDL was significantly lower in females in the Age ≥65 group than in females in the Age <65 group (Table 3). In males, BUN showed a positive correlation with adiponectin in the Age ≥65 group ($r = 0.219$, $P = 0.002$) but not in the Age <65 group. In females, BUN showed a stronger positive correlation with adiponectin in the Age ≥65 group than in the Age <65 group ($r = 0.134$, $P = 0.045$ vs $r = 0.128$, $P = 0.014$; Table 3). In multiple-regression analysis using LnAdipo as a dependent variable, BUN was selected as a significant independent variable along with sex differences, age, BMI, FPG, triglyceride and HDL in the Age ≥65 group, while BUN was not selected as a significant independent variable in the Age <65 group (Table 4).

Characteristics of subjects from the second selection are shown in Table 5. Adiponectin concentrations were 6.26±3.94 μg/ml in males and 8.84±4.71 μg/ml in females, the concentration being significantly higher in females than in males. LnAdipo correlated positively with age and testosterone in males and negatively with BMI and free testosterone in males. There was no statistical gender-based difference in age, and BMI was significantly higher in males than in females.

The mean values of testosterone, free testosterone, E1 and E2 in relation to age are shown in Figs 3 and 4. In subjects from the second selection, the changes in mean values of adiponectin in relation to age were similar to those in subjects from the first selection (Fig. 1). In males, testosterone gradually decreased in their 30s

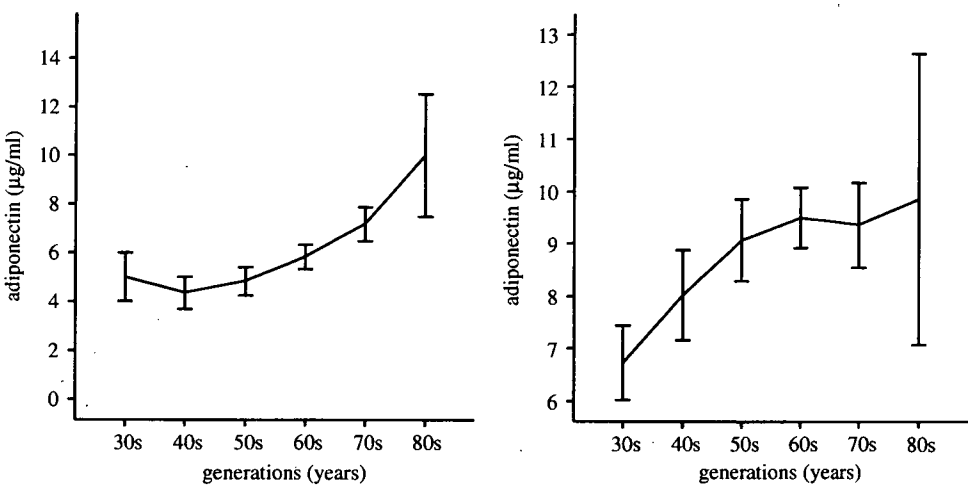

Figure 1 Mean plasma adiponectin levels for each generation in males and females. Numbers of male subjects in each age group were as follows: 30s, $n = 19$; 40s, $n = 44$; 50s, $n = 62$; 60s, $n = 130$; 70s, $n = 96$; 80s, $n = 21$. Numbers of female subjects: 30s, $n = 53$; 40s, $n = 88$; 50s, $n = 129$; 60s, $n = 209$; 70s, $n = 104$; 80s, $n = 9$.

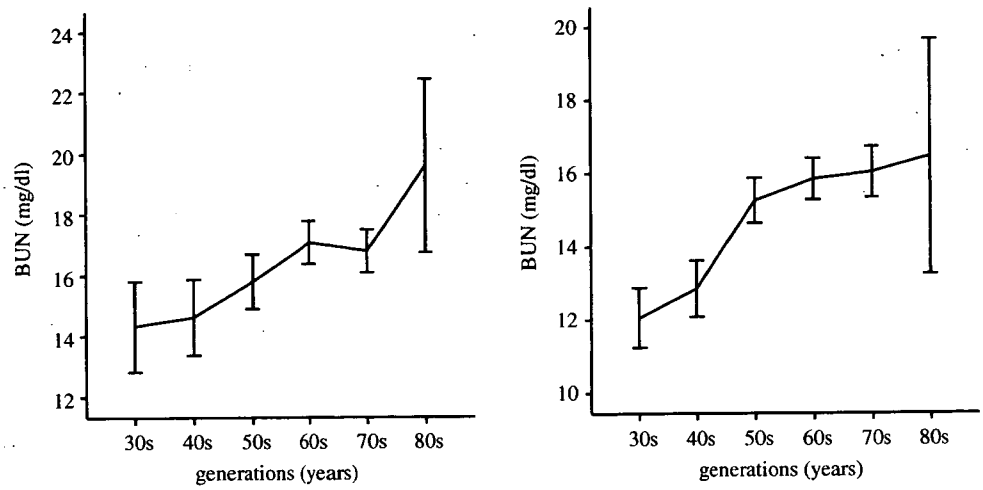

Figure 2 Mean BUN levels for each generation in males and females. Numbers of male and females subjects in each age group are given in the Fig. 1 legend. Conversion factor: mM = mg/dl × 0.357.

and free testosterone decreased almost linearly with aging, a pattern of change opposite to that of adiponectin (Fig. 3). In females, E1 and E2 sharply decreased up to the 50s age bracket, in contrast to the pattern of change in adiponectin (Fig. 4).

In multiple-regression analysis of age, BMI and sex hormones with LnAdipo as a dependent variable, free testosterone, which exhibits biological activity in humans, was not selected as a significant independent variable, whereas age and BMI were selected as significant independent variables in males. In females, E1 and E2 were also not selected as significant independent variables (Table 6).

Discussion

Previous studies showed that there is an inverse relationship between adiponectin level and creatinine clearance in essential hypertensives (10) and that aggravated renal function is one of the reasons for increase an in adiponectin level with aging (23). Another previous study showed that adiponectin level is positively associated with abnormal renal function, assessed by urinary albumin-to-creatinine ratio, in patients with diabetes (24). These studies suggest that a decrease in adiponectin clearance in the kidney may be the cause of high levels of adiponectin in the elderly, although it is unlikely to be the sole mechanism. Previous studies have shown that renal function declines with aging (26–29) and BUN is known as an indicator of renal function. It has been reported that BUN level is affected by aging (30) and that there is a significant positive correlation between BUN level and age (31). Therefore, we used BUN level as an indicator of renal function in this study.

Adiponectin increased linearly with aging in males, whereas in females it increased sharply until the 50s age bracket with a convex curve and then increased gradually (Fig. 1). The patterns of changes in adiponectin were similar to the patterns of changes in BUN (Fig. 2). In multiple-regression analysis using LnAdipo as a dependent variable, BUN was selected as a significant independent variable as well as sex differences, age, BMI, FPG, triglyceride and HDL in all subjects (Table 2) and BUN was also selected as a significant independent variable in the Age ≥65 group, whereas BUN was not selected as a significant independent variable in the Age <65 group (Table 4). These results suggest that decline of renal function with aging contributes independently to the elevation of adiponectin level. Since the biological significance of this elevation in adiponectin in the elderly is not known, further investigation is necessary to clarify the effects of increase in adiponectin in the elderly.

Studies conducted in Japan and other countries have demonstrated that sex hormone levels change with aging (25, 32–36). In Japan, the average age of

Table 2 Results of multiple-regression analysis related to LnAdipo in subjects from the first selection.

	β	*r*	V (%)	*P* value
Sex	0.331	0.373	12.3	<0.001
Age	0.240	0.170	4.1	<0.001
BMI	−0.170	−0.291	4.9	<0.001
SBP	−0.002	−0.010	0.0	0.946
FPG	−0.131	−0.197	2.6	<0.001
TC	−0.035	0.026	0.1	0.257
TG	−0.140	−0.318	4.5	<0.001
HDL	0.139	0.312	4.3	<0.001
BUN	0.086	0.078	0.7	0.002

Sex, males = 0, females = 1; BMI, body mass index; SBP, systolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; BUN, blood urea nitrogen; β, standardized regression coefficient; *r*, versus LnAdipo, Pearson's correlation; V, variation of LnAdipo, calculated by β × *r* × 100 in absolute value.

Table 3 Unpaired *t*-test between data for subjects from the first selection in the Age ≥65 and Age <65 groups and Pearson's correlation in each group.

	Males				Females			
	Age <65 (*n* = 171)		Age ≥65 (*n* = 201)		Age <65 (*n* = 368)		Age ≥65 (*n* = 224)	
	Mean±S.D.	*r*	Mean±S.D.	*r*	Mean±S.D.	*r*	Mean±S.D.	*r*
Age (years)	52.1±9.2*	0.166†	71.9±5.6	0.288†	51.5±9.5*	0.202†	70.7±4.6	0.022
BMI (kg/m^2)	24.5±3.8*	−0.197†	23.2±2.7	−0.371†	22.8±3.3*	−0.215†	23.6±3.1	−0.349†
SBP (mmHg)	126.5±17.9*	0.073	139.3±21.9	−0.175†	121.8±19.7*	−0.078	141.3±23.5	0.083
DBP (mmHg)	76.3±11.9	−0.004	75.6±12.0	−0.208†	71.7±11.8*	−0.107†	75.2±12.6	0.121
FPG (mg/dl)	96.4±15.0	−0.071	97.9±17.6	−0.193†	91.9±17.8*	−0.227†	95.8±13.6	−0.189†
TC (mg/dl)	194.8±32.2	−0.163†	191.9±33.6	−0.150†	200.1±33.6*	0.042	213.8±29.5	−0.025
TG (mg/dl)	131.4±94.3*	−0.306†	101.7±50.8	−0.348†	83.6±42.9*	−0.207†	98.6±42.9	−0.193†
HDL (mg/dl)	50.6±11.2	0.310†	52.1±12.0	0.256†	60.2±13.8*	0.204	57.7±13.1	0.226†
BUN (mg/dl)	15.9±3.9*	0.045	17.0±4.2	0.219†	14.3±3.9*	0.128†	16.0±3.8	0.134†
Cr (mg/dl)	1.07±0.12	−0.054	1.12±0.43	0.094	0.87±0.10*	0.036	0.93±0.40	0.090
Adipo (μg/ml)	4.96±2.41*		6.93±3.72		8.58±4.12*		9.45±4.27	

Age <65 group, group of subjects aged less than 65 years; Age ≥65 group, a group of subjects 65 years of age or older.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; BUN, blood urea nitrogen; Cr, serum creatinine; Adipo, adiponectin.
r, versus LnAdipo, Pearson's correlation coefficient.
* $P < 0.05$ versus the group of subjects 65 years of age or older, unpaired *t*-test.
† $P < 0.05$ versus LnAdipo, Pearson's correlation.
Conversion factors: FPG, mM = mg/dl × 0.05551; TC, mM = mg/dl × 0.02586; TG, mM = mg/dl × 0.01129; HDL, mM = mg/dl × 0.02586; BUN, mM = mg/dl × 0.3570; Cr, μM = mg/dl × 88.40.

menopause is about 50 years (35). It is known that the concentrations of adiponectin in the elderly are high (21, 22), but there has been little investigation of changes with aging. Investigation using mice revealed that androgens might inhibit the production of adiponectin (37) and that a decrease in sex hormones with aging might induce a gender difference in the process of elevation of adiponectin, because both testosterone and estrogen inhibited adiponectin, but the regulation by estrogen was weak and that by testosterone was strong (38). It has been reported that testosterone showed negative correlations with adiponectin in boys and that adiponectin levels decrease in parallel with the progression through puberty (39). Most of the subjects in the present study were middle-aged and elderly, and males tended to show a gradual decrease in testosterone in their 30s and an almost linear decrease in free testosterone from their 30s with aging (Fig. 3), whereas females showed a sharp drop in E1 and E2 in their 50s, the age of menopause (Fig. 4). Testosterone, free testosterone, E1 and E2 all changed with aging in manners consistent with previously reported findings (25, 32, 36). Adiponectin tended to increase with aging in both males and females (Fig. 1) (21, 22). It tended to increase linearly with aging in males, while it sharply increased with a convex curve in females until their 50s, the age of menopause. The patterns of changes in adiponectin seem to be mirror images of changes in free testosterone in males and changes in E1 and E2 in females. However, in multiple-regression analysis of age, BMI and sex hormones with LnAdipo

Table 4 Results of multiple-regression analysis related to LnAdipo in subjects from the first selection in the Age ≥65 and Age <65 groups.

	Age <65 group				Age ≥65 group			
	β	*r*	V (%)	*P* value	β	*r*	V (%)	*P* value
Sex	0.339	0.463	15.7	<0.001	0.337	0.317	10.7	<0.001
Age	0.199	0.153	3.0	<0.001	0.122	0.119	1.5	0.005
BMI	−0.119	−0.281	3.3	0.003	−0.248	−0.317	7.9	<0.001
SBP	−0.001	−0.081	0.0	0.975	−0.002	−0.023	0.0	0.969
FPG	−0.145	−0.216	3.1	<0.001	−0.109	−0.202	2.2	0.010
TC	−0.027	0.015	0.0	0.522	−0.037	0.024	0.1	0.445
TG	−0.153	−0.353	5.4	<0.001	−0.106	−0.271	2.9	0.027
HDL	0.140	0.345	4.8	0.001	0.131	0.291	3.8	0.007
BUN	0.046	0.006	0.0	0.231	0.127	0.127	1.6	0.003

Age <65 group, a group of subjects aged less than 65 years; Age ≥65 group, a group of subjects 65 year of age or older; Sex, males = 0, females = 1; BMI, body mass index; SBP, systolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; BUN, blood urea nitrogen; β, standardized regression coefficient; *r*, versus LnAdipo, Pearson's correlation; V, variation of LnAdipo, calculated by β × *r* × 100 in absolute value.

Table 5 Background of subjects from the second selection (mean values and correlation related to adiponectin).

	Males ($n = 123$)		Females ($n = 114$)	
	Mean±S.D.	r	Mean±S.D.	r
Age (years)	59.8±16.7	0.405†	56.6±15.6	0.182
BMI (kg/m^2)	23.9±3.4*	−0.364†	22.6±3.4	−0.138
T (ng/dl)	425.0±152.7	0.183†	–	–
Free T (pg/ml)	18.32±6.95	−0.182†	–	–
E1 (pg/ml)	–	–	37.2±30.3	−0.129
E2 (pg/ml)	–	–	58.9±76.3	−0.100
Adipo (μg/ml)	6.26±3.94*		8.84±4.71	

BMI, body mass index; T, testosterone; free T, free testosterone; E1, estrone; E2, estradiol.
r, versus LnAdipo, Pearson's correlation coefficient; –, unavailable.
* $P < 0.05$ versus females, unpaired t-test.
† $P < 0.05$ versus LnAdipo, Pearson's correlation.
Conversion factors: T, nM = ng/dl × 0.03467; free T, pM = pg/ml × 3.467; E1, pM = pg/ml × 3.699; E2, pM = pg/ml × 3.671.

as a dependent variable, free testosterone, which exhibits biological activity in humans, was not selected as a significant independent variable, whereas age and BMI were selected in males. In females, E1 and E2 were also not selected as significant independent variables (Table 6). These results indicate that the influence of bioactive sex hormones on changes in values of adiponectin with aging is not clear compared with the influence of decline of renal function on changes in values of adiponectin with aging.

One limitation in this study is the inconsistent timing of blood collection from premenopausal females, because samples were obtained from subjects undergoing periodical check-ups. For examination of female hormones in premenopausal females, blood should be collected at a certain time point of the menstrual period, such as the follicular phase (40) or luteal phase (41, 42), but there is a limitation to this in the setting of mass-screening tests. However, none of the enrolled females had a past history of gynecological disease, and since it was confirmed that E1 and E2 changed with aging in a pattern consistent with that reported previously, as shown in Fig. 4 (36), it is thought that the results reflect general changes in female sex hormones. Another limitation is that this investigation was a cross-sectional study. Therefore, more prospective studies may be necessary to clarify the relationship between aging and adiponectin.

In summary, we investigated the change in human adiponectin with aging separately in males and females and showed that there is a gender difference in the process of elevation of adiponectin. We also confirmed changes with aging in BUN in males and females and testosterone and free testosterone in males and E1 and E2 in females, which are consistent with findings reported previously (25, 32, 36). The patterns of changes in adiponectin were similar to patterns of changes in BUN and seemed to be a mirror image of patterns of changes in free testosterone, E1 and E2 on a graph. However, multiple-regression analysis showed that the decline of renal function with aging seemed to be more involved in the elevation of adiponectin with aging than were changes with aging in these sex hormones. In humans, especially in the elderly, a decrease in adiponectin clearance due to a slight decline of renal function with aging, assessed by the BUN levels, may cause increase in serum adiponectin concentrations. On the other hand, it may be because androgen inhibits the production of adiponectin that adiponectin is lower in males than in females (37). Therefore, in terms of the increase in adiponectin with aging in the elderly, adiponectin seems to be influenced more strongly by BUN than by sex hormones

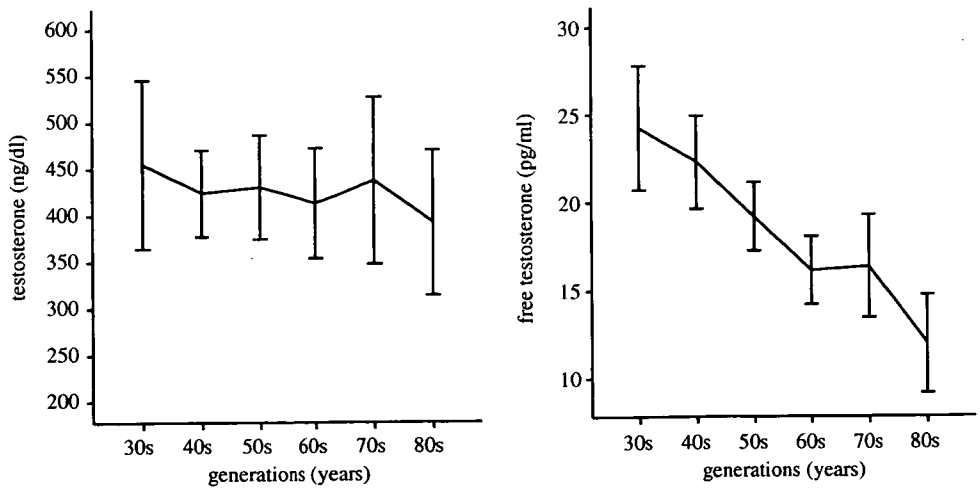

Figure 3 Mean plasma testosterone and free testosterone levels in males for each generation. Numbers of male subjects: 30s, $n = 19$; 40s, $n = 21$; 50s, $n = 21$; 60s, $n = 21$; 70s, $n = 21$; 80s, $n = 20$. Conversion factors: testosterone, nM = ng/dl × 0.03467; free testosterone, pM = pg/ml × 3.467.

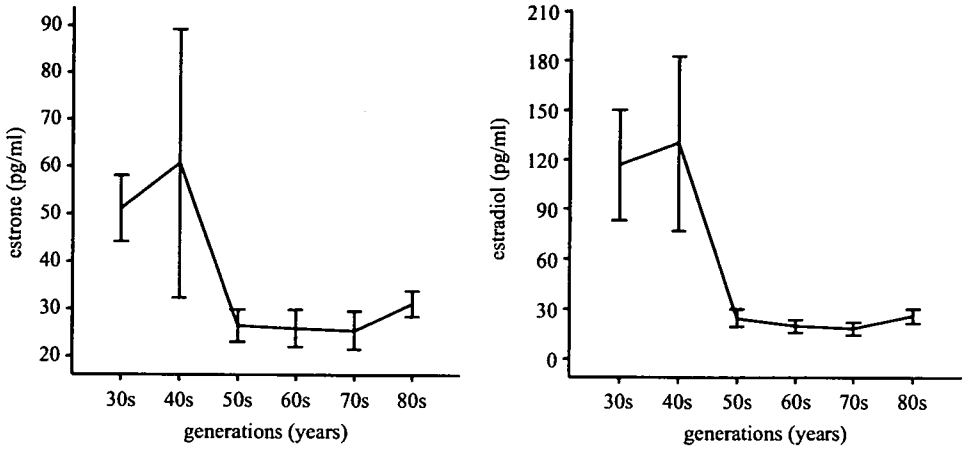

Figure 4 Mean plasma estrone (E1) and estradiol (E2) levels in females for each generation. Numbers of female subjects: 30s, $n = 21$; 40s, $n = 21$; 50s, $n = 21$; 60s, $n = 21$; 70s, $n = 21$; 80s, $n = 9$. Conversion factors: estrone, pM = pg/ml × 3.699; estradiol, pM = pg/ml × 3.671.

Table 6 Results of multiple-regression analysis related to LnAdipo in subjects from the second selection.

	Males ($n = 123$)					**Females** ($n = 114$)			
	β	*r*	V (%)	*P* value		β	*r*	V (%)	*P* value
Age	0.374	0.405	15.1	<0.001	Age	0.200	0.182	3.6	0.050
BMI	−0.253	−0.364	9.2	0.002	BMI	−0.176	−0.138	2.4	0.067
T	0.197	0.183	3.6	0.015	E1	−0.052	−0.129	0.7	0.601
Age	0.418	0.405	16.9	<0.001	Age	0.225	0.182	4.1	0.045
BMI	−0.301	−0.364	11.0	<0.001	BMI	−0.180	−0.138	2.5	0.060
Free T	0.134	−0.182	2.4	0.177	E2	0.017	−0.100	0.2	0.875

BMI, body mass index; T, testosterone; free T, free testosterone; E1, estrone; E2, estradiol; β, standardized regression coefficient; *r*, versus LnAdipo, Pearson's correlation; V, variation of LnAdipo, calculated by β × *r* × 100 in absolute value.

and to be increased by a decline in renal function with aging.

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Relation of hypertension and glucose tolerance impairment in elderly people to the development of arteriosclerosis: Investigation using pulse wave velocity*

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Background: The aim of the present study was to determine the correlation between the combination of hypertension and diabetes mellitus and arteriosclerosis using pulse wave velocity (PWV).

Methods: The subjects were 186 men over the age of 60 years (mean age: 68.8 ± 5.8 years). PWV, systolic blood pressure (SBP), diastolic blood pressure, body mass index, fasting blood sugar (FBS), total cholesterol, triglyceride and HDL cholesterol were measured in all subjects. The subjects were divided into three groups on the basis of FBS level: a normal group (FBS < 110 mg/dL), an impaired fasting glucose group (110 ≤ FBS < 126 mg/dL) and a diabetes mellitus group (FBS ≥ 126 mg/dL or taking antidiabetics). The subjects were also divided into two groups on the basis of blood pressure level: a hypertension (HT) group (SBP ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or taking antihypertensives) and a normotension group (other subjects).

Results: PWV showed positive correlations with SBP and FBS ($r = 0.499$ and $r = 0.300$, respectively). In all three groups classified by FBS level, PWV was higher in subjects with HT than in subjects with normotension ($P < 0.01$ in all three groups). In the HT group, PWV had already increased at the stage of impaired fasting glucose and was significantly higher in the diabetes mellitus group than in the normal FBS group ($P = 0.002$). In multiple regression analysis using PWV as a dependent variable, SBP and FBS were selected as independent variables.

Conclusions: Even in the elderly, strict control of blood pressure and blood sugar level may be necessary in order to prevent the development of arteriosclerotic diseases.

Keywords: arteriosclerosis, elderly people, glucose tolerance, hypertension, pulse wave velocity.

Introduction

Hypertension and impaired glucose tolerance are known as important risk factors for the progression of arteriosclerosis and also for the development of arteriosclerotic diseases. Even if both are mild, their combination is thought to promote the progression of

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arteriosclerosis. Pulse wave velocity (PWV), the speed of transmission of a pulse wave through the aorta, has been shown to be an indicator of arterial stiffness, and measurement of PWV has been used as a non-invasive method for assessing the progression of arteriosclerosis.[1,2] The simple device ABI-form (BP-203RPE; Nihon Colin, AT Co., Komaki) is now widely used for the measurement of PWV. We have used this device to measure PWV in male inhabitants of a community to evaluate the usefulness of PWV as an indicator of the progression of arteriosclerosis in impaired glucose tolerance, and the results of that study showed that PWV increased significantly in parallel with the advance of glucose tolerance impairment and suggested that arteriosclerosis is likely to have already progressed at the borderline blood sugar stage.[3]

As subjects for the present study, we selected elderly people in a general population, who are likely to already have advanced arteriosclerosis. We measured PWV using an ABI-form in each of the subjects, and we investigated the relationship between the combination of impaired glucose tolerance and hypertension and PWV in order to evaluate the progression of arteriosclerosis in elderly people.

Subjects and methods

The subjects were 186 male elderly people over the age of 60 years (mean age: 68.8 ± 5.8 years) randomly selected from 581 male residents of the towns of Tanno and Sobetsu in Hokkaido who underwent medical examinations in 2000. The design of this study was approved by our institutional ethical committee, and all subjects gave their informed consent to participate in this study.

Medical examinations were carried out between 06.00 hours and 08.00 hours after overnight fasting. After measuring body height and body weight, blood pressure was measured and then blood samples were collected. Systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), fasting blood sugar (FBS) level, total cholesterol (TC) level, triglyceride (TG) level, HDL cholesterol (HDL) level and PWV were measured in each subject. After relaxing in a sitting position for several minutes, brachial blood pressure was measured at least twice in each subject, and mean values of systolic and diastolic blood pressure were used for analysis. Blood samples were collected from the cubital vein using a vacuum blood collecting tube with the subject in a sitting position. The methods used for blood chemistry were the glucose oxidase electrode method for FBS, cholesterol-oxidase-dimethoxy-anilinehydroxy-3-sulfoproxyl (DAOS) method for TC, glycerol-3-phosphte-oxidase·DOAS method for TG, and dextran-sulfate magnesium-chloride precipitation method for HDL.

An ABI-form (BP-203RPE; Nihon Colin, AT Co., Komaki) was used for the measurement of PWV. PWV between the brachial artery and the ankle artery (brachial–ankle PWV; baPWV)[4] was measured on both sides, and the mean of the right and left values was used for analysis. The ABI-form simultaneously measures blood pressure levels in both arms and both legs by using four cuffs and also records plethysmograms of the right arm and both legs by means of sensors in the cuffs. From blood pressure levels of the arms and legs, it calculates the ankle–brachial pressure index. It stores data on the starting point of each pulse wave in the right arm and both legs in memory and records the time difference between transmission time to the arm and transmission time to the ankle as transmission time. It calculates transmission distance from the right arm to each ankle according to body height. Then it automatically computes baPWV from transmission time and transmission distance and outputs the result. The ABI-form enables simultaneous measurements of ABI and baPWV with almost no error due to the operator's technique, and the device is thus considered to be useful for mass medical examinations and epidemiological studies on many people.[3]

In the present investigation, an ABI of 0.9 or less was considered to be a positive sign of arteriosclerosis obliterans.[5] In cases of low ABI due to arteriosclerosis obliterans, baPWV is not thought to be a reliable index of arteriosclerosis because the transmission speed of the pulse wave is reduced due to a decline in blood pressure. Thus, subjects with ABI ≤ 0.9 were excluded from the present study. Subjects with a past history of arteriosclerotic diseases such as angina pectoris, myocardial infarction and cerebral infarction were also excluded.

According to the American Diabetes Association (ADA) criteria for diagnosis of diabetes,[6] the subjects were divided into three groups on the basis of FBS level: a normal (NGT) group consisting of subjects with FBS < 110 mg/dL, an impaired fasting glucose (IFG) group consisting of subjects with 110 ≤ FBS < 126 mg/dL, and a diabetes mellitus (DM) group consisting of subjects with FBS ≥ 126 mg/dL or subjects taking antidiabetics. The subjects were also divided into two blood pressure groups, a hypertension (HT) group and a normotension (NT) group, based on the standards of JNC-VI and WHO/ISH criteria for diagnosis of hypertension.[7,8] The HT group consisted of subjects with SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or of subjects taking antihypertensives, and the NT group consisted of the remaining subjects. By a combination of classification based on FBS and classification based on blood pressure, the subjects were categorized into six groups: NGT-NT (78 subjects, 68.7 ± 5.9 years old), NGT-HT (65 subjects, 69.3 ± 5.8 years old), IFG-NT (eight subjects, 67.0 ± 5.2 years old), IFG-HT (13 subjects, 67.1 ± 4.6 years old), DM-NT (15 subjects,

68.5 ± 5.1 years old) and DM-HT (seven subjects, 72.7 ± 8.0 years old). Parameters in these six groups were compared.

The Japanese edition of Windows SPSS version 10.0 (SPSS Japan Inc.) was used for statistical analysis. All numerical values are expressed as means ± standard deviations. The unpaired *t*-test was used for examination of differences between two groups. For examination of differences in three groups or more, multiple comparisons were performed using Bonferroni's method. Multiple regression analysis was performed using baPWV as a dependent variable. The significance level was set at $P < 0.05$.

Results

There were no significant differences in the six groups with regard to age, BMI, TC, TG and HDL. Significant differences were found between SBP levels in the NT and HT groups ($P < 0.05$) and between FBS levels in the NGT, IFG and DM groups ($P < 0.05$). Significant differences were also found in the levels of DBP ($P < 0.05$) except for the differences between the NGT-HT and IFG-NT groups, IFG-NT and IFG-HT groups, and IFG-NT and DM-HT groups (Table 1). The proportions of HT subjects taking antihypertensives were 53.8% in NGT-HT group, 61.5% in the IFG-HT group and 57.1% in the DM-HT group. The differences were not significant. Significant positive correlations were found between baPWV and SBP ($r = 0.499$, $P < 0.001$) and between baPWV and FBS ($r = 0.300$, $P < 0.001$) (Fig. 1), and positive correlations between baPWV and SBP and between baPWV and FBS were also found in analyses from which data from subjects undergoing therapy for hypertension and diabetes mellitus had been excluded. No correlation was found between baPWV

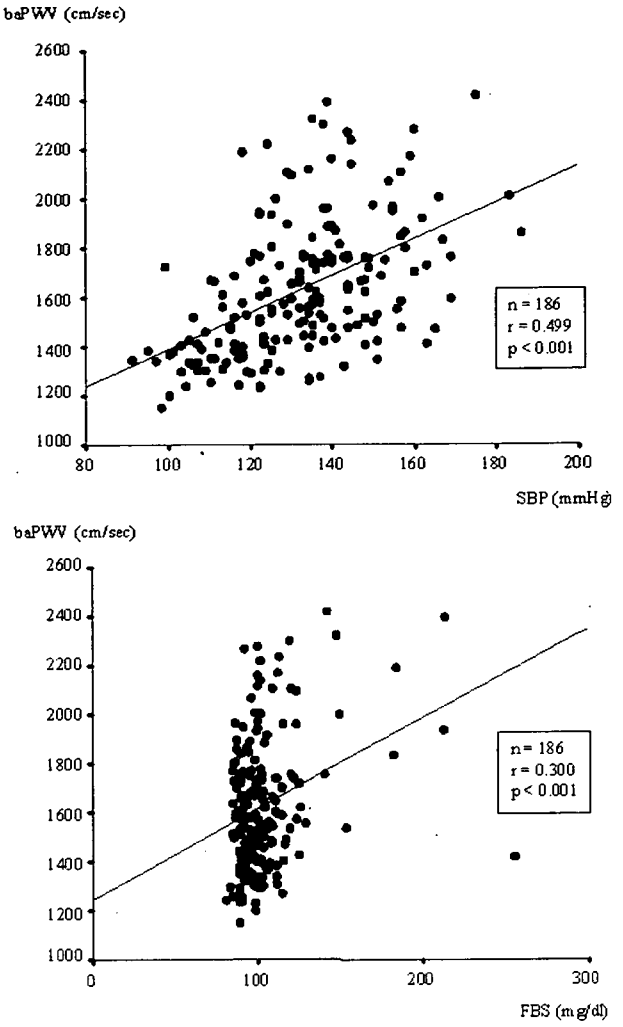

Figure 1 Correlations between systolic blood pressure (SBP) and brachial–ankle pulse wave velocity (baPWV) and between fasting blood sugar (FBS) and baPWV.

Table 1 Background of subjects

Group	NGT-NT	NGT-HT	IFG-NT	IFG-HT	DM-NT	DM-HT
n	78	65	8	13	15	7
Age	68.7 ± 5.9	69.3 ± 5.8	67.0 ± 5.2	67.1 ± 4.6	68.5 ± 5.1	72.7 ± 8.0
BMI (kg/m^2)	22.8 ± 2.8	23.7 ± 2.7	24.7 ± 3.8	24.9 ± 2.3	23.5 ± 3.1	23.4 ± 1.8
SBP (mmHg)	119.5 ± 12.1	145.1 ± 15.0	123.9 ± 13.1	147.1 ± 11.3	124.7 ± 9.2	149.6 ± 16.7
DBP (mmHg)	72.6 ± 8.7	83.2 ± 9.9	74.9 ± 8.8	81.3 ± 8.4	70.7 ± 7.4	83.9 ± 12.0
FBS (mg/dL)	94.7 ± 6.4	95.4 ± 6.3	115.6 ± 5.7	115.5 ± 4.9	137.0 ± 46.1	157.6 ± 31.2
TC (mg/dL)	183.5 ± 31.5	190.1 ± 27.5	189.5 ± 33.7	179.0 ± 35.3	194.3 ± 29.9	215.0 ± 34.4
TG (mg/dL)	106.1 ± 46.1	120.2 ± 71.9	176.6 ± 182.1	120.7 ± 44.7	131.9 ± 69.4	107.0 ± 36.4
HDL (mg/dL)	53.1 ± 14.8	54.2 ± 13.6	49.0 ± 9.1	47.2 ± 15.6	58.6 ± 11.9	50.8 ± 8.9

Values are means ± standard deviations. The proportions of hypertension subjects taking antihypertensives were 53.8% in the NGT-HT group, 61.5% in the IFG-HT group and 57.1% in the DM-HT group. There were no significant differences among three groups.

BMI, body mass index; DBP, diastolic blood pressure; FBS, fasting blood sugar; HDL, high-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

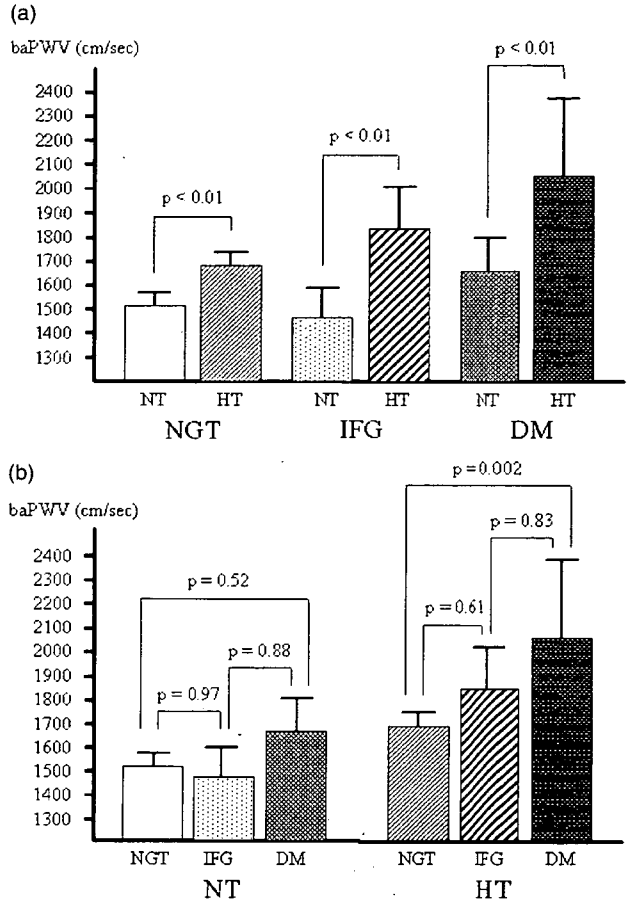

Figure 2 (a) Brachial–ankle pulse wave velocity (baPWV) for three groups classified by plasma glucose level. (NT, normal blood pressure; HT, hypertension, systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or therapy for hypertension; NGT, normal fasting glucose, fasting blood sugar < 110 mg/dL; IFG, impaired fasting glucose, 110 ≤ fasting blood sugar < 126 mg/dL; DM, diabetes mellitus, fasting blood sugar ≥ 126 mg/dL or therapy for diabetes mellitus). (b) Brachial–ankle pulse wave velocity (baPWV) for three groups classified by blood pressure. (NT, normal blood pressure; HT, hypertension, systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or therapy for hypertension; NGT, normal fasting glucose, fasting blood sugar < 110 mg/dL; IFG, impaired fasting glucose, 110 ≤ fasting blood sugar < 126 mg/dL; DM, diabetes mellitus, fasting blood sugar ≥ 126 mg/dL or therapy for diabetes mellitus).

Table 2 Multiple regression analysis related to brachial–ankle pulse wave velocity (baPWV)

	β	S.E.	*t*	*P*
Age	0.282	2.824	4.707	< 0.001
BMI	0.012	6.008	0.195	N.S.
SBP	0.463	0.904	7.669	< 0.001
FBS	0.241	0.716	4.101	< 0.001
TC	0.67	46.586	1.101	N.S.

BMI, body mass index; β, standardized regression coefficients; FBS, fasting blood sugar; SBP, systolic blood pressure; S.E., standard error of the mean; TC, total cholesterol.

and BMI ($r = 0.088$, $P = 0.234$) or between baPWV and TC ($r = 0.056$, $P = 0.446$). The values of baPWV were 1515 ± 231 cm/s in the NGT-NT group, 1687 ± 237 cm/s in the NGT-HT group, 1465 ± 154 cm/s in the IFG-NT group, 1834 ± 294 cm/s in the IFG-HT group, 1660 ± 253 cm/s in the DM-NT group and 2053 ± 350 cm/s in the DM-HT group. In the NGT, IFG and DM groups, baPWV was significantly higher in the subjects with HT than in the subjects with NT ($P < 0.01$) (Fig. 2a). The value of baPWV was significantly higher in HT subjects in the DM group than in HT subjects in the NGT group ($P = 0.002$), and baPWV increased in parallel with an increase in FBS, showing a tendency to be high even at the stage of IFG (Fig. 2b).

Multiple regression analysis was performed on baPWV with regard to age, BMI, SBP, FBS and TC, and then age, SBP and FBS were selected as independent variables. It was shown that both blood pressure and blood sugar level independently contribute to elevation of baPWV (Table 2). A significant correlation was found between pulse pressure (PP) and baPWV. PP was also selected as a significant predictor variable in multiple regression analysis performed on baPWV (data not shown).

Discussion

Measurement of PWV is a non-invasive method for estimating the progression of arteriosclerosis.[1,2] As is indicated by the results of the present study, blood pressure is one of the factors affecting PWV: Changes in blood pressure cause changes in arterial wall tension, resulting in changes in elasticity of the arterial wall, and this change in elasticity of the arterial wall causes changes in PWV. Since the magnitude of change in blood pressure depends on the condition of the arterial wall, adjustment of PWV by blood pressure is difficult. In the present study, it is thought that there was little effect of blood pressure on baPWV because baPWV was measured after the subject had relaxed in the supine position for about 5 min.

It has been reported that baPWV measured using an ABI-form and carotid–femoral PWV (cfPWV)[9] measured by the conventional method show a significant positive correlation, indicating that baPWV, as well as cfPWV, can be used as a clinical indicator of progression of arteriosclerosis.[10] ABI-form is a simple and convenient device with excellent reproducibility of measurements,[4] and it is possible that this device will be used in general medical practice in the future for estimation of progression of arteriosclerosis. The development of arteriosclerotic diseases has been used in clinical practice as an end-point indicator of the progression of arteriosclerosis. However, this indicator is not sufficient from the point of view of prevention of cardiovascular diseases. If it becomes possible to detect relatively mild